Balancing selection shapes density-dependent foraging behaviour

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The optimal foraging strategy in a given environment depends on the number of competing individuals and their behavioural strategies. Little is known about the genes and neural circuits that integrate social information into foraging decisions. Here we show that ascaroside pheromones, small glycolipids that signal population density, suppress exploratory foraging in Caenorhabditis elegans, and that heritable variation in this behaviour generates alternative foraging strategies. We find that natural C. elegans isolates differ in their sensitivity to the potent ascaroside icas#9 (IC–asc–CS). A quantitative trait locus (QTL) regulating icas#9 sensitivity includes srx–43, a G–protein–coupled icas#9 receptor that acts in the ASI class of sensory neurons to suppress exploration. Two ancient haplotypes associated with this QTL confer competitive growth advantages that depend on ascaroside secretion, its detection by srx–43 and the distribution of food. These results suggest that balancing selection at the srx–43 locus generates alternative density–dependent behaviours, fulfilling a prediction of foraging game theory.

The success of a particular foraging strategy varies according to the behaviour of competitors. Balancing selection can therefore favour the co-existence of multiple strategies within a species¹,². The pioneering example of strategic competition is the natural genetic variation seen at the foraging (for) gene in Drosophila melanogaster larvae³,⁴. Two for alleles for active (rover) or sedentary (sitter) behaviour are maintained in a population because of frequency-dependent balancing selection against larvae with the more common foraging strategy⁵. This example, and others like it, suggests that animals could benefit from detecting against larvae with the more common foraging strategy⁵. This example, and others like it, suggests that animals could benefit from detecting

individual wild-type N2 strain animals (Fig. 1a). To mimic the effects of high population density on these isolated animals, we conducted the assay in the presence of natural pheromone extracts. The pheromones strongly suppressed exploration (Fig. 1b), as did several pure synthetic ascarosides at concentrations at or below those that induced dauer larva development (Fig. 1c, d). However, ascr#5, a potent regulator of dauer development, only weakly suppressed exploration (Fig. 1d). Thus a subset of ascarosides regulates foraging behaviour at biologically relevant concentrations.

The exploration assay is an indirect measure of the relative time C. elegans spends in roaming and dwelling states⁷,⁸. Quantitative behavioural analysis of video recordings showed that the potent ascarosides icas#9 and ascr#8 decreased the fraction of time spent

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a. Schematic of the exploration assay. b. Wild-type N2 response to crude pheromone extract, showing exploration scores and a pheromone–response index, presented as mean ± s.e.m. c. Structures and names of selected ascarosides. d. N2 response to individual ascarosides, presented as mean ± s.e.m., **P < 0.01, ***P < 0.001 by ANOVA with Dunnett’s correction; NS, not significant.
A QTL for pheromone sensitivity

A variety of genetically diverse wild-type C. elegans strains, including the control N2-like strain CX12311, responded to the presence of ascarosides with a suppression of exploration12 (Fig. 2a). The wild-type German strain MY14 failed to respond to 10 nM icas#9 in the exploration assay, although it responded normally to the presence of ascr#2, ascr#3, and ascr#8 (Fig. 2a and Extended Data Fig. 2). Coupled alterations in the levels of pheromone signalling and detection ability can contribute to reproductive isolation during incipient speciation15. However, MY14 and CX12311 were found to produce similar levels of icas#9 and another 16 ascarosides (Extended Data Fig. 3), indicating that the change in the icas#9 response in MY14 was independent of icas#9 production.

To determine the genetic basis of icas#9 insensitivity, 94 recombinant inbred lines (RILs) were generated from intercrosses between MY14 and CX12311 strains. A continuous distribution of icas#9 sensitivity was observed in the exploration behaviour of the RILs (Fig. 2b), suggesting that two or more loci contribute to icas#9 sensitivity. The 94 RILs were genotyped at ~185-kb resolution across the genome by low-coverage whole-genome sequencing14 (Supplementary Table 1). QTL analysis identified a single significant QTL of genome-wide significance that accounted for 34.9% of the total variance between the RILs, which we term roam-1 (Fig. 2c). Covariate analysis failed to find additional QTLs that were either additive or interactive with roam-1 (Extended Data Fig. 4).

The impact of roam-1 on foraging was confirmed by creating near-isogenic lines (NILs) that had small genetic regions substituted between the strains. NILs in which the 2.5 Mb surrounding roam-1 were reciprocally exchanged between CX12311 and MY14 were intermediate in their icas#9 sensitivity compared to the parental strains (Fig. 2d). Depending on the direction of the introgression, the roam-1 locus accounted for 39–46.4% of the genetic variance between the two parental strains.

To simplify further mapping, the roam-1 region from MY14 (kyIR144, Fig. 2e) facilitated further mapping that localized roam-1 to 182 kb (Fig. 2e). High-density mapping of 2,600 F2 progeny of a cross between N2 and the NIL kyIR147 yielded 12 informative recombinants in this 182-kb region that mapped the roam-1 QTL to a 37-kb region on chromosome V (Fig. 2f and Methods).

roam-1 affects the icas#9 receptor SRX-43

The 37-kb roam-1 region contains 16 protein-coding genes, including 5 genes that encode predicted G-protein-coupled chemoreceptors in the srx or str gene families (Fig. 3a). We thought it possible that one or more of the chemoreceptors could be icas#9 receptors, with reduced activity in MY14. We therefore introduced N2-derived sequences that overlapped the chemoreceptor genes into the roam-1 locus in MY14 to assess the possible relationship of the chemoreceptors to icas#9 sensitivity (Fig. 3b, 3c, and Methods). The function of srx-43 was examined further by characterizing loss-of-function mutations in the endogenous srx-43 gene (srx-43(lf); Methods). We found that srx-43(lf) mutants in both N2 and roam-1(N2) MY14 genetic backgrounds were insensitive to icas#9 (Fig. 3c). Sensitivity to icas#9 in the N2 srx-43(lf) mutant was restored by the introduction of an N2 srx-43 transgene (Fig. 3c). These results indicate that srx-43 is necessary for the icas#9 response in both N2 and MY14 strains and is essential for the behavioural difference between them.

The activity of the N2 and MY14 srx-43 genes was compared by targeting a single copy of srx-43 from each strain to a defined locus. This was done using the Mos1 transposase in an srx-43(lf) mutant, so that the single-copy transgene was the sole source of srx-43. The N2 srx-43 genomic region fully rescued the icas#9 response, whereas the MY14 region did not (Fig. 3d). The different effects induced by the introduction of different single-copy transgenes indicate that MY14 srx-43 possesses reduced activity compared to N2 srx-43.

Reporter genes with N2 or MY14 srx-43 sequences driving expression of green fluorescent protein (GFP) were expressed selectively in the ASI sensory neurons (Extended Data Fig. 5a), which promote roaming...
behaviour\textsuperscript{4,15}. A genomic clone with GFP fused to the C-terminus of the SRX-43 protein was enriched in ASI sensory cilia, the site of sensory transduction (Fig. 3e), suggesting that SRX-43 is a chemoreceptor. We investigated the effects of icas\#9 on ASI activity using in vivo calcium imaging, but did not observe a response. This negative result is consistent with studies of dauer formation, in which ascarosides regulate gene expression in ASI neurons and not acute calcium levels\textsuperscript{16–19}.

To investigate whether SRX-43 was an icas\#9 receptor, srx-43 cDNA was expressed in the ASH class of sensory neurons, which are normally insensitive to ascarosides\textsuperscript{17} (Extended Data Fig. 5b). Ascaroside-induced calcium flux was then monitored using genetically encoded calcium indicators\textsuperscript{17,20}. ASH neurons expressing SRX-43 responded to 10 nM icas\#9 with calcium transients, but this was not observed in response to other ascarosides or indole (Fig. 3f). Although the MY14 strain was largely insensitive to icas\#9 in foraging assays, MY14 SRX-43 also detected icas\#9 when expressed in ASH (Extended Data Fig. 5c).

We observed expression of a MY14-derived srx-43::GFP reporter gene in ASI neurons, but this appeared to be weaker than expression of the N2 srx-43::GFP reporter (Extended Data Fig. 5a). To investigate which sequences led to the difference in N2 and MY14 srx-43 activity, we exchanged the srx-43 promoter and coding regions of both strains and tested these constructs as Mos1-mediated single-copy insertion (MosSCI) srx-43 transgenes. A transgene with the promoter region of the N2 strain and the coding region of MY14 rescued icas\#9 sensitivity in srx-43(null) mutants but the opposite did not, localizing the difference to the srx-43 promoter (Fig. 3g). Quantitative measurements of endogenous srx-43 mRNA levels demonstrated that srx-43 was expressed at a level fivefold lower in roam-1(lm104) than in N2 (Fig. 3h). Therefore, the natural variation in srx-43 promoter activity between N2 and MY14 affects srx-43 gene expression and behavioural sensitivity to icas\#9.

Ascarosides promote dauer larva development in part by suppressing the transcription of daf-7, a gene expressed in ASI neurons that encodes a secreted TGF\beta-related peptide\textsuperscript{19}. As with animals treated with ascarosides, daf-7(null) mutants exhibit reduced levels of roaming\textsuperscript{4}. Expression of a daf-7::GFP reporter was significantly reduced by treatment with icas\#9, indicating that daf-7 may be a target for icas\#9; animals bearing a daf-7 mutation were also less responsive to icas\#9 than controls (Extended Data Fig. 5d, e). Behaviour was only influenced by icas\#9 after several hours of exposure (Extended Data Fig. 5f), a delay that is in agreement with the slow transcriptional regulation of the daf-7 signalling pathway. Together, these results suggest that icas\#9 acts as a primer pheromone\textsuperscript{12,22} that regulates foraging via transcription and endocrine signalling.

Balancing selection at a foraging QTL

To understand the population genetics of roam-1, we examined the genomic sequence of a 20-kb region centred around srx-43 in 39 additional wild-type C. elegans isolates sequenced by the Million Mutation Project\textsuperscript{23}. Two discrete, highly divergent haplotypes for the roam-1 region were found: one resembling N2 that was present in 34 strains and another resembling MY14 that was present in 7 strains with different geographical origins and genetic backgrounds (Fig. 4a, b and Extended Data Fig. 6a). The N2 and MY14 haplotypes differed by 2.64\% of all positions over the 20-kb srx-43 region, a figure 12 times that of the genome-wide average\textsuperscript{24}. These data were, however, derived using Illumina sequencing, a method that can fail to align highly divergent sequences. Targeted Sanger sequencing revealed that MY14 and N2 actually differed at 19.7\% of all positions in the srx-43 promoter and coding regions (Extended Data Fig. 6b). A phylogeny constructed for srx-43 and the most closely related genes in C. elegans, C. briggsae, and C. remanei confirmed that the divergent srx-43 alleles represent the same gene (Extended Data Fig. 6c). All seven tested MY14-like strains were relatively resistant to icas\#9 compared to N2-like strains (Fig. 4c). These results suggest that naturally occurring resistance to icas\#9 is associated with a highly divergent roam-1 haplotype that includes srx-43.

The marked allelic divergence of the roam-1 region, together with the observation that most genes in the interval including srx-43 have a low $d_{S}/d_{s}$ ratio (Extended Data Table 1), suggests that these alternative haplotypes might be subject to balancing selection. To examine this possibility, we analysed a large database of wild-type strains assembled and sequenced by the Andersen laboratory at Northwestern University (CeNDR; http://www.elegansvariation.org). The MY14 haplotype was present in 21 of the 152 unique strains, with the remainder being of the N2 haplotype. Both haplotypes were found globally: the rarer MY14 haplotype was found in Europe, the United States, New Zealand and Chile. In almost all cases in which individuals with the MY14 haplotype were isolated, individuals of the N2 haplotype were isolated from proximal environments at the same time—a distribution that is consistent with balancing selection.

Sequence features of the roam-1 region also fit the criteria for a region under balancing selection. The region encompassing roam-1 had a relatively high Tajima’s D of 1.01, a figure unusual both at the genomic level (at which <3.4\% of bins had a higher value) and in the centre of chromosome V where srx-43 lies (at which <3.6\% of bins had a higher value). A phylogenetic analysis of the 152 strains revealed that the roam-1 haplotype extends by approximately 30 kb before being disrupted by recombination events (Extended Data Fig. 7). Given the low outcrossing rate in wild-type C. elegans populations\textsuperscript{25,26}, this short roam-1 haplotype suggests co-occurrence of both haplotypes within interbreeding populations over many generations.

To assess directly the possibility that selection could act on roam-1, we designed competition experiments to compare the relative fitness of N2 and roam-1(MY14) strains. Experiments were conducted under high-density conditions to permit the accumulation and detection of endogenously produced icas\#9, with competition applied by growing C. briggsae or C. remanei with roam-1(MY14) strains and monitoring the number of C. elegans offspring. The first competition experiments were conducted on a simple lawn of Escherichia coli OP50 bacteria with a population of 20 N2 and 20
dependent on exogenous icas#9 (Fig. 5d). These results thus indicate that selection on the roam-1 locus depends on pheromones. Indica
ting that natural trait variation acts explicitly at the intersection of
interactions30,31. Our results ground this abstraction in observation,
physiological responses.

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humans, is dominated by evidence for gene–environment interactions30,31. Our
results ground this abstraction in observation, showing that natural trait variation acts explicitly at the intersection of
innate circuits and environment cues, with genetic changes allowing the
differential incorporation of environmental information into innate foraging behaviours.

Discussion

Conspecific individuals are informative elements of an animal's natural
environment, in part because they represent competition for resources.
Our results demonstrate that conspecific pheromones alter long-term
foraging strategies and that natural variation in this behaviour stems
from altered expression of the icas#9 receptor SRX-43. The comple-
ment of ascarcosides produced by C. elegans varies with sex, age and
feeding status27,28. The specificity of receptors such as SRX-43 provides
a mechanism through which this information can be detected by
the nervous system, allowing it to regulate different behaviours and
physiological responses. srx-43 is expressed in ASI sensory neurons,
which are targets of internal neuromodulators that regulate roaming
and dwelling5, and represents a site of integration for internal and exter-
nal influences on foraging behaviour. Although we do not know the
suite of pheromones that are produced by C. elegans in the wild, the
presence of secreted icas#9 in dense culture supernatants at concen-
trations 100-fold above those that suppress roaming suggest that it is a
relevant regulator of foraging and that altered sensitivity to this mole-
cule could affect animals' overall sensitivity to secreted ascarcosides.

The roam-1 QTL that encompasses srx-43 has sequence features
consistent with an area under balancing selection. While srx-43 is an
essential component of roam-1, it may not be the only gene in this
QTL, or the only gene that is under balancing selection, as the haplo-
type extends for ~30 kb to include several other genes. Moreover, the
behaviour identified here need not be the most important one in natural
settings; it may represent one of several behavioural and physiological
responses that facilitate adaptation to different environments. Balancing
selection may be fairly common throughout the C. elegans genome: a recent report identified 61 highly divergent
regions consistent with balancing selection that segregate among wild
strains of C. elegans, including a second region 200 kb from roam-1
(refs 24, 29; Extended Data Fig. 8). The composition of these regions
is biased towards particular gene classes, including chemoreceptors,
which may act as evolutionary hotspots.

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Online Content Methods, along with any additional Extended Data display items and
Source Data, are available in the online version of the paper; references unique to
these sections appear only in the online paper.

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Figure 5 | Bidirectional competitive selection at the roam-1 locus. a, Diagram of 'boom–bust’ competition experiments, with food depletion
followed by 48 h of starvation before transfer. b–d, Competition on a 'simple lawn' showing allele ratio of DNA harvested at transfers 1 and 3.
N2 versus roam-1MY14 NIL (b), N2 srx-43(lf) versus roam-1MY14 srx-43(lf) (c) and N2 daf-22(lf) versus roam-1MY14 daf-22(lf), without or with 10 nM
exogenous icas#9 (d). e, ‘Patchy lawn’ competition between N2 and roam-1MY14 NIL. Grey points represent individual competition
experiments; red line indicates the mean. *P < 0.05, **P < 0.01, ***P < 0.001 compared to an expected value of 0.5 by t-test with
Bonferroni correction.

roam-1MY14 age-matched adults (Fig. 5a). These conditions resulted in
a growth advantage for the N2 genotype over roam-1MY14 in the
first cycle of competition that continued in subsequent cycles (Fig. 5b).
The two tested strains differ in the 182-kb roam-1 region, which
encompasses 81 genes. To investigate whether this competitive advan-
tage required srx-43, the experiment was repeated using N2 srx-43(lf)
and roam-1MY14 srx-43(lf) strains. The competitive N2 advantage
disappeared in this setting, indicating that the icas#9 receptor SRX-43 is
essential for the competitive effect (Fig. 5c).
We assessed the role of endogenous pheromones by repeating
the competition experiments with N2 and roam-1MY14 strains with
loss-of-function mutations in the gene daf-22, which is required for
the secretion of ascarcosides including icas#9. Mutations in daf-22
eliminated the competitive advantage of the N2 strain over roam-1MY14
(Fig. 5d). A partial advantage for N2 daf-22(lf) was recovered upon
addition of exogenous 10 nM icas#9 (Fig. 5d). These results thus
indicate that selection on the roam-1 locus depends on pheromones.
The increased roaming of roam-1MY14 animals at high population
densities could be expected to cause greater exploration of a patchy
food environment. In a second competition design, we seeded a patchy
environment consisting of 16 small bacterial lawns with 20 N2 and
20 roam-1MY14 adults (Fig. 5a). In these conditions, the N2 advantage
was lost and instead a moderate but significant selection favoured
roam-1MY14 over N2 animals (Fig. 5e). Together, these results
demonstrate that roam-1 has a bidirectional effect on fitness that is
dependent on srx-43 expression, pheromone production and food
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Supplementary Information is available in the online version of the paper.

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Author Contributions J.S.G. designed and performed the genetic, molecular biology, and behavioural experiments, together with M.B. for RIL analyses and I.G.I. for competition experiments. M.D. performed calcium imaging experiments. E.Z.M. discovered the effect of pheromones on foraging. X.Z. and R.A.B. analysed pheromone production and synthesized pure pheromones. D.J.C. and P.T.M. performed population genetic analysis. J.S.G. and C.I.B. analysed and interpreted data. J.S.G. and C.I.B. wrote the manuscript, with input from all authors.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to C.I.B. (cori@rockefeller.edu).

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Nematode culture. All strains were grown at 21–22 °C on nematode growth-medium plates seeded with *E. coli* OP50 bacteria. For OP50 cultures, a single colony was inoculated into 100 ml of LB and grown for 48 h at 21–22 °C. Transgenic lines were generated by standard injection methods and included the desired transgene, a fluorescent co-injection marker and an empty vector, bringing the total DNA concentration up to 100 ng μl⁻¹. For each transgene, three independent extrachromosomal lines that propagated the transgene at high rates were tested in parallel to account for variability typical of such strains. All mutagenized strains were back-crossed 5–7 times before characterization.

Natural isolates and origin of wild-type strains. *N2* (Bristol, UK), CX12311 *kyIR1* (chrVCB4865 > N2) V; *qgIR1* (chrX, CB4865 > N2), CB4865 (HW) (Hawaii, USA), JU258 (Riberio Frio, Madeira), MY1 (Lingen, Germany), MY14 (Mecklenberg, Germany), JU775 (Lisbon, Portugal), JU1400 (Sevilla, Spain), JU1652 (Montevideo, Uruguay), BA1 (Adelaide, Australia), MY16 (Mecklenberg, Germany), JU1171 (Concepcion, Chile), MY6 (Roxel, Germany), JU360 (Francoizville, France), ED3021 (Edinburgh, Scotland), MT2 (Roxel, Germany).

Strain CX12311 bears ancestral alleles of the *nap-1* and *gab-3* genes, which affect oxygen sensitivity and are mutated in the N2 laboratory strain; it is therefore used as a comparison strain for wild-type strains bearing the ancestral alleles.

**METHODS**

**Nematode culture.** All strains were grown at 21–22 °C on nematode growth-medium plates seeded with *E. coli* OP50 bacteria. For OP50 cultures, a single colony was inoculated into 100 ml of LB and grown for 48 h at 21–22 °C. Transgenic lines were generated by standard injection methods and included the desired transgene, a fluorescent co-injection marker and an empty vector, bringing the total DNA concentration up to 100 ng μl⁻¹. For each transgene, three independent extrachromosomal lines that propagated the transgene at high rates were tested in parallel to account for variability typical of such strains. All mutagenized strains were back-crossed 5–7 times before characterization.

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**MY14–CX12311 RILs.** CX14697–CX14712, CX14731–CX14748, CX14750–CX14757, CX14783, CX14784, CX14876–CX14820, CX14822–CX14839. Genotypes inferred from low-coverage genomic sequence and behavioural data are included as Supplementary Table 1.

**Near-isogenic lines.** CX15881 *kyIR142 (chrV:14–16.8 Mb, CX12311 > MY14), CX15878 *kyIR139 (chrV:14–16.8 Mb, MY14 > CX12311), CX15885 *kyIR144 (chrV:14–16.8 Mb, MY14 > N2), CX16075 *kyIR147 (chrV:15.661–16.6 Mb, MY14 > N2), CX16140 *kyIR153 (chrV:16.043–16.8 Mb, MY14 > N2), CX16300 *kyIR163 (chrV:15.861–16.043 Mb, MY14 > N2; rosn-1*5302, CX16294 *kyIR157 (chrV:15.861–16.066 Mb, MY14 > N2).

**Transgenic lines.** DNA lines are N2-derived unless otherwise noted. CX16884 *kyIR163; V; kyEx5851 (Parx:3r-43:3r-srx-43:2s::GFP, 2.5 μg μl⁻¹, Psry::mcherry, 5 μg μl⁻¹, V; CX17202 *kyIR163; V; kyEx6012 (Parx:3r-43:3s::nonsense::s2::GFP, 2.5 μg μl⁻¹, Psry::mcherry, 5 μg μl⁻¹, V; CX16881 srx-43 (gk2922634) V; kyEx5848 (srx-35:3r-srx-35:2s::GFP, 2.5 μg μl⁻¹, Psry::mcherry, 5 μg μl⁻¹, V; gk2922634 changes R160 to anopal codon, CX17204 kyEx6013 (Parx:3r-43:3r-srx-43:2s::GFP, 50 μg μl⁻¹, Parx:3s::GFP 2.5 μg μl⁻¹, Psry::mcherry, 5 μg μl⁻¹, V; kyEx5849 (Parx:3r-43:3r-srx-43:2s::GFP, 2.5 μg μl⁻¹, Psry::mcherry, 5 μg μl⁻¹, V; CX16425 kyIr602 (Para-6::gCAMP,0.75 μg μl⁻¹, Poed::GFP, 10 μg μl⁻¹, V; kyEx5594 (Para-6::srx-35:3r-srx-43:2s::GFP, 50 μg μl⁻¹, Poed::mcherry, 5 μg μl⁻¹, V; CX16931 kyIr602; kyEx5885 (Para-6::srx-35:3r:srx-35:11, 50 μg μl⁻¹, Poed::mcherry, 5 μg μl⁻¹, V; CX17196 kyIr606 (MosSCI Parx:3r-43:3r-srx-43:2s::GFP, 2.5 μg μl⁻¹, V; gk2922634) V, outcrossed 4×, V; CX17198 kyIr618 (MosSCI Parx:3r-43:3r:srx-43:11, 50 μg μl⁻¹, V; gk2922634) V, outcrossed 4×, V; CX17201 kyIr629 (MosSCI Parx:3r-43:3r:srx-43:11, 50 μg μl⁻¹, V; gk2922634) V, outcrossed 4×, V; CX17203 kyIr637 (MosSCI Parx:3r-43:3r:srx-43:11, 50 μg μl⁻¹, V; gk2922634) V, outcrossed 4×, V; FX1810 kls2 (papk-6::GFP + rol-6 (kls1006), CX16958 kyIr613 V, kls2 Mutants. CX16849 srx-43 (gk2922634) V, outcrossed 5× to N2, gk2922634 is 1600bp. This mutation was provided by the Million Mutation Project.
threshold was determined using 1,000 permutation tests. The effect-size of the roam-1 locus was estimated using the fitqtl function with a single QTL. The peak of the roam-1 locus (chromosome V: 16,451,686–16,579,457) was used as an additive and interactive covariate for additional one-dimensional scans, assuming a normal model. The significance threshold for these two tests was also determined using 1,000 permutation tests.

NIL mapping. Before the detailed QTL mapping by sequencing described above, the roam-1 QTL was localized to 2.5 Mb (chrV: 14.3–16.8 Mb) by examining 14 high-confidence phenotypically extreme RILs (Supplementary Table 1). This result, which was confirmed by the full analysis, guided the initial generation of NILs. The NIL kyIR142 was produced by backcrossing the RIL CX14816 nine times to MY14, maintaining N2 alleles at chrV: 14.3 and chrV: 16.8 Mb at each generation. The NIL kyIR139 was produced by backcrossing the RIL CX14708 nine times to CX12311, maintaining MY14 alleles at chrV: 14.3 and chrV: 16.8 Mb. The NIL kyIR144 was produced by crossing kyIR139 with N2 and isolating recombinants with the N2 allele of glb-5 (chrV: 5.36 Mb), the MY14 alleles at chrV: 14.3 and 16.8 Mb, and the N2 allele of npr-1 on chrX. The NILs kyIR147 and kyIR153 were created by crossing kyIR144 with N2 and identifying progeny with the N2 allele at chrV: 14.3 Mb and the MY14 allele at chrV: 16.8 Mb.

High-density recombination mapping. We crossed kyIR147 with males from CX16290, a N2 strain with an integrated fluorescent marker at chrV: 15.83 Mb. F1 progeny were identified by fluorescence, picked to growth plates, and allowed to lay eggs for 12 h. Following 3 days of growth, ~2,600 non-fluorescent F2 animals were sorted individually into wells of 96-well plates by a worm sorter (COPAS Biosort Systems, Union Biometrica). These F2 were grown in 200 µl of S Basal buffer (5.85 g NaCl, 1 g K2HPO4, 6 g KH2PO4, 5 mg cholesterol per litre) with cholesterol, supplemented with OP50 bacteria. A fraction of the F3 progeny from each isolate were lysed and genotyped at chrV: 16,069 Mb. Those with an N2 allele at chrV: 16,069 Mb were genotyped at chrV: 15,861 Mb. Twelve recombinants with an N2 allele at chrV: 16,069 Mb and a MY14 allele at chrV: 15,861 Mb were isolated and characterized behaviourally, among which were kyIR163 and kyIR157 (Fig. 2f). The N2 NIL with kyIR163 (182 kb of MY14 sequence) is referred to as roam-1srx-43.

Imaging. Calcium imaging experiments were performed and analysed as described previously30. In brief, young adult animals were placed into custom-made 3 mm2 microfluidic polydimethylsiloxane devices that permit rapid changes in stimulus solution. Each device contains two arenas, allowing for simultaneous imaging of two genotypes with approximately ten animals each. Animals were transferred to the arenas in S Basal buffer and paralyzed for 80–100 min in 1 mM (−) tetramisole hydrochloride. Experiments consisted of four pulses of 10 s of stimulation separated by 30 s of buffer, with an additional 60 s between stimulus phases. TIFF stacks were acquired at 10 frames s−1 at 5× magnification (Hamamatsu Orca Flash 4 sCMOS), with 10 ms pulsed illumination every 100 ms (Sola, Lumencor; 470/40 nm excitation).

Fluorescence levels were analysed using a custom ImageJ script that integrates and background-subtracts fluorescence levels of the ASH neuron cell body (4 × 4 pixel region of interest). Using MATLAB, the calcium responses were normalized to the mean fluorescence of the 10 s preceding the first pulse of the stimulus. Each experiment was performed a total of four times over two separate days. Animals were pooled together by strain to calculate population mean and standard error (N2 srx-43 allele, 23 animals; MY14 srx-43 allele, 30 animals; array normal control, 19 animals). Experiments were conducted on two days.

For GFP expression studies, live adult animals were mounted on 2% agarose pads containing 5 mM sodium azide. Images were collected with a 100 × objective on a Zeiss Axio Imager.Z1 ApoTome microscope with a Zeiss AxioCam MRM CCD camera. For day-7 reporter studies, expression was quantified 16–24 h after L4 animals were placed on observation assay plates. Images were processed in Metamorph and ImageJ to generate a maximum-intensity Z-projection. Reporter values were assessed as the mean grey value for a 16-pixel-radius circle centred over the cell body minus the mean background intensity. Both ASI neurons were analysed in each animal; experiments were performed over three days.

Digital PCR. Digital PCR was conducted on a QuantStudio 3D digital PCR platform (Thermo Fisher), and analysed on the QuantStudio 3D AnalysisSoftwareCloud.

The srx-43 mRNA expression studies were conducted on synchronized L4 worms 48 h after laying. RNA was collected on RNaseasy Mini columns (Qiagen) and treated with DNase (Qiagen). SuperScript III First-Strand Synthesis System (Thermo Fisher) was used to create cDNA libraries. Custom TaqMan Expression Assays (Thermo Fisher) were used for srx-43 quantification, and the tubulin gene, dha-1, was used for normalization of digital PCR.

For quantitative analysis of the competition experiments, DNA was extracted with a standard phenol–chloroform protocol. Custom TaqMan SNP Genotyping Assays (Thermo Fisher) were used to determine the relative ratio of N2 versus roam-1srx-43 DNA by digital PCR. The assay was validated with known ratios of N2 to roam-1srx-43 DNA (Extended Data Fig. 9).

Population genetics. To create the gene and organism phylogenies, we used SNV data downloaded from the Million Mutation Project (http://genome.sfu.ca/mmp/) or the CeNDR resource (http://www.elegansvariation.org). For the CeNDR dataset, MY14 was assumed to be clonal or near-clonal with MY23, as was suggested by RAD sequencing. Software was written in Python using the Biopython module to create a neighbour joining tree for the roam-1 locus, SNVs on chrV between 16,010,000 and 16,030,000 were used. For the glc-1 locus, SNVs on chrV between 16,181,000 and 16,222,000 were used. All SNVs were used to construct the whole-genome strain tree. Number of genetic variants and Tajima’s D were calculated on 5-kb bins using vcftools37. dN/dS was calculated by counting using custom Python scripts analysing variants between MY23 and the N2 reference. Phylogenies of srx-43 and closely related genes were performed using protein sequences obtained previously38.

Fitness assays. Competition experiments consisted of three boom–bust cycles. During the boom phase, population growth led to the rapid depletion of food, initiating the bust phase, which lasted for two days. Simple lawn competition experiments were conducted on 100-mm NGM agar plates with a single lawn formed from 800 µl of saturated OP50 culture. Patchy lawn competition experiments were conducted on 150-mm NGM agar plates with a 200 µl ring-shaped OP50 lawn in the centre of the plate surrounded by 15 small 40-µl lawns (Fig. 3a); at the assay start and at transfers animals were placed in the centre of the plate.

Populations were initiated from 20 N2-type and 20 roaming-1srx-43-type age-synchronized young adult animals. The initial population depleted food within 4 days, and on day 6 animals were washed into M9 media. 20% of the suspension was transferred to a new plate and the remainder was lysed for quantitative DNA analysis. For the second and third boom–bust cycle, food resources were depleted in 2 days and the plates were kept starved for an additional 2 days. Following the second bust phase, 20% of the animals were transferred to a new plate; following the third bust phase, the entire population was harvested for DNA extraction.

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Extended Data Figure 1 | Roaming and dwelling states in the presence of ascarosides. a, Roaming and dwelling behaviours scored from video analysis. \( n = 102–214 \) tracks per data point. b, c, Cumulative distribution of roaming (b) and dwelling (c) state durations for animals in a.

***\( P < 0.001 \) by log-rank test; ns, not significant. d, e, Scatter plot of average speed and angular speed (a measure of turning rate) in 10 s intervals taken from 1.5-h-long video recordings of wild-type animals in control (d) and icas#9 (e) conditions. Roaming animals move quickly and turn infrequently compared with dwelling animals. Note the bimodal distribution defining distinct behavioural states. Control, 161 tracks; icas#9, 102 tracks. f, g, Speed following a reversal (f) and the reversal rate (g) for roaming or dwelling animals. Roaming speed is slightly slower in ascarosides (e, f). Data presented as mean ± s.e.m. *\( P < 0.05 \), ***\( P < 0.001 \) by ANOVA with Dunnett correction; ns, not significant.
Extended Data Figure 2 | Roaming and dwelling behaviour of MY14.

a, Fraction of time that MY14 animals spend roaming or dwelling in control, ascr#8, and icas#9 conditions; n = 66–109 tracks per data point. Assays were conducted in 8% O2. b, c, Cumulative distribution of roaming (b) and dwelling (c) state durations for MY14 animals scored in a.

Roaming states are significantly shorter in the presence of ascr#8 (t½ = ~150 s, versus ~220 s in controls), but are not significantly affected by icas#9 (t½ = ~190 s). Roaming states may also be longer at baseline in MY14 than in N2 (see Extended Data Fig. 1). ***P < 0.001 by log-rank test.
Extended Data Figure 3 | Ascarosides produced by wild-type strains. a, b, LC–MS/MS analysis of ascarosides secreted by N2, CX12311 and MY14 strains grown on OP50 (a) or HB101 (b) bacteria. icas#9 is produced at similar levels by icas#9-sensitive and icas#9-resistant strains. \( n = 2 \) (a) or 3 (b) culture extracts per genotype.
Extended Data Figure 4 | Covariate analysis of 94 RILs. a, b. Covariate analysis controlling for the roar-I genotype, testing for additive (a) or interactive (b) QTLs at other loci. The horizontal line denotes the $P < 0.05$ genome-wide significance threshold. LOD, log likelihood ratio.
Extended Data Figure 5 | Signal transduction by SRX-43. a, Expression of Psrx-43::srx-43::SL2::GFP bicistronic reporter transgenes bearing N2 (top) or MY14 (bottom) srx-43 sequences. Arrows indicate cell bodies of ASI sensory neurons. Scale bars, 50 μm. b, ASH sensory neurons are insensitive to multiple ascarosides. ASH calcium imaging with GCaMP3 in control animals that do not express the srx-43 transgene, isolated as non-transgenic siblings of transgenic animals tested in Fig. 3f (n = 19). Ascarosides tested at 10 nM. c, SRX-43 from MY14 confers icas#9 sensitivity on ASH neurons. Compare SRX-43 from N2 in Fig. 3f. d, icas#9 decreases daf-7::GFP expression in ASI neurons of N2 but not roam-1 Mel14 adults. Bars indicate mean fluorescence intensity ± s.e.m. *P < 0.05 by ANOVA with Tukey’s multiple comparisons test. e, Responses to icas#9 of daf-7(lf) mutants are attenuated in N2 but not in roam-1 Mel14 genetic backgrounds. Modified exploration assays were conducted on strains including daf-3(lf) alleles (see methods). *P < 0.05 by t-test. Data presented as mean ± s.e.m. f, Time course for icas#9 response in exploration assay. Pheromone response expressed as mean ± s.e.m. for 2, 4, 6, 10, and 14 h following initiation of exploration assay. ***P < 0.001 by t-test with Bonferroni correction comparing squares entered in control plates versus 10 nm icas#9 plates; n = 12 for all time points.
Extended Data Figure 6 | Alternative roam-1 alleles have high sequence variability. a, The roam-1 QTL region (top). roam-1 SNPs are SNPs, when compared to the N2 reference genome, that are shared by JU360, MY2, MY14, ED3021, JU1171, MY16 and MY6 and not by any other strains, according to the Million Mutation Project. This defines the roam-1_ju360 haplotype. Other SNPs denote all other SNPs with respect to the N2 reference genome found in any of the 40 wild isolates in the Million Mutation Project. b, Polymorphisms of the srx-43 promoter and coding region revealed by Sanger sequencing. Despite the high rate of polymorphism, there are only four non-synonymous mutations in the MY14 coding sequence detected by Sanger sequencing; three of these four were detected by the Million Mutation Project (Extended Data Table 1). We confirmed that the MY14 and N2 sequences are alleles of the same gene by examining sequence reads of the MY14-like strain MY23 in the CeNDR data set (http://www.elegansvariation.org) and aligning each read to N2 and MY14 sequence for the srx-43 region as determined by Sanger sequencing. We observed that 7,272 of the MY23 (MY14) reads better matched the MY14 Sanger sequence and 4 of the reads better matched the N2 reference sequence, as would be expected if MY14 and N2 each bear one alternative allele of the gene. c, Phylogeny constructed for srx-43 and related genes in C. elegans, C. briggsae and C. remanei demonstrates that the srx-43 alleles in N2 and MY14 are closely related alleles of a single gene. Genes are colour-coded by species (green, C. elegans; blue, C. briggsae; orange, C. remanei). Protein sequences and gene names are as previously described[8].
Extended Data Figure 7 | Substantial recombination between roam-1 and surrounding regions. Top, phylogenies constructed with 152 diverse wild-type isolates revealing differences for the region surrounding srx-43 and the regions immediately to the left and right of the 30-kb haplotype. Bottom, graph showing the number of variants and Tajima’s D score calculated for 5-kb bins across a 250-kb region. The bin containing srx-43 has 250 polymorphisms and a Tajima’s D of 1.01, which is high both at the genomic level (<3.4% of bins had a higher value) and for the chromosomal location of srx-43 (<3.6% of bins had a higher value).
Extended Data Figure 8 | Recombination between srx-43 and glc-1 in natural isolates. a, The glc-1 gene has previously been shown to be subject to balancing selection and is chromosomally near srx-43. The blue line shows the number of SNPs per kb for N2 and MY14 averaged over 5-kb intervals for the region spanning srx-43 and glc-1. The large region of low heterozygosity between srx-43 and glc-1 indicates that balancing selection on glc-1 is unlikely to account for the high heterozygosity near srx-43. b, Dendrogram for the glc-1 region for the strains shown in Fig. 4a. The clades for roam-1MY14 and glc-1 are not identical.
Extended Data Figure 9 | Standard curve for digital PCR experiments.
Best-fit line of digital PCR results for known ratios of N2 to \textit{roam-1}MY14 DNA created by mixing different ratios of genomic DNA extracted from independent N2 or \textit{roam-1}MY14 populations.
Extended Data Table 1 | $d_N/d_S$ for *srx-43* and other genes in the *roam-1* region

| Gene   | Chr. | Start | Stop  | substitutions | Non-synonymous | Synonymous | $d_N/d_S$ |
|--------|------|-------|-------|---------------|----------------|------------|----------|
| str-231| V    | 16006304 | 16007605 | 36            | 9              | 27         | 0.097    |
| str-233| V    | 16008332 | 16009664 | 85            | 24             | 61         | 0.118    |
| C06C6.1| V    | 16010710 | 16012112 | 72            | 22             | 50         | 0.128    |
| T10C6.2| V    | 16012537 | 16014226 | 60            | 24             | 36         | 0.195    |
| str-232| V    | 16014509 | 16015752 | 60            | 20             | 40         | 0.145    |
| srx-43 | V    | 16017971 | 16019862 | 30            | 3              | 27         | 0.033    |
| srx-44 | V    | 16020342 | 16022652 | 25            | 5              | 20         | 0.071    |
| T10C6.6| V    | 16024326 | 16027454 | 27            | 2              | 25         | 0.025    |
| T10C6.7| V    | 16027449 | 16031713 | 12            | 10             | 2          | 1.26     |
| T10C6.16| V    | 16032471 | 16034861 | 28            | 22             | 6          | 0.943    |
| T10C6.10| V   | 16036413 | 16038293 | 39            | 22             | 17         | 0.357    |
| his-4  | V    | 16040029 | 16040548 | 6             | 0              | 6          | 0        |
| his-3  | V    | 16040771 | 16041288 | 13            | 0              | 13         | 0        |
| his-2  | V    | 16041828 | 16042283 | 3             | 0              | 3          | 0        |
| his-1  | V    | 16042486 | 16043013 | 7             | 0              | 7          | 0        |
| T10C6.15| V   | 16044296 | 16045874 | 19            | 9              | 10         | 0.228    |
| ZK265.2| V    | 16046873 | 16047446 | 8             | 3              | 5          | 0.175    |
| str-198| V    | 16047808 | 16049147 | 5             | 3              | 2          | 0.401    |
| best-12| V    | 16049492 | 16051554 | 13            | 6              | 7          | 0.227    |
| F14H3.3| V    | 16051923 | 16053510 | 32            | 22             | 10         | 0.522    |
| F14H3.4| V    | 16053900 | 16055072 | 5             | 3              | 2          | 0.388    |
| F14H3.5| V    | 16055806 | 16057054 | 11            | 10             | 1          | 2.711    |
| F14H3.6| V    | 16057322 | 16058892 | 11            | 9              | 2          | 1.24     |
| F14H3.15| V  | 16059781 | 16060235 | 11            | 9              | 2          | 1.37     |
| fbx-100| V    | 16060526 | 16062053 | 11            | 6              | 5          | 0.303    |
| F14H3.9| V    | 16067949 | 16068763 | 9             | 7              | 2          | 0.83     |
| cyp-3501| V   | 16069238 | 16071301 | 94            | 27             | 67         | 0.113    |
| nhc-176| V    | 16071325 | 16072738 | 15            | 5              | 10         | 0.127    |