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A natural mutational event uncovers a life history trade-off via hormonal pleiotropy

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SUMMARY

Environmental signals often control central life history decisions, including the choice between reproduction and somatic maintenance. Such adaptive developmental plasticity occurs in the nematode *Caenorhabditis elegans* where environmental cues govern whether larvae will develop directly into reproducing adults or arrest their development to become stress-resistant dauer larvae. Here we identified a natural variant underlying enhanced sensitivity to dauer-inducing cues in *C. elegans*: a 92 base-pair deletion in the *cis*-regulatory region of the gene *eak-3*. This deletion reduces synthesis or activity of the steroid hormone dafachronic acid (DA) thereby increasing environmental sensitivity for dauer induction. Consistent with known pleiotropic roles of DA, this *eak-3* variant significantly slows down reproductive growth. We experimentally show that, while the *eak-3* deletion can provide a fitness advantage through facilitated dauer production in stressful environments, this allele becomes rapidly outcompeted in favourable environments. The identified *eak-3* variant therefore reveals a trade-off in how hormonal responses influence both the pace of developmental timing and the way in which environmental sensitivity controls adaptive plasticity. Together, our results show how a single mutational event altering hormonal signalling can lead to the emergence of a complex life history trade-off.

INTRODUCTION

Adaptive developmental plasticity is common across diverse taxa, allowing organisms to flexibly adjust their phenotype in response to environmental variation [1–5]. There is ample evidence that such plasticity exhibits natural genetic variation and there is rapidly growing knowledge on the molecular mechanisms regulating plasticity [6–9]. Yet, we still have little information on how the degree of sensitivity to environmental cues evolves through specific changes at the molecular level. In particular, given the predominant roles of conserved, often pleiotropic pathways (*e.g.* involving endocrine signalling) in developmental plasticity [4,5], it remains unclear if modification of such genetic components is a major driver of plasticity evolution. Central questions are therefore how the genetic architecture underlying developmentally plastic phenotypes constrains their evolvability and whether evolutionary changes in plasticity come at a cost due to the pleiotropic nature of underlying genetic mechanisms.

Here we address these questions by studying natural genetic variation in adaptive developmental plasticity in the nematode *Caenorhabditis elegans*. Depending on the early larval environment, L2 larvae can either go through reproductive growth or enter a diapause stage called dauer [10]. The developmentally arrested dauer larva is morphologically and metabolically specialized, making it resistant to various environmental stressors, including prolonged starvation, desiccation or extreme temperatures [10–13]. The *C. elegans* dauer decision thus reflects a plastic mechanism for switching between alternative life histories, *i.e.* immediate reproduction versus somatic maintenance [14,15].

C. elegans dauer larvae are commonly isolated from natural populations and presumed to play a central role in dispersal via invertebrate carriers [16,17]. Dauer induction seems to be tightly associated with the boom-and-bust population dynamics of natural *C. elegans* populations in ephemeral habitats. Rapid reproductive growth allows for efficient exploitation of transitory food sources while dauer passage allows for survival and dispersal during prolonged periods of food scarcity. The dauer stage thus reflects an integral part of the natural *C. elegans* life cycle [16,17]. Entry into dauer is cued by environmental signals correlated with adverse environmental conditions, in particular, high dauer pheromone concentration (occurring at high population density), low food concentration and high temperature [12,15,18–21]. In addition, variation in microbial food source, including exposure to certain pathogenic bacteria, can trigger *C. elegans* dauer formation [22]. The most potent dauer-inducing cue appears to be the pheromone mixture composed of small signalling molecules, termed ascarosides [23–27]. Low food or starvation conditions generally enhance the propensity to enter the dauer stage when pheromone concentrations are sufficiently high. In addition, the tendency to form dauers increases at higher temperatures [18,19,28].

The regulatory network underlying the plastic *C. elegans* dauer decision couples sensory with endocrine signalling through evolutionarily conserved signalling pathways. Pheromone and other environmental cues are perceived by the nematode's sensory system via specific chemoreceptors as well as via internal sensation of nutritional status [12,15,24,29–31]. Sensory processing of such stimuli occurs via elements of the neurosensory system and guanylyl cyclase signalling. In favourable growth conditions, this will activate neuroendocrine signalling via TGF- β and insulin/IGF pathways to upregulate the production of bile acid-like steroid hormones, termed dafachronic acids (DA), in steroidogenic tissues, such as XXX cells and the hypodermis [32–35]. While low levels of DA are sufficient to block dauer entry, subsequent amplification of DA is further necessary to ensure robust reproductive growth [36]. Ultimately, the binding throughout the animal of DA with its nuclear receptor DAF-12 (orthologous to vitamin D and liver-X receptors in vertebrates) prevents dauer entry [15]. In contrast, under unfavourable conditions, DAF-12 remains unliganded and binds to its co-repressor DIN-1, thus promoting dauer entry [37]. Hence, DA levels operate as the central element regulating the plastic switch between alternative life cycles.

C. elegans wild isolates differ in the propensity to produce dauers in response to environmental cues, such as crude pheromone [38], specific pheromone ascarosides [31,39,40], population density and starvation [41–43], or high temperature [31]. Moreover, quantitative genetic analysis has identified multiple genomic regions associated with natural variation in *C. elegans* dauer induction [31,40–43]. Only one of these QTL (Quantitative Trait

Loci) has so far been resolved: assaying pheromone-induced variation in dauer formation across a world-wide panel of 157 wild isolates, Lee *et al.* [40] showed that deletion alleles of two chemoreceptors (*srg-36* and *srg-37*), reducing dauer pheromone sensitivity [24,40], segregate in natural *C. elegans* populations. Given the scarcity of such data, it is therefore unclear whether dauer induction preferentially evolves at the level of sensory perception of specific environmental cues, or downstream at the level of conserved signalling pathways (TGF- β , insulin, steroid hormone signalling).

Here we characterized the molecular basis underlying natural variation in *C. elegans* dauer induction by focusing on a wild isolate with enhanced dauer induction. Using QTL mapping and molecular genetic analysis, we identified a deletion in a regulatory region of the gene *eak-3* [44] – involved in DA steroid hormone synthesis or secretion – as the causally underlying variant. We provide evidence that this *eak-3* variant not only enhances the propensity to form dauers but also negatively affects reproductive growth due to the pleiotropic effects of altered steroid hormone production. Moreover, we experimentally demonstrate that the variant may have strong and opposite fitness consequences, depending on exposure to stressful or favourable conditions. Our findings show how a single mutational event can generate a life history trade-off via hormonal pleiotropy.

RESULTS

JU751 exhibits enhanced dauer induction in response to diverse environmental stimuli

In the laboratory, *C. elegans* is cultured on standard NGM (Nematode Growth Medium) plates seeded with the *E. coli* strain OP50, usually at temperatures of 15°C to 25°C [45]. In these conditions, as long as bacterial food is present, *C. elegans* wild-type strains, including the reference strain N2, very rarely, if ever, form any dauers. In contrast, it was observed that the wild isolate JU751, derived from a dauer larva collected from garden compost (Le Perreux-sur-Marne, France) in 2005 [46], consistently generated a small fraction of dauers despite ample food and moderate population density (M.-A. Félix, pers. comm.). Comparing dauer induction of JU751 to a set of genetically divergent *C. elegans* isolates [47], across three different population densities, we found JU751 to be the only isolate producing any dauers (Figure 1A). To further characterize JU751 dauer induction, we compared it to another wild isolate, JU1200. We chose JU1200 as this isolate is very similar, at whole-genome sequence level, to the *C. elegans* reference strain N2 [48], yet without any signs of laboratory adaptation known to have occurred in N2 [49]. JU1200 did not produce any dauers in the density assay (Figure 1A) but formed dauers on standard dauer pheromone plates at 25°C [50] (Figure 1B). Because environmental cues other than pheromone can induce dauers, we further quantified JU751 dauer induction in response to different environmental factors indicative of unfavourable environments. First, we confirmed the difference in density-induced dauer formation: across five increasing densities, JU751 increased production of dauers while JU1200 never produced any dauers (Figure 1C). Second, measuring dauer induction at four different temperatures (15°C, 20°C, 25°C and 27°C), JU751 produced an increasing proportion of dauers from 20°C to 27°C while JU1200 never produced any dauers (Figure 1D). Third, crude pheromone exposure at 20°C induced dauer formation in JU751 but not in JU1200 (Figure 1E). Fourth, at food exhaustion of culture plates (20°C), JU751 but not JU1200 produced a large number of dauers (Figure 1F). Fifth, testing for an effect of a bacterial pathogen on dauer induction [22], *Pseudomonas aeruginosa* (strain PA14) triggered dauer induction in JU751 but not JU1200 (Figure 1G). Sixth, exposure to mild oxidative stress (paraquat), likely eliciting a general stress response [51], induced dauers in JU751 but not JU1200 (Figure 1H). The *C. elegans* wild isolate JU751 thus exhibits an enhanced propensity to form dauers in response to diverse stimuli associated with unfavourable environments.

A QTL on chromosome III explains enhanced dauer induction of JU751

To identify the genetic basis of enhanced dauer induction of JU751, we used a panel of SNP-genotyped F2 RILs (Recombinant Inbred Lines) derived from a parental cross between wild isolates JU751 and JU1200 (Figure 2A) [52]. We quantified dauer production of RILs (Figure 2B) at a population density at which JU751 but not JU1200 produced dauers (Figure 1C). We then attributed a binary phenotype for each RIL, *i.e.* presence or absence of dauers (see Materials and Methods). Performing a QTL (Quantitative Trait Locus) analysis using these phenotype data, yielded a single major-effect QTL on the left arm of chromosome III (Figure 2C), spanning approximately 750kb (target region 1) (Figure 2D). This QTL explained 49% of the phenotypic variance.

We first narrowed down the QTL interval using additional SNP markers to genotype RILs, which showed recombination events in the QTL region (Figure 2E and Table S2). This restricted the presumptive target interval from 750 kb to 158 kb, located between markers III_1122kb and III_1280kb (target region 2) (Figure 2E). To confirm the phenotypic effect of the restricted QTL region (target region 2), we constructed near-isogenic lines (NILs) derived from RILs NIC649 and NIC663 (both showing consistent dauer induction) as well NIC623 (which did not form dauers). These RILs were backcrossed ten times to JU1200, selecting for the presence of JU751-specific PCR products in the target region. In response to high temperature (27°C), resulting NILs NIC1638 and NIC1639 produced dauers similar to JU751, whilst NIC1637, derived from RIL NIC623, did not form any dauers (Figure 2F). These results thus validated the effect of the restricted 158kb QTL region on dauer induction. Additional fine-mapping using NIL NIC1638 further restricted the target region to 50kb, located between markers III_1158kb and III_1208kb (target region 3) (Figure 2G and Table S3).

Identification of a rare deletion allele as the candidate variant

Analysing whole-genome sequence data for JU751 and JU1200 [53], we next aimed to identify candidate variants within the 50kb target region using SNPEff software [54] and visual inspection of the aligned sequence data. Out of a total of 201 SNPs, we identified 17 SNPs with predicted moderate effects and two deletions with potential effects on gene function (Table S4). To further restrict the list of candidate variants, we removed variants present in both JU751 and the genetically most similar isolate JU2593 based on whole-genome sequence comparison [53] (Figure S4A). JU2593 and three other closely related isolates (JU2829, JU2581, JU2587), in contrast to JU751, did not display enhanced dauer induction (Figure S4B) and should consequently lack the genetic polymorphism(s) responsible for increased dauer induction in JU751. Using this approach, we obtained nine candidate variants unique to JU751 (Table S4), including an obvious prime candidate variant: a deletion in the likely regulatory region of *eak-3* (*enhancer of akt-1*) (Figure 3A), a gene previously shown to be involved in temperature-induced *C. elegans* dauer induction via effects on steroid hormone production [44] (Figure 3B). We named this deletion *eak-3(cgb1006)*.

To assess the frequency of the uncovered *eak-3* deletion in natural *C. elegans* populations we examined whole-genome sequence data of 249 wild isolates [53]: in this dataset, the deletion allele was unique to JU751. We next examined additional *C. elegans* isolates, which were isolated from the same sampling locality (a single compost heap) as JU751 in 2004 and 2005 [46]. Of 21 isolates analysed, five other isolates carried the *eak-3(cgb1006)* deletion, which were isolated together with JU751 from the same compost heap on the same day in June 2005, and which had an identical or very similar haplotype as JU751 [46] (Figure S5). Assaying a subset of 20 isolates with different haplotypes collected throughout 2004 and 2005, only the six isolates carrying the *eak-3(cgb1006)* deletion produced significant fraction of dauers in response to high temperature exposure (27°C) (Figure S5). These results confirm that *eak-3(cgb1006)* was acquired in a natural context. Yet, this deletion allele seems to be rare and localized in natural *C. elegans* populations.

A 92bp deletion in a *cis*-regulatory element of *eak-3* causes enhanced dauer induction in JU751

eak-3 was first isolated in an enhancer screen of the weak dauer-constitutive (*daf-c*) null mutant *akt-1*, an orthologue of the serine/threonine kinase Akt/PKB [44,55]. Consistent with a role in dauer formation, *eak-3* is expressed throughout L1, L2 and dauer stages, yet no transcription was detected in (reproductive) L3 to adult stages (FPKM expression data, modENCODE, www.wormbase.org). Similar to JU751, *eak-3* loss-of-function mutants (N2 background) show an increased production of dauers in response to high temperature, suggesting an inhibitory role of *eak-3* in dauer induction [44]. Given the reported role of *eak-3* in dauer induction, we tested if the uncovered *eak-3(cgb1006)* deletion in the *eak-3* upstream region is the causal variant explaining increased sensitivity to dauer-inducing cues in JU751. This deletion spans 92bp and is located 100bp upstream of the *eak-3* transcription initiation site (Figure 3A). This likely *eak-3* cis-regulatory region is identical in DNA sequence across 90% of *C. elegans* isolates, including JU1200, N2 and closely related isolates of JU751 (Figure S6). Scanning of potential transcription factor binding sites identified 69 DNA motifs (Table S5). Consistent with a scenario in which this deletion impairs transcription, JU751 exhibited strongly reduced *eak-3* transcript levels in synchronized populations at the late L1 to mid L2 stages, *i.e.* during the developmental time window of dauer induction (Figure 3B). *eak-3* transcription was also strongly reduced in mixed-stage populations, containing larval and adult animals (Figure 3C). Therefore, *eak-3* transcription in JU751 is constitutively reduced, if not completely abrogated, irrespective of developmental stage.

To validate the *cgb1006* deletion in the *eak-3* upstream region as the causal molecular variant enhancing environmental sensitivity of JU751 dauer induction, we generated a knock-in (KI) strain, termed JU751_{*eak-3(+)*}, using CRISPR-Cas9 genome editing to insert the 92bp reference genomic fragment into JU751. Insertion of the deleted fragment restored early larval *eak-3* expression of JU751 (Figure 3D). This confirmed that the *cgb1006* deletion in JU751 removes regulatory elements essential for *eak-3* transcription. We next compared dauer induction in JU751_{*eak-3(+)*} in response to multiple environmental cues relative to JU751 (and JU1200). Consistent with restored transcription of *eak-3*, JU751_{*eak-3(+)*} showed an overall reduced sensitivity to tested dauer-inducing cues relative to JU751 (Figures 3E to 3J). In response to pheromone, high temperature (27°C), oxidative stress (paraquat), pathogen exposure (*P. aeruginosa* strain PA14), JU751_{*eak-3(+)*} did not produce any dauers at all (Figures 3E to 3H). In starvation assays (Figure 3I) and standard dauer pheromone assays (Figure 3J), JU751_{*eak-3(+)*} produced dauers, yet overall fewer than JU751. However, on standard dauer pheromone plates, dauer induction was similar across strains, including JU1200 (Figure 3J). The effect of the *eak-3* variant therefore seems less prominent in more strongly dauer-inducing conditions. In addition, increased dauer induction of JU751_{*eak-3(+)*} relative to JU1200 in response to starvation (Figure 3I) indicates that variants other than *eak-3(cgb1006)* also contribute to differences in dauer induction between JU751 and JU1200. Overall, the above data show that the 92bp deletion in a cis-regulatory region of *eak-3* underlies the evolution of enhanced sensitivity to dauer-inducing cues in JU751 by abolishing, or strongly reducing, *eak-3* transcription.

Enhanced dauer induction due to the *eak-3* deletion is caused by constitutively reduced levels of the steroid hormone dafachronic acid

eak-3 encodes a plasma membrane-associated protein expressed in the two endocrine XXX cells [44], a primary synthesis site of the steroid hormone dafachronic acid (DA), which plays a pivotal role in dauer induction together with its receptor DAF-12 [33–36,56] (Figure 3B). EAK-3, and additional proteins specifically expressed in the XXX cells, has been proposed to participate in DA synthesis or secretion [12,44,57]. Enhanced dauer induction in JU751 thus results from impaired DA signalling, most likely reflecting constitutively reduced levels of DA. The initial amount of DA released by XXX cells during the L1 stage is required to attain a critical threshold to bypass dauer entry [36]. DA levels in JU751 are thus expected to be only slightly above this threshold, so that any further small reduction of DA production can be sufficient to induce dauer formation. We therefore asked how exogenously supplied DA affects dauer induction in JU751. Applying three concentrations (1nM, 100nM, 1000nM) of synthetic Δ^7 -dafachronic acid reduced temperature-induced dauer formation of JU751 in a dose-dependent fashion (Figure 3K). Enhanced dauer induction in JU751 is thus likely

caused by reduced DA availability. These results, together with past research on *eak-3* [44] and DA [33–36,56,58] indicate that enhanced dauer induction in JU751 has occurred through modified steroid hormone signalling.

***eak-3(cgb1006)* prolongs developmental time due to pleiotropic effects of reduced steroid signalling**

DA signalling is highly pleiotropic, modulating many life history traits, including developmental transitions and timing [34–36,59]. We therefore asked if impaired DA production in JU751 caused by the *eak-3(cgb1006)* deletion could affect postembryonic developmental speed. Quantifying the developmental time from L1 larva to reproductive maturity (self-fertilizing hermaphrodites having produced their first embryos) in favourable (i.e. non-dauer inducing) conditions, JU751 showed a substantial developmental delay (3-4 hours) relative to JU751_{*eak-3(+)*} (and JU1200) (Figure 4A). This delay was already detectable around the L2/L3 moult, which occurred significantly later in JU751 relative to JU751_{*eak-3(+)*} (Figure 4B). Observed effects of the *eak-3(cgb1006)* allele on developmental timing are consistent with reported results of DA signalling [36], supporting a model in which constitutively reduced DA levels in JU751 lead to prolonged developmental time and later age at maturity in favourable environments.

To evaluate the validity of this model, we tested how exogenous supply of DA affects developmental timing in JU751. Exposure to two concentrations (100nM, 1000 nM) of synthetic $\Delta 7$ -dafachronic acid increased developmental speed: JU751 treated with either 100nM or 1000nM reached age of reproductive maturity earlier than untreated JU751 (Figure 4C). Comparing the effects of DA treatment (100nM) on developmental speed between JU751, JU751_{*eak-3(+)*} and JU1200, we found that DA fully rescued the developmental delay caused by the *eak-3(cgb1006)* deletion: developmental time to reach maturity was virtually identical between DA-treated JU751 animals and both DA-treated and -untreated JU751_{*eak-3(+)*} animals (Figure 4D). Specifically, in JU751, the developmental time point at which 80% of animals had reached reproductive maturity occurred 4-5 hours earlier when treated with DA (Figure 5D). DA treatment did not accelerate developmental speed in either JU751_{*eak-3(+)*} or JU1200 (Figure 4D). This indicates that canonical *eak-3* expression maintains DA levels above a critical threshold. This particular result is again consistent with earlier experiments showing that exogenous DA application does not accelerate growth rates beyond those of wild type *C. elegans* [36]. The above results thus support a model whereby the *eak-3(cgb1006)* deletion reduces DA levels, which in turn, cause a significant developmental delay in favourable, non-dauer inducing environments. Taken together, these observations demonstrate that the *eak-3* deletion reveals a trade-off in how steroid hormones influence both the natural pace of developmental timing and the way in which environmental sensitivity controls adaptive plasticity.

The *eak-3* variant has antagonistic fitness consequences in alternative environments

Given its pleiotropic effects on major life history traits, we aimed to evaluate fitness consequences of the *eak-3(cgb1006)* deletion allele in alternative, dauer- versus non-dauer inducing conditions. As JU751 was isolated during the summer period [46], we tested if heat exposure could favour the *eak-3(cgb1006)* deletion allele due to increased production of dauers, known to show increased stress resistance [11,12,15]. First, we quantified survival of JU751, JU751_{*eak-3(+)*} and JU1200 in response to mild heat stress (27°C) for 38 hours, followed by exposure to strong heat stress (35°C) for 15 hours (Figure 5A). The imposed heat stress killed the vast majority of animals of all three genotypes. However, small pools of surviving dauers observed in JU751 were successful in re-establishing proliferating populations during the 1-week recovery period (Figure 5B). Second, we quantified survival of JU751 and JU751_{*eak-3(+)*} in mixed populations at a 1:1 ratio in response to mild heat stress (27°C) for 38 hours, followed by exposure to high heat stress (35°C) for 15 hours (Figure 5C). Exposure to heat stress caused strong mortality with an average of only 0.3% survivors (Figure 5D) most of them in the dauer stage. Despite their drastically reduced populations size after heat exposure, all replicates had successfully regrown during the one-week recovery period, with virtually complete replacement by the JU751 *eak-3* deletion allele (Figure 5E). Enhanced

dauer induction due to the *eak-3(cgb1006)* deletion can therefore provide a competitive advantage during bouts of extreme environmental stress.

The *eak-3(cgb1006)* deletion allele caused a developmental delay in favourable, non-dauer inducing environments (Figure 4). To test potential effects of this delay on competitive fitness, we next performed competition experiments under optimal growth conditions at 20°C, culturing JU751 and JU751^{*eak-3(+)*} in mixed populations at a 1:1 ratio (total 400 L1, N=10 replicates) (Figure 5F). Quantifying *eak-3* allele frequencies at three time points across 28 days, the frequency of the *eak-3(cgb1006)* deletion allele rapidly declined and had a final frequency averaging less than 4% with 5/10 replicates having completely lost the deletion allele (Figure 5G). Thus, in favourable (non-dauer-inducing) conditions the *eak-3(cgb1006)* deletion allele seems strongly deleterious, consistent with its negative effects on developmental speed (Figure 4). Together, these results support the conclusion that the observed JU751 *eak-3* variant can have opposing, environment-dependent fitness consequences due to antagonistic pleiotropy of constitutively reduced steroid hormone levels.

DISCUSSION

We identified a natural variant in the regulatory region of the gene *eak-3* that underlies enhanced dauer induction in *C. elegans*. This variant lowers steroid hormone levels, which also causes a reduced growth rate in favourable environments. Therefore, this variant has opposing effects on fitness components in contrasting environments that are linked to alternative life histories. These results show how a life history trade-off can emerge through a single mutational event via hormonal pleiotropy.

The *eak-3(cgb1006)* deletion causes enhanced dauer induction in JU751

eak-3 was identified in an enhancer screen of the (weak Daf-c) mutant *akt-1* [44]. EAK-3 represents a novel protein with no predicted functional domains but exhibits an N-myristoylation motif [44]. *eak-3* is expressed in the endocrine XXX cells [44], together with other genes of the dauer regulatory network, some with clear roles in DA biosynthesis [55,60–62]. Amongst these, *eak-4* and *eak-7*, like *eak-3*, encode novel proteins with N-myristoylation motifs and are localized to the plasma membrane of the XXX cells [44,55,57,63]. Spatial expression patterns and genetic analyses suggest that these *eak* genes, including *eak-3*, participate in synthesis or secretion of the steroid hormone dafachronic acid (DA) [57]. The *eak-3* sequence appears to show little evolutionary conservation, and a potential orthologue was only detected in *C. inopinata* (Table S6), the sister species of *C. elegans* [64]. Loss or reduction of *eak-3* function in JU751 increases dauer induction by enhancing its sensitivity to dauer-inducing environmental cues. Similarly, previously characterized *eak-3* loss-of-function mutants display a weak Daf-c phenotype when exposed to high temperatures (25°C and 27°C) [44]. Together with *eak-3* expression analyses (Figures 3C and 3D), these data show that the *cgb1006* deletion causes complete or near-complete loss of *eak-3* transcription.

The *eak-3(cgb1006)* deletion allele causes reduced DA levels

Our experimental results are consistent with the interpretation that the *eak-3(cgb1006)* deletion causes constitutively reduced DA levels in the isolate JU751. This, in turn, explains increased sensitivity to dauer-inducing cues as well as its slower pace of development in favourable conditions (Figure 4A). This scenario is further consistent with the conclusion that basally reduced DA levels render JU751 more sensitive to (a) diverse and (b) mildly stressful environments, in which JU751 but not JU1200 (or other wild isolates) produces dauer larvae. Nevertheless, constitutive DA levels in JU751 seem sufficiently high to successfully progress through reproductive development when growth conditions are optimal. In such conditions (e.g. low population density at 20°C), we did not detect any dauers or individuals with defective development or abnormal adult morphology, which are commonly observed in mutants with strong or complete loss of DA synthesis, such as *daf-9* mutants [33–36,56]. Early DA production in XXX cells of JU751, albeit reduced, therefore seems sufficient to be

amplified to reinforce reproductive growth in optimal environments. We conclude that the *eak-3(cgb1006)* deletion leads to constitutively reduced levels of DA, which explain enhanced environmental sensitivity of dauer induction in JU751. While evolutionary shifts in environmental sensitivity of plastic phenotypes (genetic accommodation) are usually thought to have a polygenic basis, multiple examples, including ours, show that they can occur through single mutations affecting hormonal signalling [5,65–67].

The *eak-3(cgb1006)* deletion reveals a life history trade-off caused by hormonal pleiotropy

We show that the acquisition of the *eak-3* deletion in JU751 underpins a trade-off between developmental timing and environmental sensitivity of the dauer decision. Although seemingly subtle, the observed developmental delay (3-4 hours) in reaching age at maturity caused by *eak-3(cgb1006)* (Figures 4A to 4D) is considerable given the short generation time of *C. elegans* (~60 hours from egg hatching to reproductive maturity). Moreover, rapid reproductive maturation is likely a key determinant of competitive fitness in resource-limited environments [68,69], *i.e.* conditions mimicking the highly ephemeral structure of the *C. elegans* natural habitat [16,17]. The *eak-3(cgb1006)* deletion could thus carry a fitness disadvantage in environments with ample nutritional resources, implying a potential cost [70] underlying the acquisition of enhanced plasticity in dauer induction.

Our results – together with previously published research on *eak-3* [44,57] and DA signalling [58] – indicate that the proximate mechanism underlying this life history trade-off is the constitutively reduced level of the steroid hormone DA. Hence, this trade-off is due to hormonal pleiotropy (Figure 6A). In general, hormonal regulation is thought to be central for the coordinated integration of different life history traits, which suggests that hormonal pleiotropy is a plausible mechanism underlying many life history trade-offs, in particular, because hormones play a fundamental role in resource allocation [14,71–74]. Nevertheless, it is well recognized that apparent trade-offs may simply result through pleiotropy of molecular signals, most notably hormones, without necessarily reflecting a resource allocation trade-off [75,76]. The apparent trade-off between dauer induction and developmental timing due to *eak-3(cgb1006)* points to the latter scenario. However, altered DA signalling may also affect resource allocation, and in any case, the *C. elegans* dauer decision reflects in itself a process to flexibly allocate resources to either reproduction or somatic maintenance. Trade-offs between fitness components are ubiquitous, yet still little is known about their molecular genetic underpinnings and how they evolve in natural populations [73,77,78]. While life history traits are generally of polygenic nature [73], we show that seemingly complex life-history trade-offs can sometimes emerge through a single mutational event with pleiotropic effects at the hormonal level.

Fitness consequences and ecological relevance of the *eak-3* deletion allele

Evaluating whether the *eak-3* deletion has been maintained selectively in natural populations by enhancing dauer induction remains speculative: this allele has only been found in the isolate JU751 and five additional isolates (with the same or similar haplotypes) collected from the same habitat on the same day in June 2005 (Figure S5). One obvious possibility is that enhanced dauer production due to *eak-3(cgb1006)* could be advantageous when encountering sudden environmental stress so that only dauers can survive. This could also provide an advantage during subsequent population growth in a favourable environment as non-dauer forming genotypes may have been eliminated. In this situation, genotypes carrying the *eak-3(cgb1006)* deletion would experience less competition, and hence, the pleiotropic effect increasing developmental time would not matter.

The compost from which JU751 was isolated was sampled at multiple occasions between 2004 and 2005 [46]. Interestingly, no *C. elegans* were recovered from the sampling event preceding the one in which JU751 and other individuals (all carrying the *eak-3(cgb1006)* deletion) were isolated (Figure S5). This observation could indicate that *C. elegans* isolates carrying the *eak-3(cgb1006)* were able to proliferate after such a presumptive population crash, *i.e.* in a scenario resembling that of performed competition experiments during heat stress (Figures 5C to 5E). Moreover, weather records from the Paris

area (<http://meteo-climat-bzh.dyndns.org/>) indicate that an exceptional heat wave (exceeding 30°C) occurred around May 27-28 (2005), *i.e.* around ten days before the sampling of JU751. Potentially, the timing of this heat wave may have favoured the *eak-3* deletion allele. Nevertheless, during sustained periods of population expansion in favourable environments, we would expect *eak-3(cgb1006)* to become rapidly outcompeted because of its deleterious effects on growth rate (Figure 5G). This is also consistent with the observation that *eak-3(cgb1006)* was never recovered in combination with isolates that do not carry the deletion.

Natural variation of the *C. elegans* dauer regulatory network

C. elegans wild isolates differ strongly in dauer induction in response to single environmental cues [31,38–43]. One important outstanding question remains how additional environmental factors present in natural, complex environments may further modify such patterns of natural variation in dauer induction; in particular, because environmental factors affecting dauer induction are diverse and may interact, *e.g.* synergistically or antagonistically [10,20,30,79,80]. Moreover, sensitivity of *C. elegans* dauer induction – and its evolution – may be strongly cue-specific. This is supported by a study [31] showing that natural variation in dauer induction in response to ascarosides did not correlate with dauer induction in response to high temperature (27°C). Contrasting our finding of universally enhanced dauer induction in the isolate JU751, this implies that sensitivities to distinct dauer-inducing cues can be genetically uncoupled and therefore may evolutionarily diverge, *e.g.* during adaptation to different ecological niches. Yet so far, although *C. elegans* dauer formation has been characterized in great molecular detail, we still lack a basic understanding of how the interplay between different environmental factors shape dauer induction. Similarly, we are only starting to unravel the molecular basis of intraspecific variation in the *C. elegans* dauer regulatory network (Figure 6B). So far, only three genes are known to harbour natural variation modulating dauer induction: *eak-3* (this study), *srg-36* and *srg-37* [40] (Figure 6B). Multiple unresolved QTL, uncovered by linkage and association mapping, suggest the presence of many additional gene variants that affect dauer induction [31,40–43]. Future molecular analysis of natural variation in *C. elegans* dauer induction thus appears particularly promising to understand how evolution shapes an intricately regulated genetic network underlying adaptive developmental plasticity.

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AUTHOR CONTRIBUTIONS

CB conceived the study. CB and BB designed experiments. PV and BB carried out the experiments. BB analysed the data. BB and CB wrote the paper.

DECLARATION OF INTERESTS

The authors declare no competing interests.

FIGURE LEGENDS

Figure 1. JU751 exhibits enhanced dauer induction in response to diverse environmental stimuli. (A) Quantification of dauer induction in 14 *C. elegans* wild isolates (Table S1), including JU751, at different population densities (N=2-16 replicates/strain/condition). Plates were initially seeded with 10, 20 or 30 adult hermaphrodites (mid L4 + 24h) at 20°C and the number of SDS-resistant dauers was counted 72 hours later. Average population size of JU751 was not markedly higher than other examined wild isolates (Figure S1). (B-H) Quantification of dauer induction in JU751 and JU1200 in response to diverse environmental cues (N=3-11 replicates/strain/condition). (B) Dauer induction on standard dauer assay plates [50]: OP50 4%, crude pheromone, streptomycin, at identical population size (300 L1 larvae) at 25°C. JU751 produced significantly more dauer larvae than JU1200 in response to the treatment (Kruskal-Wallis Test, P=0.0032). (C) Dauer induction in response to different population densities. Plates were seeded with 5, 10, 20, 30, or 50 adult hermaphrodites (mid L4 + 24h) at 20°C (D) Dauer induction in response to different temperatures at identical population size (4000 L1 larvae). (E) Dauer induction in response to pheromone exposure at identical population size (300 L1) at 20°C. (F) Dauer induction in response to food exhaustion i.e. conditions in which nematodes experienced approximately 24-36 hours of starvation after 48 hours of population expansion on NGM plates at 20°C. (G) Dauer induction in response to the (pathogenic) bacterial food source, *P. aeruginosa* at identical population size (4000 L1 larvae) at 20°C. (H) Dauer induction in response to oxidative stress (paraquat) at identical population size (1000 L1 larvae) at 25°C.

Figure 2. A QTL on chromosome III explains enhanced dauer induction in the isolate JU751. (A) Generation of JU1200 x JU751 F2 Recombinant Inbred Lines (RILs) (yellow: JU751 genotype, blue: JU1200 genotype) [52]. (B) Quantification of dauer formation in 144 RILs and parental isolates JU1200 and JU751 at moderate population density (N=4-5 replicates/RIL). For detailed data, see Figures S2 and S3. (C) Logarithm of odds (LOD) plot of composite interval mapping of dauer presence versus absence of RILs (R/QTL), revealing a single significant QTL on chromosome III. (D) 95%-confidence Bayes interval for the QTL location on chromosome three (green). The QTL interval is delineated by markers III_908123 to III_1652552 (target region 1), spanning approximately 750kb. (E) Fine-mapping of QTL region on chromosome III using 12 additional SNP markers to genotype ten RILs with recombination events in the QTL region reducing the presumptive target region to 158kb (target region 2) (Table S2). Note that NIC732 showed an inconsistent phenotype as it failed to form dauers. (F) Quantification of dauer induction in NILs in response to high temperature (27°C) at identical population size (4000 L1 larvae): NIC1638 and NIC1639 formed dauers similarly to JU751; NIC1637 did not produce any dauers (N=4-6 replicates/strain). (G) Fine-mapping through genotyping of NILs further restricted the target region to 50kb (target region 3) (Table S3). For uncovered variants in this target region, see Table S4. See also Table S1.

Figure 3. A 92bp deletion in the upstream regulatory region of *eak-3* explains enhanced dauer induction in JU751. (A) The *eak-3* gene spans 1.45kb and is located in the intronic region of *cdh-12* (www.wormbase.org). The red bar indicates the 92bp *cgb1006* deletion upstream of *eak-3* in JU751. See also Tables S5 and S6. (B) Schematic overview of the *C. elegans* dauer network. EAK-3 participates in steroid synthesis or secretion [44] (C) *eak-3* expression in JU751 and JU1200 on NGM plates at 20°C. Measurements were made using age-synchronized L1 larvae after 11 hours of growth (L1+11h, N=3 biological replicates) and after 20 hours of growth, i.e. larvae at the late L1/early L2 stages (L1+20h, N=3 biological replicates) and mixed-stage populations (N=3 biological replicates), containing all larval stages and adults. *eak-3* expression levels were normalized using *act-1*, *cdc-42* and *pmp-3* as endogenous controls. JU1200 *eak-3* expression was set as the reference. The

error bars correspond to the standard deviations. **(D)** *eak-3* expression in wild isolates JU1200, JU751 and the KI strain JU751^{*eak-3(+)*} on NGM plates at 20°C. Measurements were made using age-synchronized larvae after 11 hours of growth (L1+11h, N=3 biological replicates). *eak-3* expression levels were normalized using *act-1*, *cdc-42* and *pmp-3* as endogenous controls. JU1200 *eak-3* expression was set as the reference. Error bars indicate standard deviations. **(D-I)** Quantification of dauer induction in JU751^{*eak-3(+)*} relative to JU751 and JU1200 in response to diverse environmental cues (N=8-11 replicates/strain/condition). **(E)** Dauer induction in response to crude pheromone at identical population size (400 L1 larvae) at 20°C. **(F)** Dauer induction in response to high temperature (27°C) at two population sizes (4000 and 8000 L1 larvae). **(G)** Dauer induction in response to oxidative stress (paraquat) at two population sizes (4000 and 8000 L1 larvae) at 25°C. **(H)** Dauer induction in response to (pathogenic) bacterial food source, *P. aeruginosa* strain PA14, at identical population size (4000 L1 larvae), at 20°C. **(I)** Dauer induction in response to starvation (food exhaustion) at 20°C. Dauer induction was significantly lower in JU751^{*eak-3(+)*} compared to JU751 (Kruskal-Wallis Test, P=0.02). **(J)** Dauer induction on standard dauer pheromone plates [50]: OP50 4%, crude pheromone, streptomycin, at identical population size (400 L1 larvae) at 25°C. Dauer induction did not differ between JU751^{*eak-3(+)*} and JU751 (Kruskal-Wallis Test, P=0.10). **(K)** Effect of different concentrations of exogenous DA (1nM, 100nM and 1000nM) on dauer induction in JU751, at identical population size (4000 L1 larvae) at 27°C. See also Figure S5 and S6, and Table S1.

Figure 4. *eak-3(cgb1006)* prolongs developmental time due to pleiotropic effects of reduced steroid signalling. Measurements of postembryonic developmental speed of self-fertilizing hermaphrodites in favourable, non-dauer inducing conditions (NGM plates with low population density and *ad libitum* food at 20°C). Developmental time points indicate hours of development from the L1 larval stage. **(A)** Developmental speed of JU751, JU751^{*eak-3(+)*} and JU1200 inferred from quantifying the proportion of individuals at reproductive maturity at different time points (N=40-218/strain/time point). The dotted line highlights the point at which 80% of the worms had reached reproductive maturity. **(B)** Early developmental speed of JU751, JU751^{*eak-3(+)*} and JU1200 inferred from quantifying the timing of the L1 and L2 moult. Measurements indicate proportions of non-lethargic, i.e. pumping, larvae. Time of moulting was estimated to occur when the lowest proportion of non-lethargic worms was observed. While the timing of the first moult (M1) did not differ between strains, the second moult (M2) occurred later in JU751 (N=40-104 animals per strain per time point). **(C)** Effect of DA treatment (100nM and 1000nM) on developmental speed in JU751 inferred from quantifying the proportion of individuals at reproductive maturity across different time points (N=79-333/strain/time point). **(D)** Effect of DA treatment (100nM) on developmental speed in JU751, JU751^{*eak-3(+)*} or JU1200 inferred from quantifying the proportion of individuals at reproductive maturity at different time points (N=78-288/strain/time point).

Figure 5. Antagonistic fitness effects of the *eak-3* deletion allele in alternative environments. **(A)** Design and **(B)** results of experiments quantifying survival of JU751, JU751^{*eak-3(+)*} and JU1200 in response to mild heat stress (27°C) for 38 hours, followed by exposure to strong heat stress (35°C) for 15 hours, after which populations were allowed to recover for one week at 20°C. The number of surviving animals was scored after 15 hours of exposure to 35°C (timepoint T1) and after another week of recovery at 20°C (timepoint T2). For each strain, NGM plates (N=5-8) were seeded with 4000 L1-arrested larvae at the beginning of the experiment. **(C)** Design and **(D,E)** results of competition experiments of JU751 versus JU751^{*eak-3(+)*}, in response to mild heat stress (27°C) for 38 hours, followed by exposure to strong heat stress (35°C) for 15 hours, after which populations were allowed to recover for one week at 20°C. **(D)** The number of surviving animals was scored after 15 hours of exposure to 35°C (timepoint T1) (N=11). **(E)** After one week of recovery at 20°C (timepoint T2), the allelic composition was assessed by genotyping eight of the 11 replicates for the presence of the *eak-3* deletion (N=43-48 animals per replicate). NGM plates (N= 11), were seeded with 3000 L1-arrested larvae of each genotype (JU751 and JU751^{*eak-3(+)*}) at the

beginning of the experiment. **(F)** Design and **(G)** results of competition experiments of JU751 versus JU751_{*eak-3(+)*}, at constant moderate temperature (20°C) and density (daily transfer of animals onto fresh plates). After one week (T1), two weeks (T2) and four weeks (T3), the allelic composition was assessed by genotyping for the presence of the *eak-3* deletion (29-40 animals per plate). Light yellow corresponds to the heterozygote genotype. NGM plates (N=10), were seeded with 200 L1-arrested larvae of each genotype (JU751 and JU751_{*eak-3(+)*}), at the beginning of the experiment. See also Figure S5.

Figure 6. Model of life history effects due to variation in DA levels and evolution of the dauer regulatory network. **(A)** Hypothetical model explaining life history consequences due to differences in DA levels caused by the *eak-3(cgb1006)* deletion. In favourable conditions, DA levels of JU1200 are sufficiently high to bypass the dauer stage and promote normal reproductive development at normal speed ($>[DA]_2$). Only strongly unfavourable conditions will induce dauer larvae in JU1200. In contrast, reduced DA levels of JU751 in favourable conditions ($<[DA]_2$) slow down reproductive development, and only mild environmental stress is required to induce dauer larvae. **(B)** Molecular variants underlying the evolution of dauer induction in *C. elegans*. Variants 1 and 2 are loss-of function deletion alleles of *srg-36* and *srg-37* (G-protein coupled receptors for ascarosides), respectively [40]. Variant 1 was found in a single wild isolate (PB303) and variant 2 was found in 57 wild isolates (out of a total of 157 isolates) [40]. Variants 3 and 4 are two deletions spanning full length (LSJ2) or partial length (CC1) of *srg-36* and *srg-37* [24]; LSJ2 and CC1 are laboratory strains that acquired these deletions during laboratory adaptation [24]. Variant 5 is the deletion upstream of *eak-3* in the isolate JU751 reported here, enhancing sensitivity to dauer-inducing cues due to its effects on DA signalling.

STAR * METHODS

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Christian Braendle (braendle@unice.fr). All worm strains generated in this study are available upon request.

DATA AND CODE AVAILABILITY

All raw data are available in Data S1 and Data S2.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All *C. elegans* strains used in this study are listed in the Key Resources Table. Additional information on strains is listed in Table S1. We used standard *C. elegans* methods [45] for strain maintenance: Stock cultures were kept on 2.5% agar nematode growth medium (NGM) petri-dishes (55mm diameter) at 20°C using live *Escherichia coli* (strain OP50) as a food source [45].

METHOD DETAILS

Quantification of dauer induction

Dauer induction in response to different environmental stimuli was measured using the different protocols outlined below.

When age-synchronized L1-arrested populations were used, embryos were recovered from young adult hermaphrodites by bleaching with sodium hypochlorite solution [45]. Embryos were washed in M9 buffer and incubated for approximately 24 hours to allow for hatching. In experiments with fixed population sizes, the number of L1 larvae was estimated from a 10µL sample to calculate the volume of the L1 solution to inoculate onto NGM plates.

At the time point of scoring the proportion of dauer larvae, populations were treated with 1% liquid solution of sodium dodecyl sulfate (SDS) for 30 minutes, *i.e.* a treatment only dauer larvae survive [10,50]. Unless noted otherwise, NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated at room temperature for three days.

Density assay (Figures 1A and 1C): 5, 10, 20 or 30 (and 50 in Figure 1C) age-synchronized adult hermaphrodites (mid L4 + 24h) were picked to standard NGM plates. After 72h of population growth at 20°C (close to food exhaustion), the number of SDS-resistant dauers was measured.

Temperature assay (Figure 1D): Standard NGM plates (55mm diameter) were seeded with 4000 age-synchronized L1 larvae and incubated at four different temperatures. The proportion of SDS-resistant dauer larvae was measured after 38h (25°C and 27°C), 48h (at 20°C) and 65h (15°C).

Starvation assay (Figures 1F and 3I): Chunks of NGM agar containing mixed-stage populations were transferred to standard NGM plates (55mm diameter). After 96h of incubation at 20°C, *i.e.* when food had been completely exhausted, the proportion of SDS-resistant dauer larvae was measured.

Pathogen assay (Figures 1G and 3H): NGM plates (55mm diameter) were inoculated with either live *E. coli* OP50 (OD₆₀₀=1) or live *Pseudomonas aeruginosa* PA14 (OD₆₀₀=1) and

incubated at room temperature for three days. Plates were then seeded with 4000 age-synchronized L1 larvae and incubated for 48h at 20°C when the proportion of SDS- resistant dauer larvae was measured.

High temperature assay (Figures 2F, 3F, S1, S4, and S5): Standard NGM plates were seeded with 3000 or 4000 age-synchronized L1 larvae and incubated for 38h at 27°C before quantifying the proportion of SDS- resistant dauer larvae.

Paraquat assay (Figures 1H and 3G): NGM plates (55mm diameter) containing 0.1mM paraquat were inoculated with an increased concentration (10X) of live *E. coli* OP50 (OD₆₀₀=1) as paraquat strongly reduces bacterial growth; plates were incubated at room temperature for three days. Plates were then seeded with 1000 or 4000 age-synchronized L1 larvae and incubated for 36h at 25°C, when the proportion of SDS- resistant dauer larvae was measured.

Pheromone assay (Figures 1E and 3E): NGM plates (35mm diameter) were inoculated with 25 µl of *C. elegans* crude pheromone extract [50] and seeded with 25 µl of live *E. coli* OP50 (OD₆₀₀=1) and incubated for one day at room temperature. Plates were then seeded with 300 age-synchronized L1 larvae and incubated for 48h at 20°C, when the proportion of SDS- resistant dauer larvae was measured.

Standard dauer assay (Figures 1B and 3J): NGM plates (35mm diameter), containing agar without bactopectone and supplemented with the antibiotic streptomycin (50 µg/ml), were inoculated with 25 µl of *C. elegans* pheromone extract and seeded with 25 µl of live *E. coli* OP50 (4% w/v) and incubated for one day at room temperature [50]. Plates were then seeded with 400 age-synchronized L1 larvae and incubated for 60-65h at 25°C, when the proportion of SDS- resistant dauer larvae was measured.

Effects of dafachronic acid (DA) on dauer induction in response to high temperature (Figure 3K): Standard NGM plates (55mm diameter) were inoculated with 20 µl of a solution containing an increased concentration (3X) of live *E. coli* OP50 (OD₆₀₀=1), as bacteria are left to grow only for 24 hours, and ethanol-solubilized (25S)-Δ7- dafachronic acid (DA) (Adipogen, CAS 949004-12-0) at a concentration of 1nM, 100nM, 1000nM or 0 nM (20 µl ethanol). The plates were incubated for 24h at 20°C. Plates were then seeded with 4000 age-synchronized L1 larvae and incubated for 38h at 27°C, at which time the proportion of SDS- resistant dauer larvae was measured.

Quantification of developmental speed

Developmental speed was assessed on NGM plates at two distinct moments of development: during early larval development (L1 to L2 and L2 to L3 larval transitions) and at transition to sexual maturity.

Transition to reproductive maturity (Figure 4A): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated for three days at 21°C. Approximately 500 age-synchronized L1 larvae were transferred to each plate and incubated at 20°C. From 49 to 63 hours after L1 plating, the proportion of gravid hermaphrodites (presence of embryos in utero) was measured every two hours by microscopy observation. The time window varied between assays, depending on the time when the first strain produced their first embryos. Scoring was performed on live animals.

Early larval development (Figure 4B): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated for three days at 21°C. Approximately 500 age-synchronized L1 larvae were transferred to each plate and incubated at 20°C. From 11 to 29 hours after L1 plating, the proportion of lethargic larvae (i.e. non-pumping and motionless) was inferred every two hours by microscopy observation. The average time point of moulting was estimated to occur when the proportion of lethargic larvae was highest [81].

Effects of dafachronic acid (DA) on developmental speed (Figures 4C and 4D): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1), increased 3-fold in concentration, as bacteria on NGM plates were allowed to grow only for one instead of three days. NGM agar was supplemented with ethanol-solubilized 25S-delta7-Dafachronic acid (DA) (Adipogen, CAS 949004-12-0) at final concentrations of 100nM, 1000nM and 0nM (in 20uL ethanol). After 24 hours of plate incubation at 20°C, approximately 500 age-synchronized L1 larvae were picked per plate and incubated at 20°C. From 49 to 63 hours after L1 plating, the proportion of gravid hermaphrodites (presence of embryos in utero) was measured every two hours. Scoring was performed on methanol-fixed animals.

Fitness and competition assays

Survival and population recovery assays after heat stress (Figures 5A and 5B): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated for three days at room temperature. 4000 age-synchronized JU1200, JU751, or JU751_{eak-3(+)} L1 larvae were plated per dish, separately, and placed at 27°C for 38 hours and then transferred to 35°C for 15 hours. After this heat shock, plates were chunked in two halves and put onto two fresh 55mm NGM plates seeded with live OP50 *E. coli* bacteria and incubated at 20°C for one week (recovery period). The number of alive (moving) individuals was counted after 15 hours (T1) and after one week of recovery (T2) (N = 5 to 8 replicates).

Competition assay in heat stress conditions (Figures 5C, 5D, and 5F): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated for three days at room temperature. 3000 age-synchronized JU751 L1 larvae and 3000 JU751_{eak-3(+)} L1 larvae were plated together per dish, placed at 27°C for 38 hours, and then transferred to 35°C for 15 hours. After this heat shock, plates were chunked into two halves and put onto two fresh 55mm NGM plates seeded with live OP50 *E. coli* bacteria and incubated at 20°C for one week (recovery period). The number of alive (moving) individuals was counted after 15 hours (T1). After one week of recovery (T2), a randomly selected fraction of individuals (N=38-43 per replicate) was PCR-genotyped, using primers flanking the *eak-3* deletion site (N=8 replicates).

Competition assay in favourable conditions (Figures 5F and 5G): NGM plates (55mm diameter) were seeded with live *E. coli* OP50 (OD₆₀₀=1) and incubated for three days at room temperature. 200 age-synchronized JU751 L1 larvae and 200 JU751_{eak-3(+)} L1 larvae were plated per dish and chunked daily for four weeks at 20°C. After one week (T1), two weeks (T2), and four weeks (T3), a randomly selected fraction of individuals (N=29-40 per replicate) was PCR-genotyped, using primers flanking the *eak-3* deletion site (N=10 replicates).

Generation of F2 Recombinant Inbred Lines (RILs)

The establishment of JU751 X JU1200 F2 RILs has been previously described (Figure 2A) [52]. In brief, reciprocal parental crosses were performed, *i.e.* JU751 hermaphrodites were mated to JU1200 males and JU1200 hermaphrodites were mated to JU751 males. For each cross direction, 72 F1 worms were singled and inbred by self-fertilization for 12 generations. The resulting 144 RILs were genotyped at 147 SNPs distributed across the genome [52]. Additional genotyping of RILs (Table S2) was done using primers listed in Data S2.

RIL phenotyping

Across 15 experimental blocks, we quantified dauer production of RILs at a population density, which induced dauers in JU751 but not in JU1200 (Figures 2B, S2, and S3). For each RIL, 4-5 replicate plates were seeded with 30 adult hermaphrodites (mid L4 + 24h) at 20°C and the number of SDS-resistant dauers was counted 72 hours later (Figures 2 and S2). For each block, JU751 and JU1200 were scored in parallel in the same fashion (Figure S2). Of 144 RILs scored, 94 RILs did not form any dauers while 50 RILs produced highly variable proportions of dauers, with strong replicate and block effects, which were also

observed for the parental strain JU751 (Figures 2B, S2, and S3). Given this high variability, we decided to attribute a binary phenotype for each RIL, *i.e.* presence or absence of dauers, rather than the actual number of induced dauers (Data S1).

QTL mapping

Raw dauer phenotyping data was Boolean-transformed (1 = presence of dauers, 2 = absence of dauers) for QTL analysis (Figures 2C and 2D), performed using the R/qtl package version 3.3.2 [82,83]. The 5% significance threshold for the QTL mapping was calculated using 1000 permutations. The confidence interval for the QTL on chromosome III was calculated using the bayesint function in R/qtl using a probability of 0.95.

Generation of Near-Isogenic Lines (NILs) and fine mapping

Three Near-Isogenic Lines (NILs) were derived from three different RILs to validate the implication of the target region identified by the QTL analysis (Figures 2E and 2G). RILs that had different recombination patterns inside the QTL were chosen to generate the NILs. We identified a total of 11 RILs that were polymorphic for the QTL region, and they were genotyped for 11 additional markers within this region (Figure 2E). RILs NIC649, NIC663 and NIC623 were used to construct NILs NIC1638, NIC1639 and NIC1637, respectively (Table S1). Each RIL was backcrossed ten times to JU1200 by selecting for the presence of JU751-specific PCR products in the QTL region. The following primers were used for the selection step: 1176KbF-R (for NILs NIC1638 and NIC1639) and III_1323F-R (for NIC1637). The NIL genotype in the QTL region was further characterized by genotyping 13 markers between III_900kb and III_1380kb (Table S3). To confirm that NILs were of JU1200 genotype outside the QTL region, we genotyped six additional markers outside the QTL on chromosome III and all other chromosomes. For a complete list of primers used, see Data S2.

Expression analysis

For each biological replicate, total RNA was extracted from six plates of mixed-age JU1200 or JU751 populations grown at 20°C (Figure 3C), and from synchronized population of JU1200, JU751 and JU751^{*eak-3(+)*} at L1 + 11h and L1 + 20h grown at 20°C (Figure 3D), using Trizol/Chloroform protocol. RNA samples were purified and concentrated using RNeasy MinElute Cleanup Kit (Qiagen). Random hexamers and SuperScript™ III Reverse Transcriptase (Invitrogen) were used to synthesize cDNA from the 5 µg of total extracted RNA, according to the manufacturer's protocol. Measurement of *eak-3* expression by qPCR was done using Master Mix Select SYBR (ThermoFisher) on a StepOne Plus Real-Time PCR System (ThermoFisher). Quantification of relative expression was determined by the $\Delta\Delta$ -CT method [84]. *act-1*, *cdc-42* and *pmp-3* were used as endogenous control to normalize *eak-3* expression. JU1200 samples were set as the reference to calculate fold changes in *eak-3* expression. All RT-qPCR were done in biological triplicates and each qPCR reaction was done in technical triplicates. Primers (and their efficacies) used for qPCR experiments are listed in Data S2.

Genome editing

Knock-in of the deleted sequence into JU751 at the *eak-3(cgb1006)* locus was generated by Sunybiotech using CRISPR-Cas9 technology [85]. The KI JU751^{*eak-3(+)*} strain and allele are named PHX705 and *eak-3(syb705)*, respectively. The inserted sequence corresponds to the reference sequence obtained from JU1200 and was verified by sequencing JU751^{*eak-3(+)*} using the primers listed in Data S2. Sequences of the *eak-3* regions in JU751^{*eak-3(+)*} and JU751 are shown below; the donor template sequence is shown in purple and the sgRNA target sequence is highlighted in yellow. The synonymous mutation preventing re-cutting is shown in cyan.

JU751^{*eak-3(+)*}

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CTGCCTCTTGCGATTCTGCTCAATAACATTAACCGTCAGGAAATATGTGCTCTTAGTCTC  
CCGGTCCAATGGCTCAGTTGTGTAGATTTGACCACTCTGGGAGTCAATCTCGAATTCCT  
CTGAAAAAATTCGAAAATTGATCTGAAAATTTGTAAAACACTCACTGCTCTGCGTATGGAA
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CGCGTATCTGAATTTCTTCTGGCAACCGGCTTGAACCTCGGACTACAGCGTCGTGGGCTC
CGGAATTTTCCATAACCTGGGCTCTGTACACTTTCTGTTGGAATTGGCAGGAAGTTGAT
GGGGTGTGGTTGATGATAGTCGGCCTTCCACGAAGATTGGTAGCTGAAAAATGGCCAT
AACTTTGTTGACTTAAAGATTTTTAAAAATTGTCAACCGTGAATTTGTAGCCCTTGAAT
TTTACACATTTTGATAGTTGGAATTTTTCTAGCTCTGTTCCGAAAGGTGCTATGCGC
CTTTAAAGTGCGCACCCCAAACAGACACAGTCTCCGTCTCTCCCATGTTTTTTTCGTA
CGGCGCCGGTCTACAAGCTTTAATTTTCAATTTTTTTGAGAAAGTCACCCCAATTTCA
CAAATTGTAGACTCTGCAATAGTCTATAAATTGTCAAACACCAGAAATCCCACGCTAA
TTGACATGTTATTGTGTCTCCGCGAAAAAATATGCAAATTTACAGTACCCCGCCCCCCC
CCTTTCTGTACAATTTGTATAAAAAATAGGGGCTCTGGTTGTTTTTACTCTATTTTTAG
GACTCTATGGGAAATTGCTTGAAAAACGCTCGAGGCGTCCGATCTTGACGATGATAG
CTTTATGATCAATTCTTGTTTGTGTTGAGTGTGAGTCAGAAACTATTTTTTGTGCAAATA
TAAAGCTAAAAATTCTACACATTTTGGCAGTTGAAAATTTTTTGTATAGCACTTTTCCCG
GAAG

JU751

CTGCCTCTTGCGATTCTGCTCAATAACATTAACCGTCAGGAAGTATGTGCTCTTAGTCTC
CCGGTCCAATGGCTCAGTTGTGTAGATTTGACCACTCTGGGAGTCAATCTCGAATTCCT
CTGAAAAAATTCGAAAATTGATCTGAAAATTTTGTAAAACCTCACTGCTCTGCGTATGGAA
CGCGTATCTGAATTTCTTCTGGCAACCGGCTTGAACCTCGGACTACAGCGTCGTGGGCTC
CGGAATTTTCCATAACCTGGGCTCTGTACACTTTCTGTTGGAATTGGCAGGAAGTTGAT
GGGGTGTGGTTGATGATAGTCGGCCTTCCACGAAGATTGGTAGCTGAAAAATGGCCAT
AACTTTGTTGACTTAAAGATTTTTAAAAATTGTCAACCGTGAATTTGTAGCCCTTGAAT
TTTACACATTTTGATAGTTGGAATTTTTCTAGCTCTGTTCCGAAAGGTGCTATGCGC
CTTTAAAGTGCGCACCCCAAACAGACACAGTCTCCGTCTCTCCCATGTTTTTTTCGTA
CGGCGCCGGTCTACAAGCTTTAATTTTCAATTTTTTTGAGAAAGTCACCCCAATTTCA
TGCAAATTTACAGTACCCCGCCCCCCCCCTTTCTGTACAATTTGTATAAAAAATAGGGG
TCTGGTTGTTTTTACTCTATTTTTAGCTGACTCTATGGGAAATTGCTTGAAAAACGCTC
GAGGCGTCCGATTTTGACGATGATAGCTTTATGATCAATTCTTGTTTGTGTTGAGTGT
GAAACTATTTTTTGTGCAAATAAAAAATGTAAGCTAAAAATTCTACACATTTTGGCAGTT
AAAATTTTTTGTATAGCACTTTTCCCGGAGAAG

Bioinformatic analyses

Identification of candidate variants in QTL region using whole-genome sequence data (Tables S4A, S4B, Figure S4): Variants of JU751, JU1200 and JU2593 in the target region 3 (Figure 2G) of the interval III:1156748 - III:1208151 (WS260) were identified based on VCF files and manual examination of BAM files using IGV [86]. BAM and VCF files were obtained from the *Caenorhabditis elegans* Natural Diversity Resource (CeNDR) [53].

Scan for transcription binding sites corresponding to region of the *eak-3(cgb1006)* deletion (Table S5): The identification of potential transcription binding sites (TFBS) in the deleted sequence upstream of *eak-3* was performed using the CisBP (Catalog of Inferred Sequence Binding Preferences) *C. elegans* database [87], using the PWMs-LogOdds model; significance threshold was set to 8 (<http://cisbp.cabr.utoronto.ca/index.php>). The DNA scan results are listed in Table S5.

Analysis of *eak-3* orthologues (Table S6): We ran a BlastP of the predicted EAK-3 amino acid sequence (wormbase.org) against the *Caenorhabditis* protein database (<http://blast.caenorhabditis.org/>) [88]. The significance E-value threshold was set to 1.0e-10. Blast results are listed in Table S6.

QUANTIFICATION AND STATISTICAL ANALYSIS

Dauer assay results are graphically represented by Tukey box-plots showing the IQR (box), the first and last quantiles (whiskers), the median (horizontal line). Individual data are plotted as dots on top of the box plots. In Figure 2B, only the outliers ($>1.5 \times \text{IQR}$) are plotted as dots.

Statistical analyses were performed using R or JMP 14.0. Sample sizes and replication information are provided in figures and figure legends. For exact information on replication and sample sizes, see raw data provided in Data S1.

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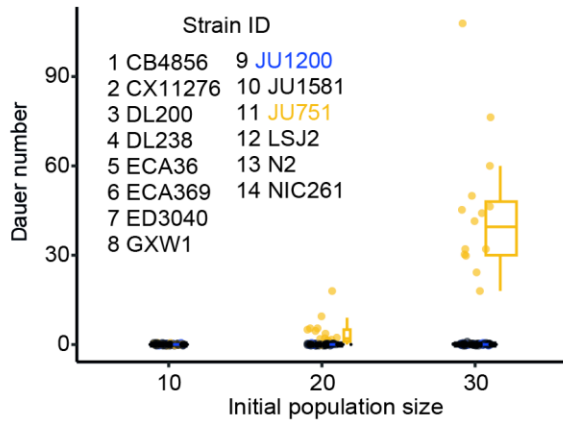
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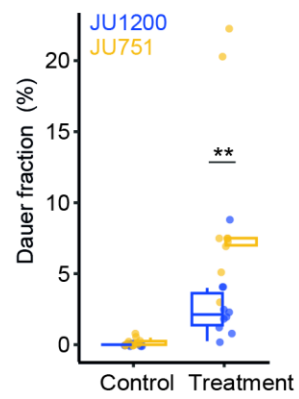
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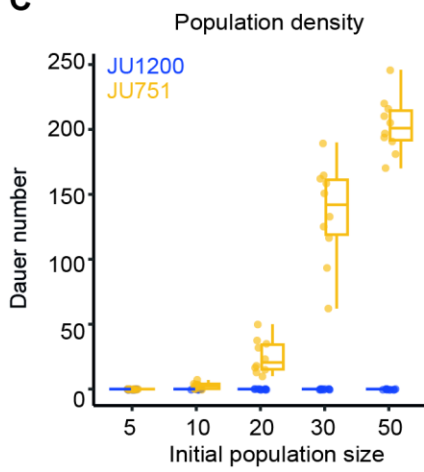
A Population density



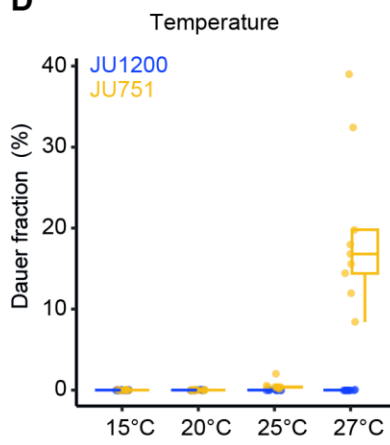
B Standard dauer assay



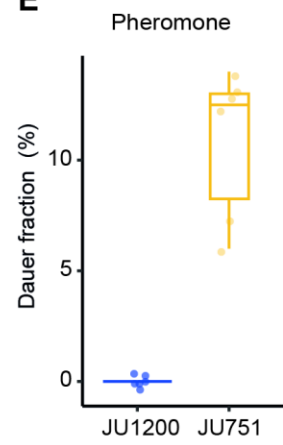
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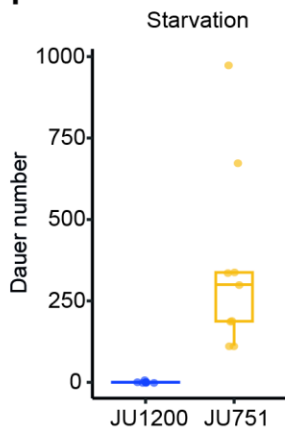
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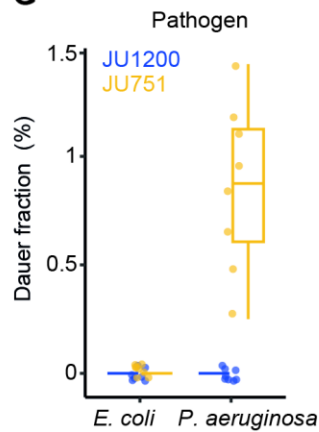
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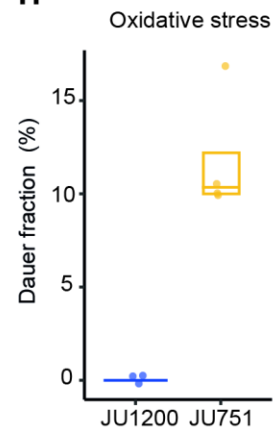
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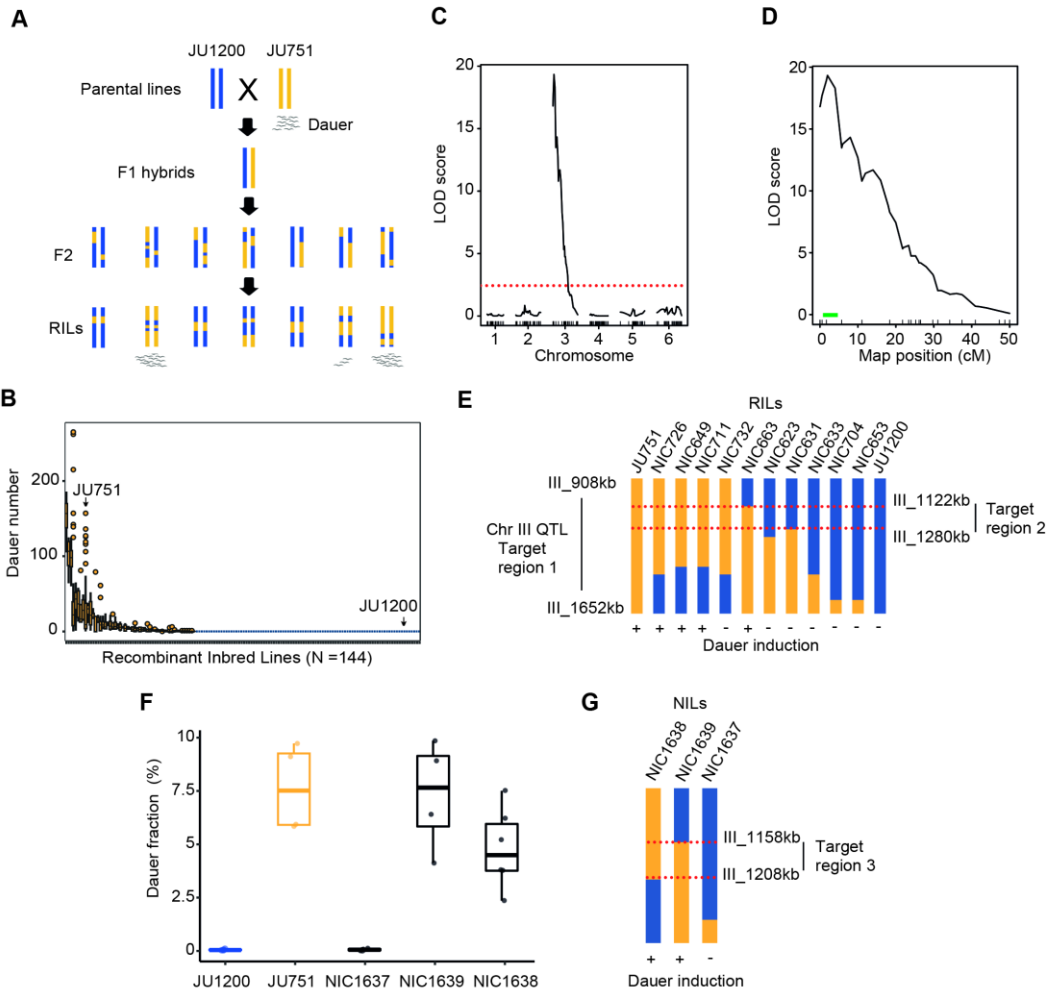


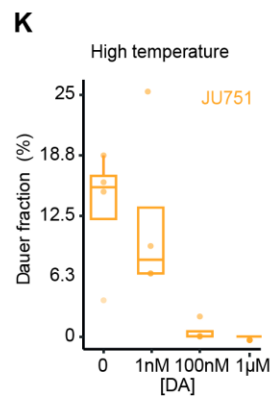
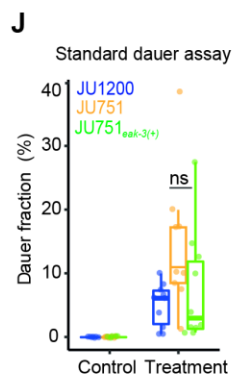
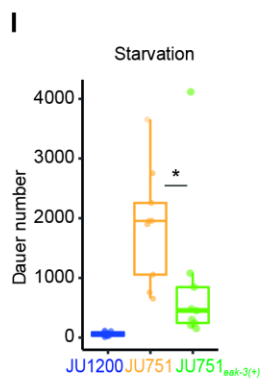
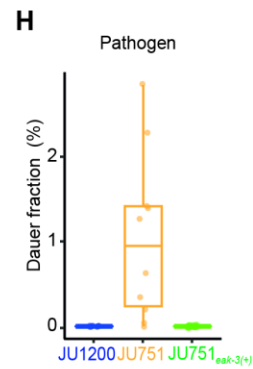
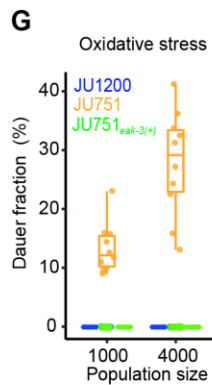
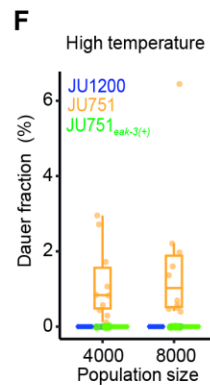
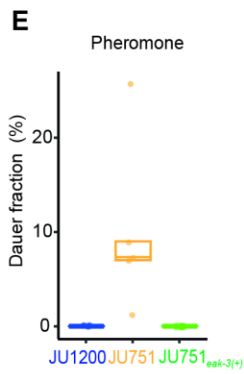
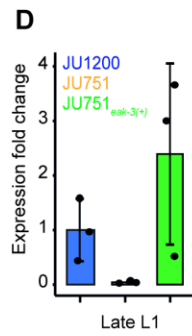
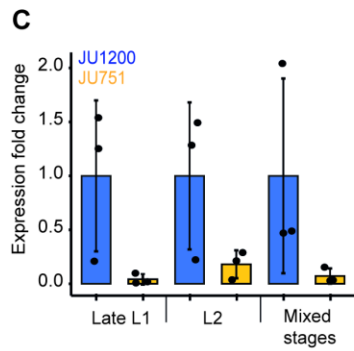
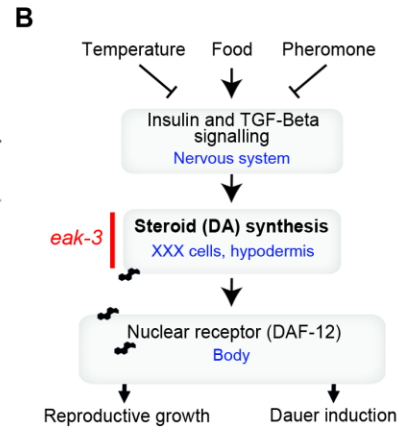
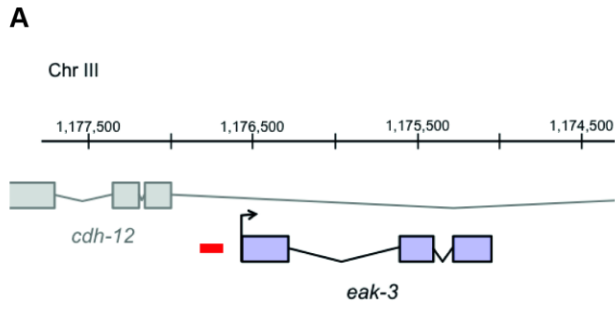
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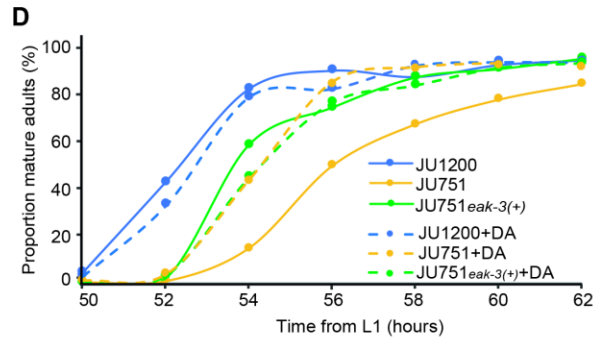
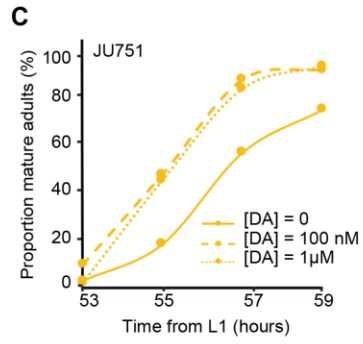
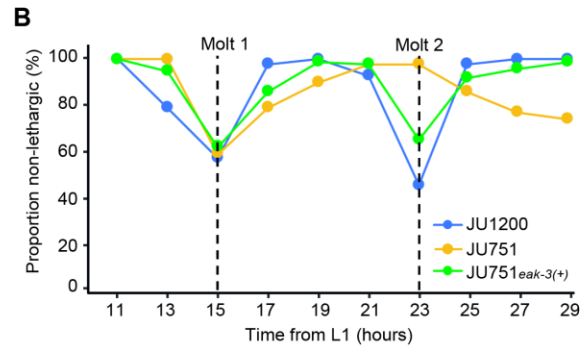
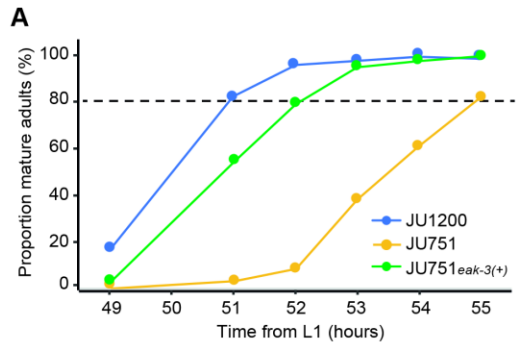


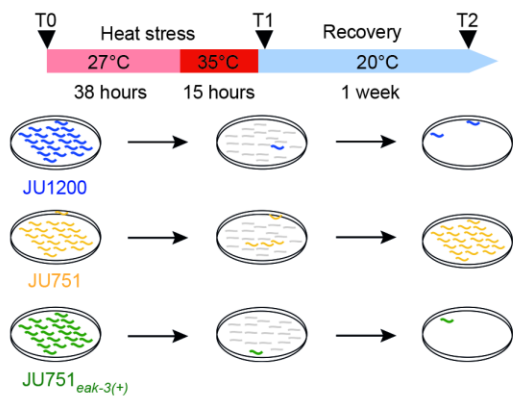
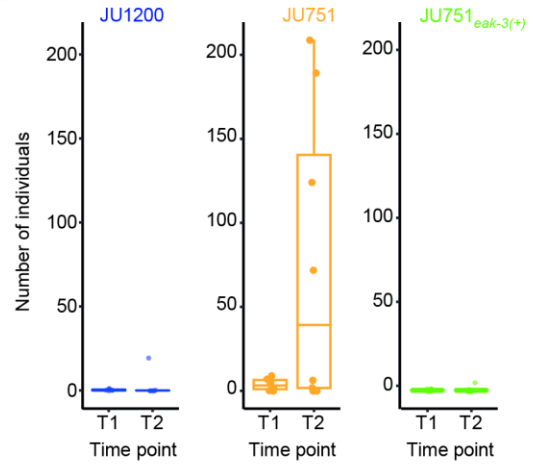
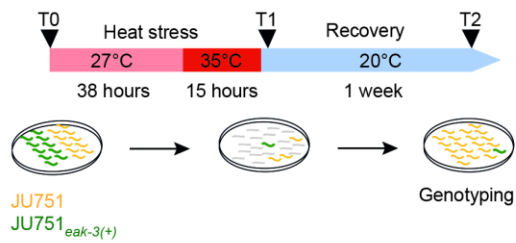
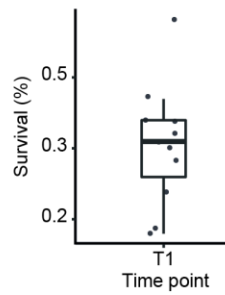
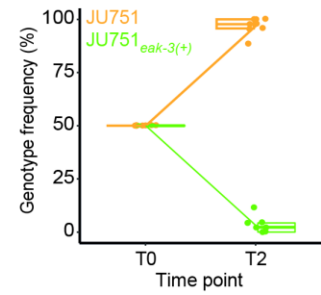
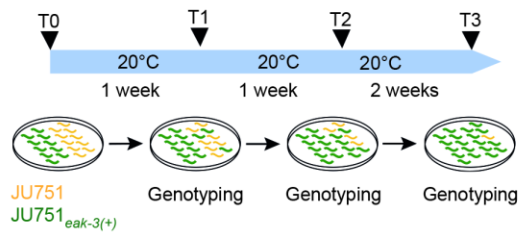
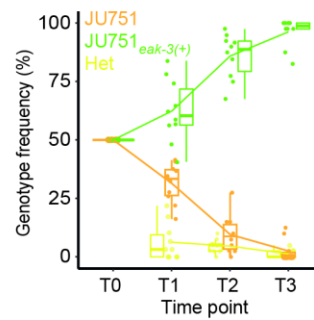
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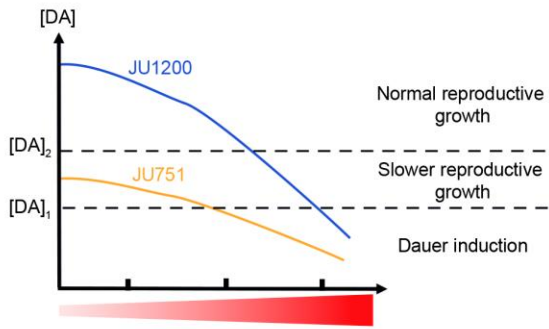








A**B****C****D****E****F****G**

A**B**