

Constitutive expression of methyl jasmonate-inducible responses delays reproduction and constrains fitness responses to nutrients in *Arabidopsis thaliana*

Don Cipollini

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Abstract The analysis of a suite of traits under a range of environmental conditions is necessary to fully examine costs of resistance. I compared phenological, morphological and reproductive traits of several independent lines of a transgenic methyl jasmonate-overproducing *Arabidopsis thaliana* plant (JMT plants) to that of vector controls in high and low soil nutrient environments. JMT plants constitutively express a suite of responses normally inducible by wounding or jasmonate. JMT plants showed a marked delay in flowering time, and were larger with more leaves at bolting than vector controls. These traits also responded more positively to increased nutrients in JMT plants than in vector controls. At the end of the season, total seed mass and seed number was lower in JMT plants than in vector controls overall, and these traits responded much less positively to nutrient addition in JMT plants than in vector controls. This study revealed delayed phenology as a novel cost of resistance in *Arabidopsis* and that overproduction of methyl jasmonate and associate responses can substantially constrain fitness responses to nutrients. These results establish a mechanism whereby costs of resistance can be more apparent under high resource conditions, rather than under low resource conditions, as is widely assumed.

Keywords *Arabidopsis thaliana* · Costs of resistance · Jasmonic acid carboxyl methyl transferase · Methyl jasmonate · Phenology · Phenotypic plasticity

Introduction

Costs and benefits of plant resistance to herbivores and pathogens have been studied intensively for over 30 years. While still debated, a wealth of evidence has accumulated that the expression of plant resistance incurs costs to fitness in the absence of benefits (Heil 2002; Heil and Baldwin 2002; Cipollini et al. 2003). Such costs can be seen as direct

D. Cipollini (✉)
Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Highway, Dayton,
OH 45435, USA
e-mail: don.cipollini@wright.edu

reductions in growth and fitness that are independent of the environment, or indirect, mediated by alterations in the ways that defended plants interact with their environment. Much of the recent evidence for costs has stemmed from the study of inducible defenses in single plant genotypes, or the study of mutant and transgenic plants with altered defense expression, each of which has allowed careful control over sources of variation, such as genotypic background, environmental conditions, and degree of defense expression (e.g., Zavala et al. 2004; Cipollini 2007). Such studies have generally supported the view that, in contrast to constitutive expression, employment of inducible defenses is a cost-saving strategy whereby costly defenses are only produced in environments where they may confer fitness benefits to the individual. However, exploring a wide range of plant traits in a range of environmental conditions can reveal novel sources of costs of resistance (Koricheva 2002).

An additional cost of inducible defense production that has been infrequently studied is the potential interactions of the induction of defense (a plastic trait) with the expression of other forms of phenotypic plasticity that minimizes the potential fitness benefits of the plastic response. This sort of cost may stem from resource constraints imposed by allocation to defense production or through interactions between signaling pathways for defense production and developmental or hormonal responses necessary for the induction of appropriate phenotypic responses (Cipollini 2004). This type of cost is not detectable without altering multiple aspects of the growth environment of a plant, but some evidence for it exists. After demonstrating that induction of defenses in *Arabidopsis thaliana* (hereafter *Arabidopsis*) with repeated jasmonic acid applications (a hormone treatment intended to simulate herbivore attack) was costly to fitness (Cipollini 2002), I showed that induction of defenses in *Arabidopsis* with jasmonic acid constrained shade-induced increases in petiole length of leaves, a potentially important part of the shade avoidance response (Cipollini 2005). Izaguirre et al. (2006) observed the inverse in wild tobacco, *Nicotiana longiflora*, in which induction of shade-avoidance responses inhibited induction of herbivore resistance traits. In turn, overproduction of methyl jasmonate and constitutive expression of jasmonate-inducible responses in a transgenic genotype of *Arabidopsis* was directly costly to fitness and also constrained the fitness benefits of a release from competition (Cipollini 2007). This indicated that both exogenous hormone-induced and constitutive expression of jasmonate-inducible plant responses apparently constrained the ability of the plant to respond phenotypically to resource opportunities.

It is widely held that costs of defense production in plants (in the absence of benefits) should be more apparent in low resource environments where tradeoffs with growth would have severe fitness consequences (Bazzaz et al. 1987; Herms and Mattson 1992). Despite this conventional wisdom, experimental studies addressing this hypothesis have produced mixed results (e.g., Cipollini 2002; Marak et al. 2003; Donaldson et al. 2006; Osier and Lindroth 2006; Stevens et al. 2007) and a meta-analysis revealed that phenotypic correlations between resistance and fitness in plants were more likely to be negative at high nutrient levels than at low nutrient levels (Koricheva 2002). If expression of constitutive or inducible defenses constrains plant phenotypic responses to resource opportunities, as evidence with *Arabidopsis* indicates, then fitness differences between defended and undefended plants (i.e., costs) may actually be more apparent in high resource environments where undefended plants have a higher “unconstrained” growth potential.

To examine whether the expression of inducible defenses constrains plant responses to nutrients, I compared phenological, morphological and reproductive traits of several independent lines of a transgenic methyl jasmonate-overproducing *Arabidopsis* plant to

that of vector controls in high and low soil nutrient environments. The transgenic plants used here constitutively express a variety of defense responses that are normally inducible by wounding or jasmonate (Seo et al. 2001; Jung et al. 2003). I predicted that the methyl jasmonate-overproducing genotypes would express dampened phenotypic responses to nutrient availability and benefit less from increased nutrients than vector controls. In so doing, costs of the overproduction of methyl jasmonate and associated jasmonate-mediated responses would be more apparent at high resource levels.

Materials and methods

The transgenic JMT genotypes and vector controls used in this experiment are described in detail in Cipollini (2007). Briefly, JMT plants have been transformed to overexpress *S*-adenosyl-L-methionine: jasmonic acid carboxyl methyltransferase (JMT), which specifically methylates jasmonic acid (Seo et al. 2001; Schaller et al. 2005). As a consequence, JMT plants constitutively produce threefold higher methyl jasmonate and twofold higher total jasmonate levels in their leaves than wild-type plants (Seo et al. 2001), and exhibit constitutively elevated methyl-jasmonate responsive genes in their leaves, including defensins, various PR proteins, and oxidative stress related genes (Seo et al. 2001; Jung et al. 2003, 2007). In turn, JMT plants are more resistant than wild-type plants or vector controls to attack by the fungus *Botrytis cinerea* (Seo et al. 2001), the bacterium *Pseudomonas syringae* pv. *tomato* (Jung et al. 2003), and the generalist herbivore, *Spodoptera exigua* (D. Cipollini, unpublished data). In this study, I grew three independent lines of JMT plants and three independent empty vector controls (all in the Columbia-0 background) from seed in Pro-Mix BX potting soil (Grace-Sierra, Red Hill, PA) in the greenhouse, as in Cipollini (2007). Replicate plants of each line were exposed to one of two soil nutrient environments, including a complete fertilizer treatment and an unfertilized treatment. Plants receiving fertilizer received a solution of Peter's 20-20-20 Complete plus micronutrients fertilizer (Grace-Sierra, Milpitas, CA) in water during weeks 3, 5, 7, 9, and 11 of growth. Each fertilized plant received 50 ml of fertilizer at a concentration of 0.57 g/l during the first three treatments and 50 ml at a concentration of 0.85 g/l during the last two treatments. Unfertilized plants received no additional fertilizer beyond that in the starter medium, but received an equivalent volume of distilled water at the same times. There were eight replicate plants in each treatment combination, but three replicates were destroyed during the experiment. As plants grew, the number of days from initial planting until bolting started was recorded for each plant. Rosette diameter was measured and the number of leaves was counted when plants began to bolt, and main stem height was measured when the stem ceased elongating. Siliques were collected as they matured, and the lengths of the three longest siliques on each plant were measured. When all siliques were collected, seeds were separated from silique walls and total seed mass was determined for each plant. Seeds from each line were then pooled across nutrient treatments and the mean mass of individual seeds for each line was determined by randomly drawing an aliquot (~500–1,000) of seed from each line, weighing it, and counting the number of seeds in the aliquot. Total seed number of plants of each line in each nutrient treatment was calculated by dividing the total seed mass of each plant by the mean individual mass of a seed of that line. Data on all traits were first included in a fixed factor nested MANOVA with genotype (JMT, vector), replicate line nested within genotype, and nutrient treatment (high or low), as main effects, including all interactions. This was followed with univariate nested ANOVA for each trait using the same factors. Means were compared with Tukey's

tests. Variation among replicate lines within the JMT and vector control genotypes was accounted for in the statistical analyses and means for all treatment combinations are shown in the figures, but the results and discussion emphasize the mean response of replicate lines within a genotype to the nutrient treatments. All data analyses were conducted using SAS (Version 9.1, SAS Institute).

Results

The multivariate phenotype of *Arabidopsis* varied significantly between genotypes, nutrient environments, and lines within genotype, and was significantly affected by the genotype \times nutrient and the lines within genotype \times nutrient interactions (Table 1).

Univariate analyses revealed that JMT plants took over 20 days or 45% longer to bolt than vector controls on average across nutrient treatments, but fertilization reduced the time to bolt by \sim 20 days in JMT plants and by \sim 5 days in vector controls (Table 2; Fig. 1a). Fertilization substantially increased rosette diameter at bolting in both genotypes, but rosette diameter was slightly smaller in JMT plants than vector controls without fertilizer, and slightly larger in JMT plants than vector controls when fertilized (Table 2; Fig. 2b). JMT plants had more leaves at bolting than vector controls across nutrient treatments, and fertilization increased leaf number at bolting by \sim 8 leaves over unfertilized plants for JMT plants, and by \sim 6 leaves over unfertilized plants for vector controls (Table 2; Fig. 1c.).

Table 1 Results from nested MANOVA of the effects of genotype (JMT vs. vector controls), replicate lines nested within genotype, nutrients (+ or –) and all interactions on the multivariate phenotype of *Arabidopsis*

Source	Wilk's lambda	F-value
Genotype	0.052	186***
Line (genotype)	0.092	8.72***
Nutrients	0.037	264***
Genotype \times Nutrients	0.068	140***
Line (genotype) \times Nutrients	0.218	4.89***

* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P \leq 0.001$

Table 2 F-values from univariate ANOVA on the effects of genotype, nutrients, and all interactions on fitness-related traits of *Arabidopsis*

Source	Days to bolt	Rosette diameter	Leaf number	Stem height	Silique length	Total seed mass	Total seed number
Genotype	151.56***	4.07*	34.09***	4.38*	23.28***	49.53***	181.39***
Line (genotype)	28.74***	1.75	25.01***	2.51*	3.51*	4.24**	5.59***
Nutrients	60.72***	1582.36***	71.16***	76.92***	47.84***	574.22***	626.53***
Genotype \times Nutrients	19.18***	19.18***	4.63*	4.25*	0.39	45.84***	155.46***
Line (genotype) \times Nutrients	7.86***	4.39**	3.43*	7.89***	1.34	3.54*	4.57**

* $0.05 > P > 0.01$, ** $0.01 > P > 0.001$, *** $P \leq 0.001$

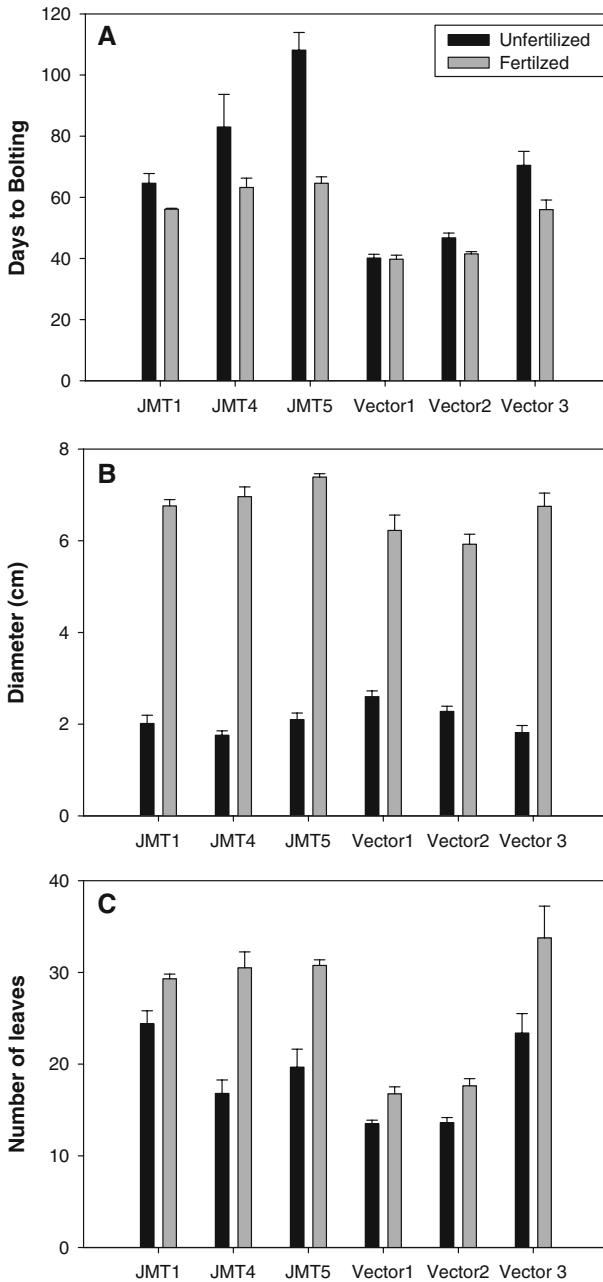


Fig. 1 Phenological and morphological traits of JMT and vector control lines of *Arabidopsis* at bolting exposed to low and high levels of soil nutrients. **a** Number of days until bolting, **b** rosette diameter at bolting, **c** leaf number at bolting. Each bar represents the means (\pm SE) of one replicate line within each genotype, with eight replicates in each line

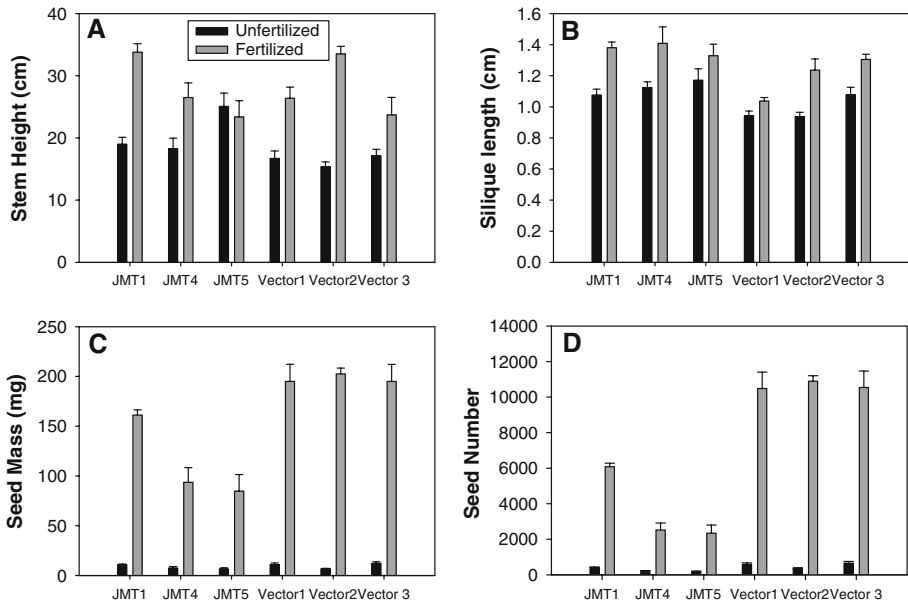


Fig. 2 Morphological and reproductive traits of JMT plants and vector control lines of *Arabidopsis* at the end of the season exposed to low and high levels of soil nutrients. **a** Main stem height, **b** mean length of the three largest siliques, **c** total seed mass. **d** Total seed number. Each bar represents the means (\pm SE) of one replicate line within each genotype, with eight replicates in each line

Fertilization substantially increased main stem height measured at the end of the season in both genotypes, but vector controls showed a 1.75-fold increase in height in response to fertilization, while JMT plants showed only a 1.35-fold increase (Table 2; Fig. 2a). The mean length of the three largest siliques was greater in JMT plants than in vector controls, and was increased by fertilization equally in both genotypes (Table 2; Fig. 2b). Total seed mass was increased substantially by fertilization in both genotypes, but vector controls showed a nearly 20-fold increase in total seed mass in response to fertilization, while JMT plants showed only a 13.5-fold increase (Table 2; Fig. 2c). Total seed number followed a very similar pattern as total seed mass (Table 2; Fig. 2d). Differences in total seed number between JMT and vector control lines were even more pronounced than differences in total seed mass, because individual seeds of JMT plants were over twice as heavy as individual seeds of vector control lines (data not shown).

Discussion

Past work has revealed inconsistencies in the environmental dependence of costs of resistance (Cipollini 2002; Marak et al. 2003; Dietrich et al. 2005). Here, I show that soil nutrient availability and methyl jasmonate phenotype independently and interactively affected the multivariate phenotype of *Arabidopsis*. In particular, phenotypic responses to nutrients varied between vector controls and genotypes of *Arabidopsis* that overproduce methyl jasmonate and constitutively express a suite of jasmonate-mediated responses. One such effect led to the appearance of greater differences in fitness between defended and undefended plants at high resource availability than at low resource availability.

One of the most striking independent effects of the overproduction of methyl jasmonate was the substantial delay in bolting exhibited by JMT plants. This phenological trait was not monitored in earlier studies of fitness traits in JMT plants (Cipollini 2007), but it may represent an important ecological cost of the expression of jasmonate-inducible responses (Heil 2002; Cipollini et al. 2003). Delays in the onset of reproduction in response to jasmonate application have been observed in some studies using crucifers or other species. Relative to uninduced controls, Agrawal et al. (1999) observed a 6% delay in the onset of flowering in jasmonic acid-induced wild radish while Redman et al. (2001) observed an 8% delay in fruit set in tomato treated with a slightly higher concentration of jasmonic acid. These delays were of much smaller magnitude than the 45% delay in the onset of bolting observed in JMT plants, which is likely due to the constitutive overproduction of jasmonate-inducible responses in JMT plants, rather than transient increases in these responses that would be observed in experiments using jasmonate applications. For *Arabidopsis*, earlier flowering is often correlated with increased fitness in even highly controlled conditions (Tonsor and Scheiner 2007). In the field, substantially delayed reproduction as a result of defense production may place plants in a maladaptive environment including increased competition, altered herbivore exposure, and unfavorable climatic conditions (Boege and Marquis 2005). Although not important for highly selfing species such as *Arabidopsis*, altered exposure to pollinators caused by delays in flowering may have particularly important fitness consequences for outcrossing species dependent on pollinator services (Strauss et al. 1999). The relationship between methyl jasmonate overproduction and flowering time seen here suggests that the distinction between “early” and “late” flowering *Arabidopsis* ecotypes may be associated with the methyl jasmonate phenotype of individual ecotypes. If so, then relationships between resistance phenotypes and flowering time phenotypes may also exist (i.e., late flowering ecotypes may be more resistant than early flowering ecotypes). This would make sense ecologically, as late flowering ecotypes would presumably have greater exposure to herbivores than early flowering ecotypes. However, while fertilization reduced the number of days to bolting in both genotypes, it did so more strongly in JMT plants. This indicates that the phenotypic response to nutrient availability in this trait was not constrained by constitutive expression of jasmonate-inducible responses. Rosette size and leaf number at bolting were higher in JMT plants than in vector controls, owing to the fact that JMT plants were about 20 days older on average than vector controls when they reached this stage. Like bolting time, there was no evidence that changes in these traits in response to nutrient availability were constrained by constitutive expression of jasmonate-inducible responses, as JMT plants responded more strongly to nutrients than vector controls.

Traits measured at the end of the season showed a different pattern of response among genotypes and nutrient treatments. In particular, main stem height was increased by fertilization less strongly in JMT plants than in vector controls, indicating a constraint on height growth responses to nutrients associated with constitutive expression of jasmonate-inducible responses. Recently, a gene that mediates growth suppression by jasmonate in *Arabidopsis* was identified (Yan et al. 2007). This discovery indicates that some of the “costs” of defense production may not only be due to passive resource constraints of defense production on growth, but instead are part of an adaptive program by induced plants to divert resources away from certain aspects of growth toward defense production (Hermsmeier et al. 2001; Jung et al. 2007). Length of the three largest siliques was greater in JMT plants overall, but it was increased by fertilization in a similar fashion in both genotypes; the only trait in which phenotypic responses to nutrients did not vary among

genotypes. Since siliques were not measured randomly, it is not clear whether average silique length varied among JMT and vector control lines.

Perhaps more important than responses of vegetative traits, total seed mass produced by JMT plants was lower than vector controls overall. This was expected on the basis of physiological costs of the overexpression of jasmonate-mediated responses seen previously in JMT plants (Cipollini 2007) or in Arabidopsis plants induced with exogenous jasmonates (Cipollini 2005, 2007). Like total seed mass, total seed number of JMT plants was only 40% of that of vector control lines in the absence of fertilization. Although individual seeds of JMT plants were over twice as heavy as individual seeds of vector control lines, seeds of JMT plants germinate at a lower percentage (Cipollini 2007). In addition to genotypic differences in production, total seed mass and seed number responded much less positively to fertilization in JMT plants than in vector controls. It was in these traits (most closely associated with fitness) that constraints on phenotypic responses to nutrient availability associated with constitutive expression of jasmonate-inducible responses were most apparent. It is not known whether this effect is unique to Arabidopsis, or unique to methyl jasmonate-mediated responses. However, the phenomenon observed here is similar to that observed in competing tobacco plants, where costs of jasmonate-induced defenses were more apparent under high nitrogen supply than at low nitrogen supply because uninduced plants produced many more seed capsules at high nitrogen supply than at low (van Dam and Baldwin 2001). Whether alterations in the response to nutrients caused by JMT overexpression of any of the other phenotypic responses measured here caused this effect was not clear. Other features of methyl jasmonate overproduction could have contributed directly to this finding, but this seems unlikely. Jasmonate is required for proper pollen development in Arabidopsis, and mutant plants lacking jasmonate are male sterile (McConn and Browse 1996). However, jasmonate is only required for pollen maturation, not the commitment to pollen production, and there is no evidence as of yet that elevated levels of jasmonate directly affect floral development (J. Browse, personal communication).

In addition to the phenotypic traits measured here, variation in root-related morphological traits or physiological responses of any sort may be responsible for variation in response to nutrients among JMT and vector controls. For example, it is well known that exogenous jasmonate treatment decreases root length in Arabidopsis, and it lowers photosynthetic rates in a number of species (Staswick et al. 1992; Metodiev et al. 1996). These traits have not been examined in JMT plants, but some jasmonate-mediated responses that are altered in JMT plants include reduced expression of several photosynthesis-associated genes (Jung et al. 2007). Such effects are all part of the costs of methyl jasmonate overproduction and may physiologically constrain the ability of JMT plants to fully respond to increases in soil nutrient availability. If jasmonate accumulation, which commonly accompanies induction by herbivore damage in a range of plant species (Schaller et al. 2005), also reduces root length as a rule across plant species, then constraints on responses to soil quality should be robust across species. Other inducible defense mechanisms controlled by hormones such as salicylic acid or ethylene may not elicit the same negative interactions with phenotypic responses to nutrient availability (e.g., Dietrich et al. 2005).

The finding that fitness of JMT plants responded less strongly to increased nutrient availability than in vector controls supports the notion that costs of resistance can be more apparent at high resource availability than at low resource availability owing to constraints on the ability of highly defended plants to express phenotypic responses to resource opportunities (Cipollini 2007). This effect may explain cases in the literature where costs of resistance were no more apparent (or often less apparent) at low resource levels than at high resource levels (Koricheva 2002). The magnitude of this effect may depend on the

nature of the defenses examined (e.g., constitutive vs. inducible, carbon- vs. nitrogen-based, restricted to particular biosynthetic pathways, etc.). The magnitude may in turn depend on what resources are altered, which in past studies of environmental effects on costs has focused largely on soil based resources or the presence of competitors (but see Osier and Lindroth 2006). Regardless, trade offs between defense production and the expression of plasticity to resource opportunities may be an important selective factor favoring inducible defense production, rather than constitutive production. This tradeoff may be particularly important for fast-growing early successional species (like *Arabidopsis* and wild tobacco) that rely on rapid colonization and growth responses to resource opportunities in their environment. Delays in phenology associated with defense production, as seen here, may also be particularly important in these environments. Understanding the full range of costs of resistance and the expression of specific resistance genes requires placing plants in a broader range of environmental conditions and assessing a broader range of traits associated with fitness.

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