selection (*3*). Selection on *P. obtusospinosa* colonies may have incorporated induced supersoldier-like anomalies by increasing their frequency through modification of the JH system (fig. S12) and by inhibiting the formation of any wing vestiges (fig. S13 and S14). Army ant raids may have been a selective pressure that incorporated these anomalies, because *P. obtusospinosa* supersoldiers currently use their extra-large heads to defend against these raids (*13*).

Selection for re-evolving supersoldiers may generally be reduced, because almost all *Pheidole* species lack a supersoldier subcaste (*10*, *11*). *P. hyatti* provides insight into how this selective pressure can be reduced: Although *P. hyatti* retains the developmental potential (Fig. 4) and lives in an ecological environment similar to that of *P. obtusospinosa*, it has not re-evolved a supersoldier subcaste (*11*). Instead, *P. hyatti* uses nest evacuation behavior when attacked by army ants (*21*). The retention of this potential in *P. hyatti* and other *Pheidole* species that lack a supersoldier subcaste may therefore be due to a clade-specific constraint (*22*). This constraint may have arisen from having the same hormone (JH) mediate the determination of both soldiers and supersoldiers in the common ancestor of all *Pheidole*. Soldiers and supersoldiers are both defined by their larval size and the development of their vestigial wing discs, which indicates that their developmental programs share many modules. Therefore, the ancestral potential to produce supersoldiers cannot be lost without compromising the developmental program of soldiers.

Recurrent phenotypes reflecting ancestral potentials have long been recognized as widespread in plants and animals (*6*, *19*, *23*–*28*). Because of the lack of empirical evidence, however, the evolutionary significance of these recurrent phenotypes has been underappreciated (*19*, *29*). We uncovered an ancestral developmental potential to produce a novel supersoldier subcaste that has been retained throughout a hyperdiverse ant genus that evolved ~35 to 60 million years ago (*10*) (Fig. 4). Our results suggest that the recurrent induction of ancestral developmental potential is an important source of adaptive variation for selection that facilitates the adaptive and parallel evolution of novel phenotypes.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6064/79/DC1
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Fitness Trade-Offs and Environmentally Induced Mutation Buffering in Isogenic *C. elegans*

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Mutations often have consequences that vary across individuals. Here, we show that the stimulation of a stress response can reduce mutation penetrance in *Caenorhabditis elegans*. Moreover, this induced mutation buffering varies across isogenic individuals because of interindividual differences in stress signaling. This variation has important consequences in wild-type animals, producing some individuals with higher stress resistance but lower reproductive fitness and other individuals with lower stress resistance and higher reproductive fitness. This may be beneficial in an unpredictable environment, acting as a "bet-hedging" strategy to diversify risk. These results illustrate how transient environmental stimuli can induce protection against mutations, how environmental responses can underlie variable mutation buffering, and how a fitness trade-off may make variation in stress signaling advantageous.

A specific mutation can have different consequences in different individuals. For example, even in "Mendelian" human diseases, such as cystic fibrosis, an inherited mutation can result in severe disease in one individual but a milder phenotype in another (*1*). Incomplete penetrance is also observed in isogenic model organisms and is poorly understood (*2*–*4*).

Many mutations have outcomes that depend on the activity of molecular chaperones—proteins that aid the folding of other macromolecules (*5*–*14*). More generally, molecular mechanisms that promote environmental robustness (survival after environmental challenges) also tend to increase mutational robustness [the extent to which an organism's phenotype remains constant in spite of mutation (*15*–*17*)].

We investigated whether genetically increasing environmental stress resistance could modify mutation penetrance in the model organism *Caenorhabditis elegans*. We used a transgene to overexpress the transcription factor heat shock factor 1 (HSF-1), a master regulator of the environmental stress response. Transgenic animals are more resistant to a range of environmental challenges (*18*, *19*) and show a delayed age-dependent reduction in protein-folding homeostasis (*20*). We crossed the *hsf-1* transgenic animals with strains carrying diverse mutations that affect development but with outcomes that vary across individuals (table S1).

In 8 out of 11 tested cases, mutation penetrance was reduced in the transgenic animals (Fig. 1, fig. S1, and table S2). Protection was observed for mutations affecting both embryonic (Fig. 1A) and postembryonic (Fig. 1B) development. For example, embryonic lethality caused by a deletion in the intermediate filament protein gene *ifb-1* reduced from 33% to 17% (48% of animals that would have died were protected, $P = 5.7 \times 10^{-12}$) (Fig. 1, fig. S1, and table S4). The buffered mutations are molecularly diverse and act in distinct

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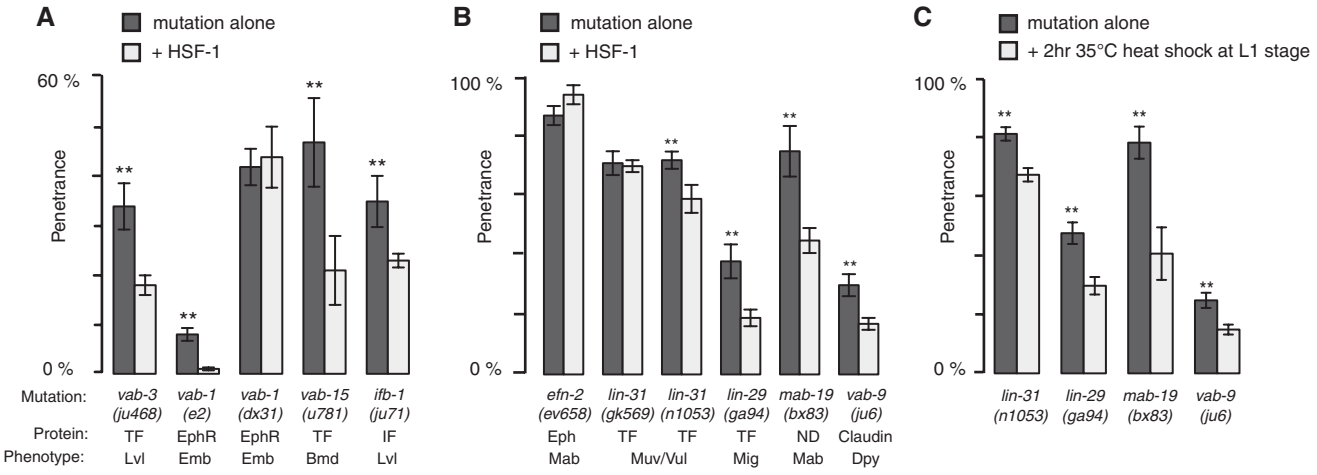

Fig. 1. Genetic and environmental stimulation of mutation buffering during the development of *C. elegans*. Increased expression of HSF-1 reduces the penetrance of mutations acting early (**A**) or late (**B**) in development. Similarly, a 2-hour 35°C heat shock at the L1 stage of development reduces the penetrance of late-acting mutations (**C**). $^{**}P < 0.01$, Fisher exact test; error bars indicate SEM. Phenotypes: Lvl, larval lethal; Emb, embryonic lethal; Bmd, body morphology defect; Muv, multivulva; Vul, vulvaless; Mig, male gonad migration defect; Mab, male abnormal (male tail ray defect); Dpy, dumpy; TF, transcription factor; EphR, ephrin receptor; IF, intermediate filament; ND, not determined. See also fig. S1, tables S1 to S5.

pathways and tissues (table S1). Protection ranged from 18 to 88% in the different cases. All of the buffered mutations had temperature-sensitive outcomes, whereas those refractive to buffering did not, and they likely represent genetic nulls (tables S1 and S5).

These observations suggest that, at least in *C. elegans*, a stimulated stress response can reduce the penetrance of partial loss-of-function mutations. We next tested whether the environmental stimulation of a stress response can have a similar effect. A mild environmental stimulus induces chaperone expression and promotes survival in subsequent environmental challenges, a response referred to as "hormesis" (*21*). We subjected animals to a transient heat shock as larvae to induce a stress response, allowed them to develop to adults, and examined the proportion of individuals affected by late-acting mutations. When a mutation was chaperone-dependent, a mild environmental challenge stimulated a reduction in penetrance (Fig. 1C). For example, an inactivating mutation in the zinc finger transcription factor LIN-29 caused abnormal male gonad migration in 46% of controls but only in 30% of animals receiving a prior heat stress ($P = 5 \times 10^{-4}$, a 35% reduction) (tables S3 and S4).

Beyond genetic differences, therefore, a second cause of variation in mutation outcome can be variation in the prior exposure of individuals to transient environmental stimuli (Fig. 1C and fig. S1, C to K). Although severe environmental stress can reduce mutation buffering (*8*, *9*), transient environmental stimuli can promote it.

Isogenic individuals often show substantial variation in their response to a common environmental challenge (*21*–*24*). We tested whether this interindividual variation also affects the outcome of mutations. We applied a transient heat shock and sorted animals according to their induction of one of the stress-responsive reporters *hsp-16.2p::GFP* (green fluorescent protein) or *hsp-16.2p::mcherry* (*22*). We found that animals in which a stronger stress response was induced had reduced mutation penetrance (Fig. 2, A and B; fig. S2; and table S6). Quantifying the response in individual animals confirmed this finding: Animals that had a stronger stress

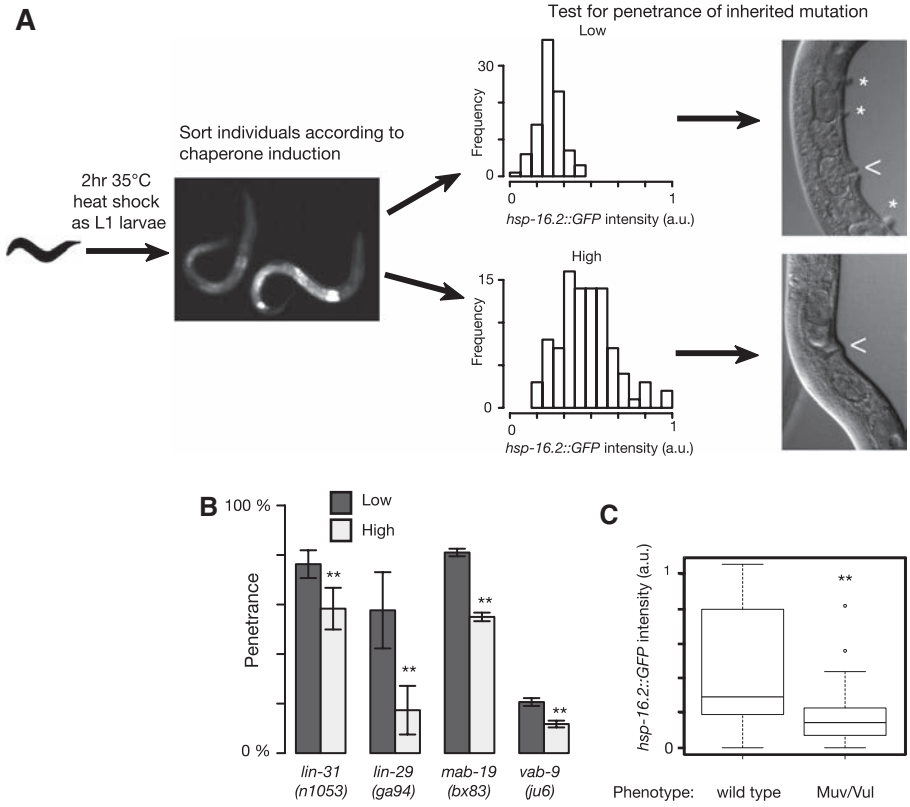

Fig. 2. Interindividual variation in a stress response predicts variation in mutation outcome. (**A**) Animals received a 2-hour 35°C heat shock as L1 larvae and were sorted 1 day later into "high" (right worm) and "low" (left worm) populations, according to the induction of an *hsp-16.2* chaperone promoter reporter. Histograms show representative GFP intensity distributions of sorted populations. Images on the right show the midbody region of *lin-31(n1053); hsp-16.2::GFP* animals. Asterisks mark pseudo-vulvae and arrowheads the vulva. (**B**) Mutation penetrance in the sorted populations for *lin-31(n1053); hsp-16.2::GFP*, *lin-29(ga94); hsp-16.2::GFP*, *mab-19 (bx83); hsp-16.2::mcherry, and vab-9(ju6); hsp-16.2::GFP* (raw data in table S6). (**C**) GFP intensity in individual *lin-31(n1053); hsp-16.2::GFP* animals 12 hours after heat shock in larvae that did (right) and did not (left) ultimately develop an abnormal vulva phenotype. GFP expression levels are scaled between 0 and 1 in each panel.

response were less likely to be affected by an inherited chaperone-dependent mutation (Fig. 2C and table S7).

Isogenic individuals that induce higher chaperone levels are therefore more resistant to the effects of certain inherited mutations. These individuals are also more resistant to heat stress and live longer (*22*). Why, therefore, is a strong stress response not induced in all individuals in a population? Mutations that increase stress resistance can also have a pleiotropic effect on fitness: Perturbing the insulin-like signaling pathway in *C. elegans* can reduce fecundity (*25*), and increased chaperone expression in *Drosophila* can be detrimental (*26*). We reasoned, therefore, that a fitness trade-off might occur across isogenic individuals, with those having a stronger stress response also incurring a fitness cost.

Using the *hsp-16.2p::GFP* reporter to separate individuals after a heat stress, we observed that, although individuals that had a stronger response were more resistant to a subsequent severe heat stress (fig. S3 and table S8), they had a significantly reduced early fecundity (fig. S3D and table S9). If conditions remain benign, therefore, individuals that have a stronger stress response incur an important fitness cost (fig. S3D and table S9).

In addition to HSF-1, a second key regulator of the stress response is the FOXO transcription factor DAF-16. DAF-16 relocalizes from the cytoplasm to the nucleus after a heat stress and, with HSF-1, induces the expression of target genes, such as *hsp-16.2* (*27*, *28*). Using a DAF-16::GFP fusion protein, we observed that the nuclear translocation of DAF-16 is rapid but that variation occurs across individuals in the time that DAF-16 remains in the nucleus (Fig. 3). For example, DAF-16 had been exported from the nucleus in half of animals ~100 min after a 3-hour heat shock (Fig. 3A). Sorting animals according to the nuclear residency time of DAF-16, we found that higher chaperone induction (fig. S4 and table S21), increased stress resistance (Fig. 3C, table S8), and reduced early fecundity (Fig. 3D and table S10) were all associated with prolonged DAF-16 signaling.

We next used a transcriptional reporter for an endogenously expressed chaperone, DAF-21 (Hsp90) to test whether variation in the stress response relates to preexisting variation in chaperone levels. Consistent with this idea, animals with higher *daf-21p::mcherry* expression before a heat shock developed greater thermotolerance (fig. S3G and table S8) and incurred a reproductive fitness cost after a mild heat stress (fig. S3H and table S11). Thus, interindividual variation in the response to a heat stress is, at least partially, due to preexisting molecular variation in a population.

We reasoned that this preexisting variation in chaperone expression might also affect the outcome of mutations. We used an RNA interference screen to define the individual chaperone dependence of different mutations: The results of this screen revealed that individual mutations differ in their chaperone dependence and provide a resource for future mechanistic work (fig. S5 and tables S13 to S20). In this screen, we identified the mutation *lin-31(n1053)* as strongly dependent on *daf-21* (*Hsp90*) activity (fig. S5D). We tested whether endogenous variation in *daf-21* expression predicted interindividual variation in the outcome of this mutation, by separating animals as larvae by their expression of the *daf-21p::mcherry* reporter and after the development of mutant phenotypes. Specifically for the *lin-31(n1053)* mutation, higher expression of the *daf-21* reporter was associated with a reduced mutation penetrance (Fig. 4 and table S22). This illustrates that even in the absence of a macroscopic environmental perturbation there can be predictable interindividual variation in the capacity to buffer mutations.

In summary, we have shown that, after a mild environmental stimulus, a trade-off occurs in *C. elegans* between the development of stress resistance and reproductive fitness. Preexisting

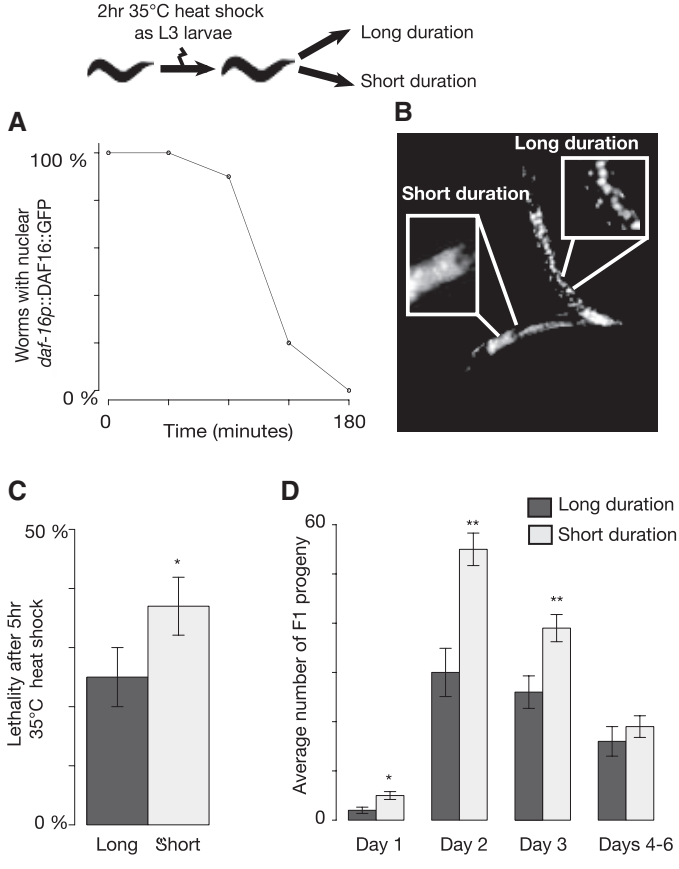

Fig. 3. A trade-off between the development of thermotolerance and reproductive fitness in an isogenic population. (**A**) Proportion of animals with nuclear DAF-16::GFP after a 2-hour 35°C heat stress at the L3 stage. (**B**) Two worms 50 min after heat shock: In the right individual ("long duration"), DAF-16::GFP is predominantly localized in the nucleus, whereas in the left ("short duration") individual, it is also in the cytoplasm. (**C**) Survival after a 5-hour heat shock at 35°C for the first (short duration) and last (long duration) 10 to 20% of individuals in which DAF-16::GFP returns to the cytoplasm. (**D**) The number of embryos laid as adults. $*P < 0.05$, $**P < 0.01$, Fisher exact test for lethality, Wilcoxon rank sum test for fecundity (see also tables S8 and S10).

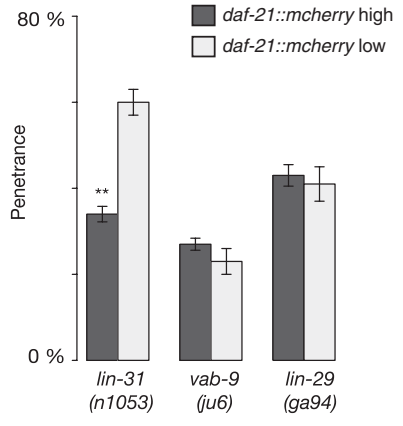

Fig. 4. Interindividual variation in the expression of an endogenous chaperone during larval development predicts mutation outcome. *lin-31(n1053); daf-21p::mcherry*, *lin-29(ga94);daf21p::mcherry*, and *vab-9(ju6);daf-21p::mcherry* animals were sorted into high- and low-expressing populations as L4 larvae and scored for abnormal phenotypes as adults. Variation in the *daf-21* reporter predicts variation in the outcome of the *lin-31(n1053)* mutation. $**P = 5.7\times10^{-4}$, Fisher exact test (table S22). Of these three mutations, only the penetrance of *lin-31(n1053)* is enhanced when *daf-21* is inhibited by RNA interference (fig S5D).

molecular variation in an isogenic population means that, after an environmental challenge, stress signaling is prolonged in some individuals, and these individuals develop increased stress resistance but a lower reproductive potential. *C. elegans* is a naturally self-fertilizing species and, in the wild, is likely to experience highly variable environmental conditions. In a dynamic and unpredictable environment, the generation of such phenotypic diversity can be beneficial, acting as a "bet-hedging" strategy to diversify risk (*29*–*33*). We suggest that interindividual variation in stress signaling may therefore be beneficial, as it resolves a trade-off between the development of stress resistance and rapid reproduction.

We have also shown that an environmental stress response can stimulate genetic buffering and so protect individuals from inherited mutations. Variation in stress signaling, however, means that this induced capacity to buffer mutations differs predictably across individuals. The conservation of stress responses and their variability (*21*, *24*) suggests that these concepts may apply quite widely, including perhaps in human genetic disease.

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Supporting Online Material

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Molecular Mimicry Regulates ABA Signaling by SnRK2 Kinases and PP2C Phosphatases

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Abscisic acid (ABA) is an essential hormone for plants to survive environmental stresses. At the center of the ABA signaling network is a subfamily of type 2C protein phosphatases (PP2Cs), which form exclusive interactions with ABA receptors and subfamily 2 Snfl-related kinase (SnRK2s). Here, we report a SnRK2-PP2C complex structure, which reveals marked similarity in PP2C recognition by SnRK2 and ABA receptors. In the complex, the kinase activation loop docks into the active site of PP2C, while the conserved ABA-sensing tryptophan of PP2C inserts into the kinase catalytic cleft, thus mimicking receptor-PP2C interactions. These structural results provide a simple mechanism that directly couples ABA binding to SnRK2 kinase activation and highlight a new paradigm of kinase-phosphatase regulation through mutual packing of their catalytic sites.

Abscisic acid (ABA) is a vital plant hormone and a central regulator that protects plants against abiotic stresses such as drought and salinity. The core of the ABA signaling network comprises a subfamily of type 2C protein phosphatases (PP2Cs) and three Snf1-related kinases, SnRK2.2, 2.3, and 2.6 (*1*, *2*), whose activities are tightly controlled by ABA. In the absence of ABA, SnRK2 kinases are inactivated by PP2Cs, including ABI1, ABI2, and HAB1 (*2*–*5*), which physically interact with SnRK2s and dephosphorylate a serine residue in the kinase activation loop (S175 in SnRK2.6) whose phosphorylation is required for kinase activity (Fig. 1A) (*6*–*8*). ABA binding to the PYR/PYL/RCAR family of ABA receptors promotes the receptors to bind to the catalytic site of PP2Cs and inhibit their enzymatic activity (Fig. 1A) (*3*–*5*, *9*–*12*). In turn, ABA-induced inhibition of PP2Cs leads to SnRK2 activation by activation loop autophosphorylation (*6*, *7*), which allows the SnRK2s to relay the ABA signal to downstream effectors (*13*, *14*). In plants, SnRK2 activation loop phosphorylation may also involve unidentified upstream kinases (*7*, *15*).

Recent structural studies have revealed a gate-latch-lock mechanism for PP2C inhibition by ABA receptors (*9*–*12*, *16*). The ABA-binding pocket of receptors is flanked by two highly conserved loops that serve as a gate and latch.

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