Selection and gene flow shape niche-associated variation in pheromone response

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From quorum sensing in bacteria to pheromone signalling in social insects, chemical communication mediates interactions among individuals in local populations. In *Caenorhabditis elegans*, ascaroside pheromones can dictate local population density; high levels of pheromones inhibit the reproductive maturation of individuals. Little is known about how natural genetic diversity affects the pheromone responses of individuals from diverse habitats. Here, we show that a niche-associated variation in pheromone receptor genes contributes to natural differences in pheromone responses. We identified putative loss-of-function deletions that impair duplicated pheromone receptor genes (srg-36 and srg-37), which were previously shown to be lost in population-dense laboratory cultures. A common natural deletion in srg-37 arose recently from a single ancestral population that spread throughout the world; this deletion underlies reduced pheromone sensitivity across the global *C. elegans* population. We found that many local populations harbour individuals with a wild-type or a deletion allele of srg-37, suggesting that balancing selection has maintained the recent variation in this pheromone receptor gene. The two srg-37 genotypes are associated with niche diversity underlying boom-and-bust population dynamics. We hypothesize that human activities likely contributed to the gene flow and balancing selection of srg-37 variation through facilitating the migration of species and providing a favourable niche for the recently arisen srg-37 deletion.

To maximize reproductive success, organisms must respond to changing environmental conditions. In a fluctuating environment, each response will likely have a fitness trade-off with reproductive success now or in the future. *Caenorhabditis elegans* can either grow to a reproductive adult in three days or delay maturity for months by entering the dauer diapause stage1, Food supply and pheromone signals act oppositely to promote either further reproductive growth or the development of a stress-resistant and long-lived dauer stage2. *C. elegans* secretes sugar-based pheromone compounds called ascarosides3, and individuals must measure the amount of remaining food and the ascaroside pheromones to determine whether it is advantageous to continue reproductive growth or to enter the dauer stage, disperse and hopefully encounter a new food source. Therefore, dauer formation decreases reproductive success in the short term in favour of future survival success. Decades of research have provided insights into the chemical and genetic bases of the dauer-pheromone response4. However, most studies used a single laboratory-adapted strain (N2), which has limited our understanding of the natural processes that have shaped the dauer-pheromone response.

After decades of focused laboratory research on *C. elegans* as a model organism, the natural history of this species has only recently been described from extensive field research5. These field studies have revealed that the dauer stage is important for the population dynamics in their natural habitat1. These dynamics are typified by a boom phase after the initial colonization of a nutrient-rich habitat, followed by a bust phase when resources are depleted. At the end of the boom phase when the local population size is large and nutrients are limited, individual animals enter the dauer stage. Dauers exhibit a stage-specific behaviour called nictation, which facilitates interspecific interactions between dauer larvae and more mobile animals to disperse to favourable environments6,7. Because dauer larvae are presumed to play a crucial role in the survival and dispersal of the species, it is likely that the genetic controls of dauer formation are under natural selection. Although differences in dauer development among a small number of wild *C. elegans* strains have been described previously8,9,10, no underlying natural genetic variant has been identified. Here, we integrate laboratory experiments, computational genomic analyses and field research to further our understanding of the genetic basis underlying intraspecific variation in pheromone-mediated developmental plasticity. We identify natural genetic variation in responses to dauer pheromones and characterize a pheromone receptor allele that has spread around the globe.

**Results**

Natural variation of the dauer-pheromone response was measured using a high-throughput dauer assay. To explore the effects of natural genetic variation on the ability to enter the dauer stage, we developed a high-throughput dauer assay (HTDA) to quantify the dauer-pheromone responses of wild *C. elegans* strains. The HTDA takes advantage of the observation that dauer larvae have no pharyngeal pumping11. We treated animals with fluorescent microspheres that can be ingested, and we then quantified both the fluorescence and size of individual animals using a large-particle flow...

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Fig. 1 | An HTDA measures the natural variation of the dauer-pheromone response. a, The workflow for the HTDA using a COPAS BIOSORT (see Methods for further description). G, generation. The bottom plot shows relative animal length measured by time-of-flight (µm) on the x axis and bead-derived green fluorescent intensity (arbitrary units, a.u.) on the y axis. Animal size and fluorescence-intensity traits are used as variables to build a model that differentiates dauer (blue) and non-dauer (orange) populations. Each point corresponds to the measurement of an individual animal, coloured by the stage. b, Measurements of the laboratory wild-type strain (N2) (top) and a Daf-c mutant, daf-2(e1370) (bottom), are shown under control (left) and ascr#5-treated (ascr#5 800 nM) conditions (right) at 25 °C using the HTDA. Animal size and fluorescence-intensity traits are used as variables to build a model that differentiates dauer (blue) and non-dauer (orange) populations. Relative animal length measured by time-of-flight (µm) is shown on the x axis, and bead-derived green fluorescent intensity (arbitrary units, a.u.) is shown on the y axis. c, Tukey box plots of the dauer fraction quantification from b are shown with data points plotted behind. Box plots are coloured by assay conditions (control (red) and ascr#5 800 nM treatment (blue)). The genotypes are shown on the x axis, and fractions of dauer larvae are shown on the y axis. d, Tukey box plots of the ascr#5 dose response at 25 °C for four divergent strains are shown with data points plotted behind. In c and d, the horizontal line in the middle of the box is the median, and the box denotes the 25th to 75th quantiles of the data. The vertical line represents 1.5× interquartile range.

cytometer (COPAS BIOSORT, Union Biometrica). These data facilitated computational classification of dauers (Fig. 1a,b and Methods) and recapitulated the known differences in the dauer-pheromone responses between N2 and a constitutive dauer mutant (Daf-c), daf-2(e1370), as well as the dauer-inducing effect of synthetic pheromone (Fig. 1b,c). To determine whether genetic variation within C. elegans causes differential dauer-pheromone responses, we applied the HTDA to four genetically divergent C. elegans strains (Fig. 1d and Supplementary Fig. 1). Among the conditions we tested, we found that 800 nM ascr#5 maximizes the among-strain variance and minimizes the within-strain variance in the dauer-pheromone response. These results enabled us to survey the effects of genetic variation on the dauer-pheromone response across C. elegans.

Genome-wide association mapping reveals multiple loci underlying natural variation in the ascr#5 response. Next, we quantified dauer induction of 157 wild strains that have been isolated from diverse habitats across six continents (Supplementary Fig. 2)\(^\text{17,18}\). We found significant variation in the ascr#5 response with a broad-sense heritability estimate of 0.29 (H\(^2\), s.e.m. = 0.14) and a narrow-sense heritability estimate of 0.18 (h\(^2\), s.e.m. = 0.12) (Fig. 2a and Methods). The two strains that represent the phenotypic extremes of the ascr#5 response are EG4349 and JU2576: EG4349 did not enter dauer and was completely insensitive to ascr#5 treatment, and a large fraction of the JU2576 individuals entered the dauer stage
The QTL that explained the most variation in pheromone-induced dauer induction (15.9%) is on the right arm of the X chromosome. Strains that have the non-reference (ALT) allele at the peak marker (X:14145335) of this QTL were less responsive to ascr#5 treatment than strains that have the reference (REF) allele (REF mean: 0.46; ALT mean: 0.30; log(P) = −5.851505). The remaining QTL on chromosomes II, III and IV explain 8.4%, 15.1% and 5.4% of the variation in the ascr#5 response, respectively. Because population structure can drive the mapping of loci that are in interchromosomal linkage disequilibrium (LD) with causal QTL, we checked the LD among the four QTL. We did not detect any obvious LD among these QTL (Supplementary Fig. 3), suggesting that multiple independent genomic loci underlie natural variation in the ascr#5 response.

A putative loss-of-function allele in an ascr#5 receptor gene is associated with reduced dauer formation. We focused our efforts on the QTL with the largest effect, which we named dauf-1 (dauerformation QTL #1). The 469 kb surrounding the dauf-1 peak marker contains 82 protein-coding genes (Supplementary Fig. 4), including the duplicated genes srg-36 and srg-37, which encode ascr#5 receptors14. Both genes are expressed in the same pair of chemosensory neurons (ASI), which play an essential role in the dauer-pheromone response15–17. Previous studies reported that both srg-36 and srg-37 are repeatedly deleted during long-term propagation of two independent laboratory-domesticated C. elegans lineages in high-density liquid cultures18–20.

To evaluate whether similar mutations in these two genes underlie the dauf-1 QTL, we investigated the genome sequences of 249 wild strains available through the C. elegans Natural Diversity Resource (CeNDR)22,23. Although we could not find a large deletion that removes both srg-36 and srg-37, we found only one strain with a 411-base-pair (bp) deletion in srg-36 and many other strains with an identical 94-bp deletion in srg-37 (Fig. 3a and Supplementary Fig. 5). We named these deletions srg-36(ean178) and srg-37(ean179). To test whether these deletions can explain the dauf-1 QTL effect, we analysed the association between the ascr#5 response and the two deletions. First, we found that srg-36(ean178), which is a deletion found only in the PB303 strain and removes the fourth and fifth exons, is associated with an insensitivity to a high dose of ascr#5 (2 μM) (Supplementary Fig. 6). Because this deletion allele was not found in any other wild strains, srg-36(ean178) cannot explain the population-wide differences in dauer formation. By contrast, we found that all wild strains with the srg-37(ean179) deletion belong to the dauf-1(ALT) group and had reduced ascr#5 sensitivity (Fig. 3b; Welch’s t-test, $P = 3.152 \times 10^{-4}$), suggesting that this deletion allele might cause a reduction in the ascr#5 response.

The srg-37(ean179) deletion removes 31 amino acids surrounding the pocket structure of the G protein-coupled receptor and causes a frameshift mutation for the 46 C-terminal amino acids, together removing 23% (77/324) of the predicted SRG-37 amino acid sequence. Thus, this deletion likely impairs SRG-37 function, which could cause lower ascr#5 sensitivity. We hypothesized that, if srg-37(ean179) causes loss of gene function, removal of additional srg-37 coding sequences would not further reduce the ascr#5 sensitivity of srg-37(ean179) wild strains. Using clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9 (CRISPR-Cas9) genome editing24,25, we removed most of the coding sequences of srg-37 from wild strains with both wild-type (reference-like) srg-37 and the natural srg-37 deletion (Fig. 3a). We observed that a large deletion in srg-37 did not change the ascr#5 sensitivities of two wild isolates with the natural deletion, but reduced the ascr#5 sensitivities of five wild isolates with reference-like srg-37 (Fig. 3c and Supplementary Fig. 7), indicating that the natural deletion is likely a loss-of-function allele. Taken together, these results show that deletion of an ascr#5 receptor gene underlies natural variation in the ascr#5 response.

**Fig. 2 | GWA mapping reveals four major loci underlying natural variation in the dauer-pheromone response.** a, A bar plot for the natural variation of ascr#5-induced dauer formation at 25 °C across 157 C. elegans wild isolates (one-way analysis of variance, log(P) = −49.6598). Each bar represents the phenotypic response of a single wild isolate to 800 nM ascr#5. b, A Manhattan plot for single-marker-based GWA mapping of the ascr#5-induced dauer formation trait from a. Each dot represents a single-nucleotide variant (SNV) that is present in at least 5% of the 157 wild strains. The genomic position in Mb, separated by chromosome, is plotted on the x axis, and the statistical significance of the correlation between genotype and phenotype is plotted on the y axis. Two significance thresholds are shown. The dashed horizontal line denotes the Bonferroni-corrected P value threshold using all markers, and the solid horizontal line denotes the Bonferroni-corrected P value threshold using independent markers correcting for LD (genome-wide eigen-decomposition significance threshold). SNVs are coloured red if they pass the second threshold. The region of interest for each QTL is represented by vertical blue dashed lines. c, Tukey box plots of phenotypes split by peak marker position of the four QTL. Each dot corresponds to the phenotype of an individual strain, which is plotted on the y axis as the normalized dauer fraction phenotype. Strains are grouped by their genotype at each peak QTL position, where REF (blue) corresponds to the reference allele from the laboratory N2 strain and ALT (red) corresponds to the alternative allele. The horizontal line in the middle of the box is the median, and the box denotes the 25th to 75th quantiles of the data. The vertical line represents 1.5x interquartile range.

in the same condition. Overall, we observed a continuous distribution of dauer-pheromone responses among these wild strains (mean = 0.41, s.d. = 0.20), indicating that natural variation in this trait is likely not explained by a single gene.

To characterize the quantitative trait loci (QTL) associated with variation in the ascr#5 response, we performed genome-wide association (GWA) mappings and identified four QTL (Fig. 2b,c).
natural variation in the dauer-pheromone response across the
*C. elegans* population.

**Selection has shaped the genetic variation of the two duplicated *C. elegans ascr#5*-receptor genes.** We performed population genetic analysis across the srg-36 and srg-37 region by analysing the genome sequences of 249 wild strains. Natural selection and demographic change can shift the allele frequency spectrum from neutrality, as measured by Tajima’s *D* \(^3\). Purifying selection, a selective sweep or a subsequent population expansion can cause accumulation of rare alleles at a given locus, indicated by a negative Tajima’s *D* value. We found that the Tajima’s *D* values were lowest across the promoter and coding regions of srg-36 and increased back to background neutrality rates in the promoter region of srg-37 (Fig. 3d and Supplementary Fig. 8). Differences in deletion allele frequencies between srg-36 and srg-37 suggest stronger purifying selection at srg-36. The 411-bp deletion allele, srg-36(ean178), is found only in a single wild isolate (PB303), whereas 18.4% (46/249) of wild isotypes (genome-wide) shows a stronger purifying selection at srg-36. The differences in deletion allele frequencies between srg-36 and srg-37 are shown in Fig. 3d and Supplementary Fig. 8. Differences in deletion allele frequencies between srg-36 and srg-37 suggest stronger purifying selection at srg-36. The 411-bp deletion allele, srg-36(ean178), is found only in a single wild isolate (PB303), whereas 18.4% (46/249) of wild isotypes (genome-wide genotypes) carry the 94-bp deletion allele, srg-37(ean179).

Although srg-36 and srg-37 are duplicated genes that are activated by the same ligand and are expressed in the same cells, differences in non-coding and coding sequences between the two genes can cause differences in gene expression levels and receptor activities. Previous studies report that transgene expression of srg-36 showed a stronger effect than srg-37 on the ascr#5 response \(^4\). To test whether srg-36, which is likely under stronger purifying selection than srg-37, plays a larger role in the ascr#5 response, we performed loss-of-function experiments. We removed the entire srg-36 coding region in two wild strains: JU346 with wild-type (reference-like) srg-36 and NIC166 with the natural 94-bp deletion allele (Fig. 3e). We found that the loss-of-function allele, srg-36(lf), reduced ascr#5 sensitivity more than the loss of srg-37, suggesting that srg-36 plays a larger role than srg-37 in the ascr#5 response.

The higher activity of srg-36 could be explained by differences in gene expression levels. We investigated the relative levels of
srg-36 and srg-37 at the first larval stage (L1), when these genes play critical roles in the dauer-pheromone response, and found that the expression levels of both genes are not significantly different (Supplementary Fig. 9; paired t-test, P = 0.1981; Methods). It is more likely that differences in protein-coding sequences cause the functional differences in the ascr#5 response. Although SRG-36 and SRG-37 show similarities in size and transmembrane structures (Supplementary Fig. 10), only 46.4% of the amino acid residues are conserved between both receptors. The molecular differences between the two ascr#5 receptors could cause quantitative differences in ascr#5-receptor activities. We therefore hypothesized that srg-36 is the primary ascr#5-receptor gene and is maintained across C. elegans through purifying selection. By contrast, the redundancy of these two genes might allow srg-37 variation, and a loss-of-function allele can arise and spread across the population.

The srg-37 deletion has spread globally and outcrossed with diverse genotypes. We investigated the locations where wild strains with the natural srg-37 deletion were isolated and found that 46 wild isolates with this allele were isolated from all six continents (Fig. 4a,b). Given the low probability of acquiring the same 94-bp deletion, we hypothesized that this allele did not independently arise across multiple global locations but originated from a single ancestral population and spread throughout the world. To test this hypothesis, we analysed the haplotype composition of C. elegans wild isolates across the X chromosome. We reproduced previous studies that showed a recent global selective sweep on the X chromosome (Supplementary Figs. 11 and 12). We found that all 46 isolates with the srg-37 deletion exclusively shared the swept haplotype at the srg-37 locus (Fig. 4c). By contrast, none of 203 isolates with wild-type srg-37 carries the swept haplotype at the srg-37 locus. This result not only demonstrates that this allele arose at a single location, but also implies that it has spread throughout the world along with the recent selective sweep. Because the srg-37 locus is far from the most swept part of the X chromosome, many strains must have outcrossed, suggesting that srg-37 is unlikely the driver of the X chromosome sweep. Specifically, we found that 34.1% (85/249) of wild isolates have an X chromosome that is swept more than 50% of its length but have diverse non-swept haplotypes at the srg-37 locus (Supplementary Fig. 13). Additionally, the genome-wide tree of 249 wild C. elegans isolates shows that the srg-37 deletion is not present in many subpopulations (Fig. 4d). These results suggest that the srg-37 deletion spread globally with the selective sweeps but has not been purged after more recent outcrossing.

Two different srg-37 genotypes coexist and associate with different niches. These signatures of multiple outcrossing events imply the co-occurrence of wild strains with and without the srg-37 deletion in the same habitats. Indeed, we found that many local populations across the world harbour distinct individuals with either the wild-type srg-37 or the deletion allele (Supplementary Fig. 14; see Methods). Because each genotype can be adaptive to different
environmental conditions, we analysed the allele frequencies of the srg-36 deletion among three subpopulations sampled from animals, compost and rotting fruits across geographic locations where both srg-37 alleles were isolated. Because reduction of the dauer-pheromone response can promote reproductive growth, we investigated whether wild strains with the srg-37 deletion were sampled more often from substrates with proliferating populations. These populations are often found in nutritious habitats, such as rotting vegetation. By contrast, C. elegans were sampled predominantly in the dauer stage from animal and compost substrates. We found that wild strains with the srg-37 deletion were 67% enriched in rotting fruits (Fig. 4e and Supplementary Data 1; hypergeometric test, \( P = 0.0026 \)). Thus, this allele is associated not only with lower dauer-pheromone responses but also with natural substrates that are known to support reproductive growth. We also analysed \( F_{ST} \) statistics of the entire X chromosome for subpopulations from different substrates across shared geographic regions. Consistent with the niche association pattern of srg-37 genotypes, we found the highest genetic divergence between the subpopulation from rotting fruit and the subpopulation from animal substrates at a genomic locus around the srg-37 gene (Supplementary Fig. 15).

Discussion

Dauer pheromones are chemical signals that are perceived by sensory neurons using chemoreceptors and cyclic guanosine monophosphate-mediated signalling. In the absence of dauer-pheromone signalling, the insulin/insulin-like growth factor 1 and transforming growth factor beta signalling pathways promote reproductive growth through the production of steroid hormones (dafachronic acid). Genetic variation in the genes that mediate pheromone perception or downstream signalling likely alter an individual’s dauer-pheromone response. However, because the signalling pathways that act downstream of pheromone perception are involved in various biological processes, mutations in these pathways might cause deleterious pleiotropic effects. Previous studies have shown that the ascr#5 receptors SRG-36 and SRG-37 were lost in two independent laboratory lineages of C. elegans, suggesting that selection more readily acts at the pheromone perception step of this developmental pathway. In this study, we provide further support for this hypothesis by showing that 18% of wild C. elegans strains harbour a putative loss-of-function deletion in only the ascr#5 receptor SRG-37, and that these individuals are more likely to be found in nutrient-rich habitats. Modification of pheromone-receptor activity might thus be favoured in both laboratory and natural conditions to fine-tune dauer-pheromone responses with few pleiotropic effects. However, we identified additional dauer-pheromone response QTL, suggesting that multiple loci are involved in ascr#5 responses. Interestingly, SRG-36 and SRG-37 are the only two known ascr#5 receptors involved in dauer-pheromone signalling. The presence of three additional ascr#5-response QTL suggests that natural genetic variants could affect uncharacterized ascr#5 receptors, novel or known factors that regulate receptor activity, or downstream signalling components.

Insights into the redundant functions of srg-36 and srg-37 were first gained from the observation that both genes were deleted from two independent laboratory-domesticated C. elegans lineages. We did not find a single wild strain in the C. elegans population that carries a deletion of both srg-36 and srg-37. Investigations of neutrality statistics (Tajima’s D) suggest that selection acts on these two genes differently. Our results indicate that the srg-36 and srg-37 genes might not be functionally equivalent in the wild population. The loss-of-function experiments suggest that srg-36 plays a larger role in the ascr#5 response than srg-37. Substantial differences in amino acid sequences between SRG-36 and SRG-37 suggest that the SRG-37 protein is likely to have less ascr#5 binding affinity or weaker signal transduction activity than SRG-36. It is also possible that redundancy between SRG-36 and SRG-37 has been reduced since the time of gene duplication, and SRG-37 could gain sensitivities to other ascarosides while SRG-36 has maintained its ascr#5 specificity. Given the important role of the dauer stage in the long-term survival and dispersal of the species, purifying selection might act to conserve the primary ascr#5 receptor (SRG-36) in the C. elegans population to maintain the responsiveness to the dauer-inducing pheromone ascr#5.

In contrast to the rare deletion of srg-36, we identified a common deletion allele (18% allele frequency) of srg-37 in the global C. elegans population. We discovered that strains harbouring different srg-37 genotypes (wild-type and deletion) have been found often in close proximity at various locations across the world, suggesting that balancing selection might have maintained both genotypes in local habitats. Previously, features of balancing selection were also reported for a locus with other pheromone-receptor genes (srx-43 and srx-44) that underlie differences in C. elegans density-dependent foraging behaviour. Differences in food distribution can exert bidirectional fitness effects on foraging behaviour. Similar to these effects, dauer formation can be disadvantageous during the population growth phase (boom phase) but beneficial during the dispersal phase (bust phase). Therefore, we hypothesize that the loss of srg-37, which reduces dauer formation, has trade-off effects between the boom and bust phases. Niche association patterns of srg-37 genotypes support this hypothesis. We found that wild strains with the srg-37 deletion are enriched in a rotting fruit niche, where ample bacterial food can support population growth during the boom phase. By contrast, the srg-37 deletion is not enriched in wild strains isolated from animal carriers, which is consistent with known behavioural ecology during the bust phase when dauer larvae can readily hitchhike on other animals for their dispersal. Our \( F_{ST} \) analysis also demonstrated significant genetic divergence at the srg-37 locus between wild strains isolated from rotting fruit and those isolated from animal carrier substrates. These observations suggest that the boom-and-bust population dynamics in wild habitats likely drive balancing selection of srg-37.

Population genomic analyses of the srg-37 locus imply that the srg-37 deletion arose recently and balancing selection might have occurred only for a short period. We found that strains with the srg-37 deletion all share the same swamp haplotype at the srg-37 locus, which is estimated to have spread worldwide in the last few centuries. Because mutation and recombination decrease LD between a selected allele and the surrounding variants over time, this haplotype homogeneity suggests that the deletion allele arose recently. Moreover, we found no genomic signatures of long-term balancing selection. Tajima’s D statistics for the srg-37 locus did not show typical features of long-term balancing selection (that is, \( D \) was not much greater than 0). We also found that genetic diversity (\( \pi \)) is reduced at the srg-37 locus in strains that carry the srg-37 deletion compared to strains that carry the wild-type allele (Supplementary Fig. 16). This result is a signature of a recently established balanced situation. We hypothesize that this recent balancing selection is related to human activities, which were also suggested to be drivers of the recent global selective sweeps. Agriculture could have provided nutritious niches and therefore expanded boom phases spatiotemporally, which is likely to cause an increase in selective pressures to maintain the srg-37 deletion. Furthermore, human migration could facilitate the worldwide gene flow of the srg-37 deletion allele. Our study implies that human civilization might exert a large impact on the natural selection and evolution of wild species.

Methods

C. elegans strains. Animals were cultured at 20 °C on modified nematode growth medium seeded with the Escherichia coli strain OP50. Before each assay, strains were passaged for at least four generations without entering starvation or encountering dauer-inducing conditions. For the GWA studies, 157 wild isolates...
from CeNDR (version 20170531) were used^{22}. All strain information can be found in Supplementary Data 2.

High-throughput dauer assay. Strains were propagated for four generations on agar plates, followed by bleach synchronization. Approximately 50 embryos were placed into each well of a 96-well microtiter plate filled with 50 μl of K medium with modified salt concentrations (10.2 mM NaCl, 32 mM KC1, 3 mM CaCl2, 3 mM MgSO4), 30 μM kanamycin, 5 mg/ml HB101 bacterial lysate (Pennsylvania State University Shared Fermentation Facility) and synthetic ascorbate dissolved in 0.4% ethanol or 0.4% ethanol. Animals were cultured for 52h at 25°C until they reached the young adult stage or arrested at the dauer stage. Animals were exposed to 0.5 μm fluorescent microspheres (Polysciences, cat. no. 19507-5) at a final concentration of 7.28 × 10^9 particles ml^-1 and 5 μl of 1 mg/ml HB101 bacterial lysate to promote feeding for 20 min. After this exposure, 5 μl of 0.5 μM sodiu ammonium azide was added to each 3.9 ml of PBS to kill the animals, stop feeding and straighten the animals. Using the COPAS BIOSORT large particle flow cytometer (Union Biometrica), optical parameters of animals, including fluorescence intensity, TOF (animal length) and extinction (optical density), were measured. Measured parameters were used to build a model that can differentiate dauer and adult stages of the population in each well through the R package EMCluster. One cluster with lower fluorescence and smaller body size was assigned to the dauer population and the other to the non-dauer population. The dauer fraction was calculated per well as a fraction of dauer animals among total animals, which is shown as a single data point in each plot. From the control experiments, both the false-positive ratio (false dauer detection in a wild-type sample without pheromone treatment) and the false-negative ratio (false non-dauer detection in Da-c mutant sample) were 5%, indicating 95% accuracy of the sample without pheromone treatment and the false-negative ratio (false non-daruer fraction were calculated using a linear model, dauer fraction ~ batch. Genotype data were acquired from the latest VCF release (Release 20180527) from CeNDR (Polysciences, cat. no. 19507-5) at a final concentration of 7.28 μl of 0.5 μM Cas9 protein (IDT, product no. 1074181) and was added and incubated at room temperature (95°C for 5 min. After cooling to room temperature, 2.87 μl of 60 μM Cas9 protein (IDT, product no. 1074181) was titred and placed into each well of a 96-well microtitre plate filled with 100 μM dpy-10 crRNA (IDT) and was incubated at 95°C for 15 min. After cooling to room temperature, 50 ml of 100 μM dpy-10 crRNA (IDT) were thawed at room temperature (95°C for 5 min). Finally, 0.5 μl of 100 μM dpy-10 snoDN (IDT) required template and 3.9 μl of nuclelease-free water were added. Ribonucleoprotein injection mixtures were microinjected into the germline of young adult hermaphrodites (Polysciences, cat. no. 19507-5) and, injected animals were singled to fresh 6 cm NGM plates 18 h after injection. Two days later, F1 progeny were screened, and animals expressing a Rol phenotype were transferred to new plates and allowed to generate progeny (F2). Then F1 animals were genotyped by PCR. Deletion of srg-36 was detected with primers oECA1460-1463, and deletion of srg-37 was detected with primers oECA1429, oECA1430 and oECA1435. Non-Rol progeny (F2) of F1 animals positive for the desired deletion were propagated on separate plates to generate homozygous progeny. F2 animals were genotyped afterwards with same primer sets, and PCR products were banger sequenced for verification. All crRNA and oligonucleotide sequences are listed in Supplementary Table 1.

Gene expression analysis of srg-36 and srg-37. Gene expression levels of srg-36 and srg-37 at the L1 larval stage (WBts:00000024) in the N2 strain were analysed from published whole-animal (WBIb:0007833) RNA-seq datasets (ERPP03471, SRRP00401, SRP003783, SRP003786, SRP034352, SRP040623, SRP058023)22. To equally weight datasets with different numbers of replicates, mean values of parameters per kilobase of transcript per million mapped reads for each dataset were used for gene expression comparisons.

Population genetics. Sliding window analyses of population genetic statistics ( Tajima’s D, Fst, and π) were performed using the PopGen PACKAGE in R. All sliding window analyses were performed using the imputed SNV VCF available on the CeNDR website with the most diverged strains, XZ1516, set as the outgroup22. The LD of the QTL markers, which can be measured as the square of the correlation coefficient (r^2), was calculated using the genetics package in R. The formula for the correlation coefficient is r = √(p(a)×p(a)×p(b)×p(b)), where D is the coefficient of linkage disequilibrium, p(a) = the observed probability of allele ‘a’ for marker 1, p(b) = the observed probability of allele ‘b’ for marker 1 and p(b) is the observed probability of allele ‘b’ for marker 1. Haplotyplic composition of each wild isolate was inferred by applying IBDesc with variants calls by RCFOols and the following parameters: filtering: depth (DP) > 10; mapping quality (MQ) > 40; variant quality (QUAL) > 10; (alternate allele depth (AD) / total depth (DP)) < 0.5; <10% missing genotypes; <10% heterozygosity. To generate the genome-wide tree, a whole-population relatedness analysis was performed using RAXML-ng with the GTR+F+ substitution model (https://github.com/20210531/zenodo.930797). SNVs were LD-pruned using PLINK (v1.9) with the indel-pairwise command ‘indep-pairwise 50 1 0.95’. We used the vcf2phylpp.py script (https://doi.org/10.5281/zenodo.1257058) to convert the pruned VCF files to the PHYLIP format22 required to run RAXML-ng. To construct the tree that included 249 strains, we used the GTR evolutionary model available in RAXML-ng22. Trees were visualized using the ggtree (version 1.10.5) R package22.

Substrate specificity analysis in the cosampling zone. The cosampling zone was defined as a location where both srg-37(+)/srg-37(-/+)44,45 were isolated (Supplementary Fig. 11). Collection information available on the CeNDR website was used to analyse correlations between the whole substrate and the srg-37 genotype of each isolate. Isolations of wide strains that shared the same genome-wide genotypes (isotype) were counted as independent isolations if they were sampled from different locations or from different substrate types. We found that 95 isolates were isolated in the cosampling zone from at least 119 independent isolations. Three substrates (animals, compost and rotting fruit) with more than ten independent isolated strains were selected for the substrate enrichment test. In total, 82 wild strains (66 isotypes) were grouped into three subpopulations by the substrate where they were isolated, and allele frequencies of each subpopulation were calculated. Significant enrichment of srg-37(-/+)44,45 in each subpopulation was determined by hypergeometric tests using the stats R package22.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All datasets, including HTDA raw data, for generating figures are available on GitHub (https://github.com/AndersenLab/DauerSRG3637).
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**Author contributions**

D.L. and E.C.A. conceived and designed the study. D.L. performed the high-throughput assay, CRISPR–Cas9 genome editing, population genomic analyses and niche enrichment tests. S.Z. performed the GWA mapping, identified genetic variants in the *dauf-1* locus, generated the genome-wide tree of 249 wild *C. elegans* strains and edited the manuscript. D.E.C. analysed the haplotype composition of 249 wild strains. L.F., J.C.H., M.G.S., J.A.G.R., J.W., J.E.K., C.B. and M.-A.F. contributed wild isolates to the *C. elegans* strain collection. F.C.S. provided the dauer pheromone. D.L. and E.C.A. analysed the data and wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

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