Supporting Information

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SI Text

Chemoattraction Assays. To test for the effects of cGMP on NILs, we used 15 nl of ETDA as attractant for lines in California background (same as in Fig. 3), and 1.5 nl of ETDA was used for *NIL WA1* in the Washington background because of the already strong chemoattraction to 15 nl of ETDA in *NIL WA1* without cGMP treatment (Fig. S1). Because only well fed nematodes were used in our assays, and that mock, cGMP, and adaptation treatments all involved soaking worms in M9 buffer for at least 1 h, differences to normal chemotaxis assay (<30 min in buffer) may incur as the result of response to prolonged starvation (17). In particular, the chemoattraction of *NIL CA* lines and wild-type California to ETDA and pentanedione were enhanced by such incidental starvation treatments, respectively.

RIL Contruction. Randomly picked single F_2 larvae from crosses between California and Washington strains were transferred to new plates for at least 10 generations before analysis (19). The high rate of RIL extinction (18%) is higher than other RILs constructed from similar crosses between California and Hawaii, or California and a Japanese strain (ref. 1 and G. Bento unpublished data), suggesting the existence of several heterozygous incompatible loci between California and Washington, which contain ≈4.5% nucleotide polymorphism between their genome sequences (D. Dieterich, unpublished results). We assayed 200 surviving F11 RILs and selected 22 lines most insensitive to 15 nl of ETDA. After confirming insensitivity for three generations ($F_{11–13}$), the 22 selected line were mapped by using standard SSCP mapping markers (single-stranded conformation polymorphism) (2). We scored markers at ≈20–50 cM intervals. The two regions with the most California haplotypes (≈15 or 16 of 22 lines having the homozygous California alleles in regions I and II, respectively) mapped to the bottom of chromosome IV, with region I delineated as S286–S587 (150–166 cM) and region II as >S290 (>176 cM).

NIL Contruction. *egl-4* was genotyped with the intragenic SSCP marker S591, and after the eighth introgression cross, also selected for the desired reciprocol background genotype at the flanking markers with markers S34 and S587 (≈7 cM interval). For *NIL WA1*, which we could not reduce the donor *egl-4* allele to a smaller interval similar to *NIL CA1*, we found no recombinants between the markers S591(*egl-4*) and S587 after examining 197 individuals in the F_{10} and F_{12} generations. Although we were able to isolate individuals that were heterozygous for S591 and homozygous Washington for S587, these heterozygotes segregated only for Washington or heterozygous genotypes, but not the desired California genotype, suggesting possible regions of incompatibility. The expected region of donor parent genome retained in the recurrent parent background after 12 introgression crosses is 15.4 ± 11 cM on chromosome IV (7.6% of 203 cM) and 0.10 cM in all nonlinked chromosomes (0.01% of 880 cM total) as calculated by the formula: $2((1 - e^{-tLM/2})/tL_M)$, where t is the number of backcrosses and L_M is the length of the marker chromosome (3) (summarized in Table S2)

Molecular cloning of *egl-4*. A BLASTX search, using *C. elegans* Wormpep160 freeze with various contigs of the finished 9× coverage *P. pacificus* California genome, identified contig85.28 as a clear 1–1 ortholog for the *C. elegans egl-4* gene (www.pristionchus.org). *Ppa-egl-4* contains a ≈2.4-kb coding region with 24 exons spanning ≈19 kb of genomic sequence. Full-length *Ppa-egl-4* from PS312 and PS1843 strains were obtained by using RH12031/RH11818 (first round); RH12032/RH11819 (second round) primers from random hexamer primed (N_6) cDNA. Overlapping PCR products containing the 3rd–24th exons were used to amplify *egl-4* cDNA from JU138 (Hawaii) and RS106 (Poland) strains (RH11820/RH12550; RH11819/RH12589 for the 5′ 1.6 kb and RH11818/RH12548; RH11821/RH12162 for the 3′ 700-bp fragments). No alternative splice forms from either N6 or polyT primed cDNAs were ever detected in the four *P. pacificus* strains using these primers. 5′ and 3′ RACE reactions were performed by using the SL1 and polyT primers as described in ref. 4. By contrast, at least three splice forms were detected from *Pristionchus* sp. 11 cDNAs, using similarly positioned RH14744/RH14746 (first round) and RH14745/RH14747 (second round) primers. However, the N_6 cDNA contains a longer transcript detected only by RH12587/RH13990 primers. The cDNA of the paralog of *Ppa-egl-4, Ppa-C09G4.2*, was isolated with RH13113/RH13114 (first round) and RH13115/RH13116 (second-round) primers. The accession numbers for the genes mentioned are EU375876–EU375890.

EGL-4 Protein Expression. *C. elegans* anti-EGL-4 antibody corresponding to amino acids 35–138 was a gift from M. Fujiwara and Y. Ohshima (Kyush University, Kyushu, Japan) (5, 6). Approximately 30 adult *P. pacificus* hermaphrodites were washed briefly in M9 and lysed at 80°C in 80 μl of Laemmli lysis buffer for 5 min. Five to 15 μl of the lysates were loaded onto 8% SDS/PAGE gels, electroblotted onto nitrocellulose membranes, and immunostained with the EGL-4 antibody. The membrane was incubated in TPBS (PBS buffer without $MgCl_2$, 0.05% Tween-20, and 2% BSA) for 1 h followed by incubation with a 1:5,000 dilution of EGL-4 antibody for >12 h at 4°C with gentle rocking. The primary anti-ALPHA-TUBULIN antibody (human) (Dianova) was used as a loading control to detect 57kD antigen. The membrane was then incubated with 1:1,000 dilution of anti-rabbit IgG alkaline phosphotase conjugate (Dianova) and washed three times in 1 h. Color visualization was done with BCIP/NBT solution (Sigma). EGL-4 immunostaining of whole California strain J4 to adult stage nematodes was done by using the Finney-Ruvkun protocol as modified for *P. pacificus* (7, 8) available under "Protocol" on www.pristionchus.org/wikionchus.

***egl-4* Deletion Mutant.** To obtain a loss-of-function deletion mutant, we mutagenized with trimethylpsoralen-UV and screened ≈1.06×10^6 genomes in the PS312 background (9), using the primers RH11962/RH11964 (first round) and RH11963/RH11965 (second round), targeting 1817 bp of the genomic region containing the first two exons (94°C for 30 s, 58°C for 20 s, 72°C for 3 min, 30 cycles, with 1:3 dilution of template for second round PCR). We isolated a mutant with a 779-bp deletion of the entire second exon and flanking introns that resulted in a putative early stop. This line *tu374* was outcrossed to PS312 four times, using PCR genotyping before commencing analyses. See Table S5 for primer sequences.

Quantitative Reverse Transcriptase-PCR (qRT-PCR). Developmentally staged RNA was obtained from various strains of *P. pacificus* J3 and J4/young adults using synchronizing eggs by bleaching or letting gravid adults lay eggs for 24 h at 20°C. J4/young adult samples do not contain laid eggs and J1 embryos. RNA was isolated with TRIZOL, treated with RQ1 DNase (Promega), and reverse transcribed with random hexamer (N_6) or polyT (Q_t)

primers and Stratagene SuperScript II. Approximately 1–2 μg of RNA from ≈3,000 worms per sample were used for synthesizing cDNA in 20-μl reactions, resulting in ≈1:20 dilutions used for each qRT-PCR. qRT-PCR samples were derived from at least two cDNA synthesis reactions.

Real-time quantitative PCR was performed by using the Roche LC480 Light Cycler, SYBR Green PCR mix, and the manufacturers software (Roche). *Ppa-egl-4* primers RH12548 and RH12549 were used to amplify a 86-bp *egl-4* coding fragment from cDNA samples (165 bp in gDNA) with a annealing temperature of 58°C for 45 cycles. AG11112 and AG11113 were used to amplify beta-tubulin as a control gene. *Egl-4* is less abundant in N_6 primed than Q_t primed cDNA but both type of cDNAs gave similar results. Relative expression level of *egl-4* is defined as: ([level of *Ppa-egl-4*/*Ppa-beta-tubulin*]x100). Inter-strain comparisons were calculated from the mean ratios of relative expression of *egl-4* between a non-California and a California strain sample within the same PCR runs (fold over California expression). 8-bromo-cGMP treated worms were incubated with cGMP for 1 h, washed in 8× volume of M9 buffer, and then RNA extracted after another hour to allow for transcription. Mock-treated worms undergo the same procedure in buffer without cGMP.

Egg Count and Body Length Measurements. PS312 (California) and *Ppa-egl-4* deletion mutant (*tu374*) young adult hermaphrodites were picked onto NGM plates seeded with one-day old OP50. These individuals were picked onto fresh plates every 18–24 h two times at 20°C and their eggs counted. Larvae that hatch and survive to J4 stage after three days were scored as the number of progeny. Body size measurements were performed with MetaMorph software (Universal Imaging) on a Zeiss AxioPlan 2 Nomarski microscope. Wild-type and mutant worms were grown concurrently and their body lengths (mouth tip to anus) were measured from age days 4–6 at ≈24-h intervals.

Morpholino Knockdown. 0.5 mM morpholino oligo (Gene Tools) targeting translational initiation in *egl-4 WA* was injected into ETDA(+) *P. pacificus* Washington young adult hermaphrodites. We observed low incidences of the small body phenotype reminiscent of the null *Ppa-egl-4* allele in the F_1 progeny within the first 48 h of injecting (45 ± 7% of *egl-4* morpholino injected parents had small F_1 progeny, with 7 ± 2% F_1 penetrance for small body, n = 104 injected animals). Later progeny of these morpholino injected worms and coinjected morpholino and RNAi (5′ 900 bp of *egl-4 WA*) worms, and the negative control Ppa-LET-23 morpholino injected worms did not show such small body phenotype.

Statistical Analyses. Statistical analyses, using two-sampled Student's t test and One-Way ANOVA, were done in Microsoft Excel. Differently treated nematodes were always derived from the same population pool and analyzed by two-sampled t test, i.e., cGMP-treated versus mock-treated. JMP software 5.1 (SAS Institute) was used to perform Wilcoxon rank sums test for discrete data. After a significant ANOVA with multiple comparisons ($P < 0.05$), we used post hoc tests to identify pairwise differences of means between specific groups (Dunnett's test for comparing to a control group or Tukey–Kramer HSD test). Alpha was set at 0.05.

1. Zauner H, Sommer RJ (2007) Evolution of robustness in the signaling network of Pristionchus vulva development. *Proc Natl Acad Sci USA* 104:10086–10091.
2. Srinivasan J, *et al.* (2002) A bacterial artificial chromosome-based genetic linkage map of the nematode *Pristionchus pacificus*. *Genetics* 162:129–134.
3. Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Sunderland, MA).
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5. Fujiwara M, Sengupta P, McIntire SL (2002) Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron* 36:1091–1102.
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7. Finney M, Ruvkun G (1990) The unc-86 gene product couples cell lineage and cell identity in *C. elegans*. *Cell* 63:895–905.
8. Kolotuev I, Podbilewicz B (2004) *Pristionchus pacificus* vulva formation: Polarized division, cell migration, cell fusion, and evolution of invagination. *Dev Biol* 266:322–333.
9. Zheng M, Messerschmidt D, Jungblut B, Sommer RJ (2005) Conservation and diversification of Wnt signaling function during the evolution of nematode vulva development. *Nat Genet* 37:300–304.

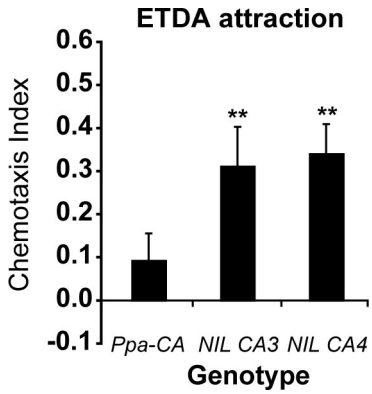

Fig. S1. Chemoattraction of additional *NIL CA* lines (CA3 and CA4) containing the *egl-4* WA locus also showed enhanced ETDA attraction compared with the parental California strain. (**, $P < 0.01$, Dunnet's *posthoc* multiple comparisons test). Error bars denote SEM.

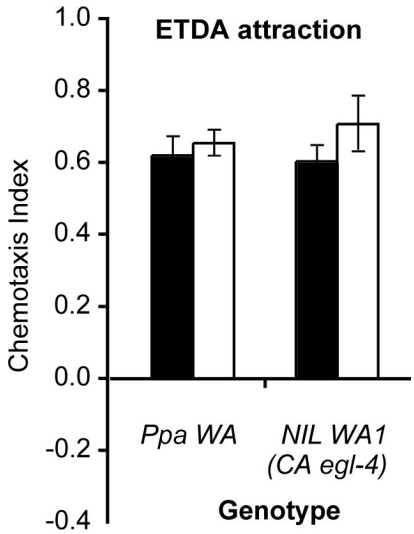

Fig. S2. Chemoattraction of NIL of *Ppa-egl-4* in Washington (WA) background having the *egl-4* CA allele showed no difference to parental Washington strain. >15 replicate assays were performed for each genotype on at least four separate days.

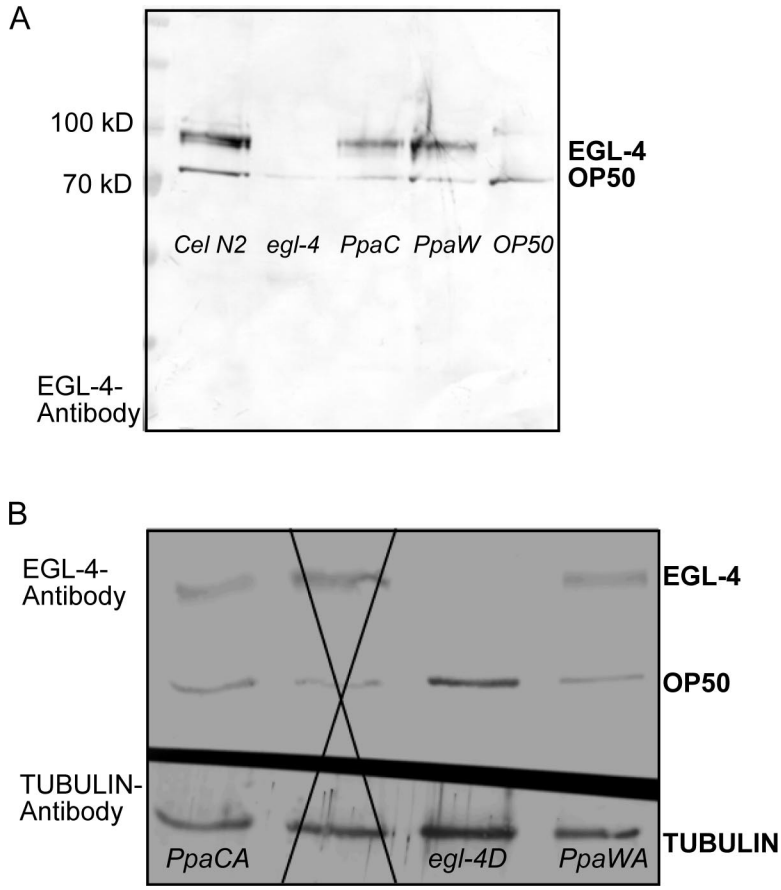

Fig. S3. Western blots of whole adult hermaphrodite protein extracts ($n = 30$ for each genotype), using an anti-*Cel*-EGL-4 antibody. (*A*) The EGL-4 antibody is specific for ≈95-kDa doublet bands found in *C. elegans N2* wild-type (lane 1) but not the loss-of-function *egl-4* mutant *n479* (lane 2; a smaller band <70 kD is visible) (14) or the OP50 *Escherichia coli* food source (lane 5, nonspecific ≈80-kDa and >100-kDa bands). The EGL-4 antibody cross hybridizes with a single ≈95-kDa protein in *P. pacificus* strains California and Washington (lanes 3–4). (*B*) (*Upper*) Using the EGL-4 antibody, no 95-kDa antigen corresponding to the *Ppa*-EGL-4 was detected in the *Ppa-egl-4* deletion mutant (*egl-4D*, lane 3) compared with the *P. pacificus* wild-type strains (lanes 1 and 4). Lane 2 is not relevant. *Bottom*: A protein loading control of the same blot, using a human *alpha-tubulin* antibody (57kD antigen) showed approximately equal loading in all lanes.

ETDA ATTRACTION	STRAIN	SEQUENCE	
-ETDA	CALI	TATTGTTAGATCAGATAAA	(-1840 bp)
-ETDA	CHNA	TATTGTTAGATCAGATAAA	
+ETDA	POLD	TATTGTTAGATCAGATAAA	
+ETDA	HAWA	TATTATTAGATCAGATAAA	
+ETDA	WASH	TATTATTAGATCAGATAAA	
+ETDA	BOLI	TATTATTAGATCAGATAAA	
+ETDA	JAPN	TATTATTAGATCAGATAAA	
+ETDA	MADG	TATTATTAGATCAGATAAA	
-ETDA	CALI	TCCGACTGAATAGCAGACGAAGAAAC	(-1660 bp)
-ETDA	CHNA	TCCGACTGAATAGCAGACGAAGAAAC	
+ETDA	POLD	TCCGACTGAATAGCAGACGAAGAAAC	
+ETDA	HAWA	TCCGACTGAATAGCAGACGATGAAAC	
+ETDA	WASH	TCCGACTGAATAGCAGACGATGAAAC	
+ETDA	BOLI	TCCGACTGAATAGCAGACGATGAAAC	
+ETDA	JAPN	TCCGACTGAATAGCAGACGATGAAAC	
+ETDA	MADG	TCCGACTGAATAGCAGACGATGAAAC	
-ETDA	CALI	TCGTAATTTC-------CGGAAAGGA	(-1617 bp)
-ETDA	CHNA	TCGTAATTTC-------CGGAAAGGA	
+ETDA	POLD	TCGTAATTTC-------CGGAAAGGA	
+ETDA	HAWA	TCGTAATTTCCGGCCTTCGGAAAGGA	
+ETDA	WASH	TCGTAATTTCCGGCCTTCGGAAAGGA	
+ETDA	BOLI	TCGTAATTTCCGGCCTTCGGAAAGGA	
+ETDA	JAPN	TCGTAATTTCCGGCCTTCGGAAAGGA	
+ETDA	MADG	TCGTAATTTCCGGCCTTCGGAAAGGA	
-ETDA	CALI	ATTTAGACGGAGAGAATGA	(-1518 bp)
-ETDA	CHNA	ATTTAGACGGAGAGAATGA	
+ETDA	POLD	ATTTAGACGGAGAGAATGA	
+ETDA	HAWA	ATTTAGACGGAGAGAGTGA	
+ETDA	WASH	ATTTAGACGGAGAGAGTGA	
+ETDA	BOLI	ATTTAGACGGAGAGAGTGA	
+ETDA	JAPN	ATTTAGACGGAGAGAGTGA	
+ETDA	MADG	ATTTAGACGGAGAGAGTGA	
-ETDA	CALI	CCGACCATTATAT---------CTGTACATAC	(-691 bp)
-ETDA	CHNA	CCGACCATTATAT---------CTGTACATAC	
+ETDA	POLD	CCGACCATTATAT---------CTGTACATAC	
+ETDA	HAWA	CCGACCATTATATTTTCCACATCTGTACATAC	
+ETDA	WASH	CCGACCATTATATTTTCCACATCTGTACATAC	
+ETDA	BOLI	CCGACCATTATATTTTCCACATCTGTACATAC	
+ETDA	JAPN	CCGACCATTATATTTTCCACATCTGTACATAC	
+ETDA	MADG	CCGACCATTATATTTTCCACATCTGTACATA	

Fig. S4. Differences in putative regulatory 1.9-kb upstream sequences from *P. pacificus* strains. Only common differences between ETDA-insensitive strains (California and China) and ETDA-attractive strains (Hawaii, Washington, Bolivia RS5275, Japan RS5195, and Madagascar) are shown. Poland strain is the most genetically similar strain to California based on nuclear and mitochondrial sequences of 84 isolated strains.

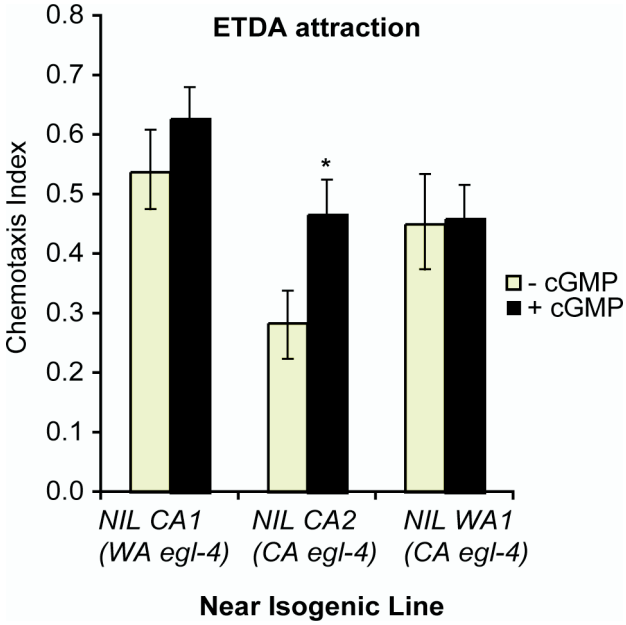

Fig. S5. The effects of 500 μM cGMP on the chemoattraction to ETDA in *NILs*. 15 nl of ETDA for *NILs CA1* (*egl-4 WA*) and and *CA2* (*egl-4 CA*) and 1.5 nl of ETDA for *NIL WA1* (*egl-4 CA*) were used. 15 replicate assays were performed for each genotype on at least three separate days. *, significant difference between mock and cGMP treated populations, $P < 0.05$ by two-sampled *t* test.

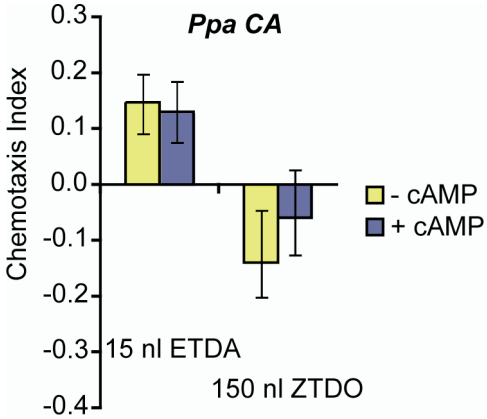

Fig. S6. 500 μM exogenous 8-bromo-cAMP did not enhance pheromone attraction in *P. pacificus* California (in contrast to 8-bromo-cGMP).

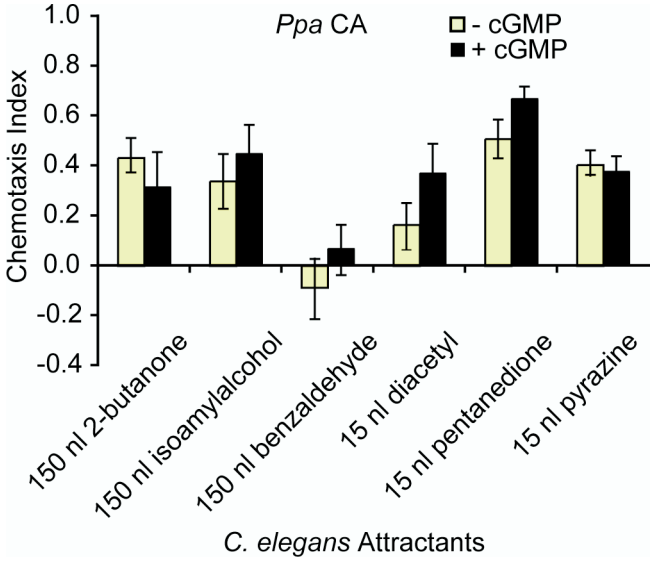

Fig. S7. Exogenous cGMP treatment of *P. pacificus* California did not enhance attraction to 150 nl of known *C. elegans* attractants 2-butanone, isoamyl alcohol, benzaldehyde, or 15 nl of diacetyl or 2,3-pentanedione.

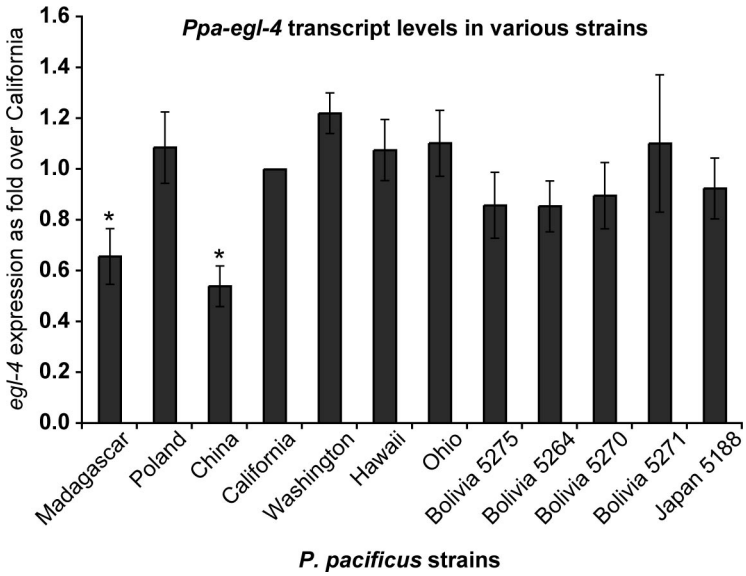

Fig. S8. qRT-PCR measurements in various *P. pacificus* strains. Average *Ppa-egl-4* transcript levels (*egl-4*/*tubulin*) are indicated relative to that of California ($n = 13$ reactions). *, expression of *egl-4* in Madagascar and China strains are significantly different from all other strains by ANOVA followed by pairwise comparisons, using Student's *t* test, $P < 0.05$.

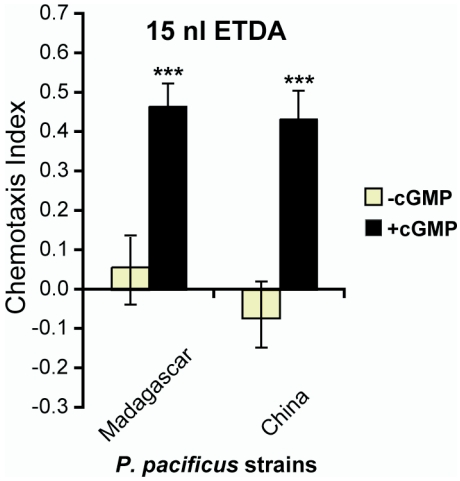

Fig. S9. Exogenous cGMP treatment can also enhance ETDA attraction of *P. pacificus* strains Madagascar and China, which showed low endogenous *egl-4* expression levels and ETDA attraction. ***, significant difference between mock and cGMP treated populations, $P < 0.001$ by two-sampled *t* test.

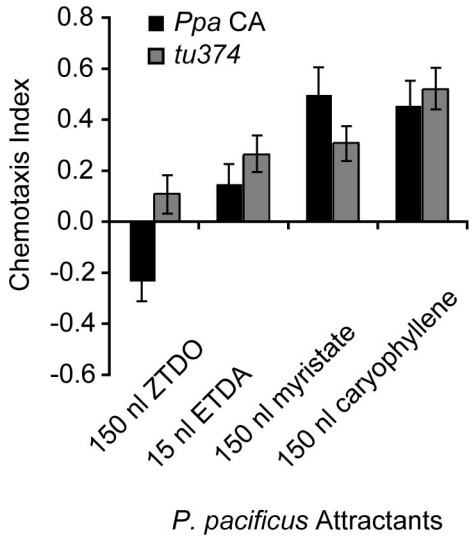

Fig. S10. *tu374* retained wild-type chemotaxis to *P. pacificus* attractants ZTDO, ETDA, myristate, and β-caryophyllene.

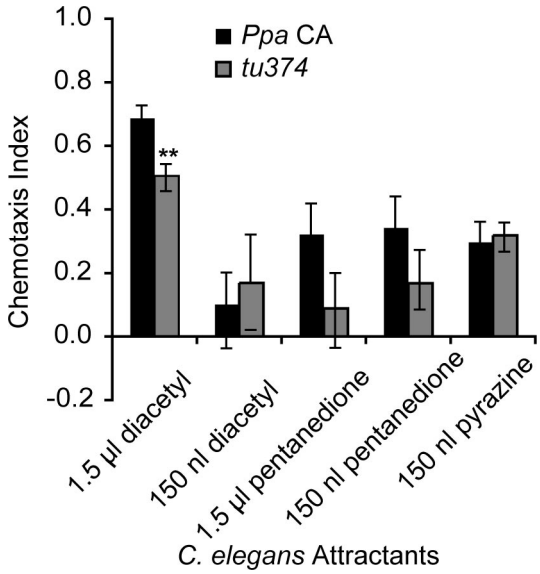

Fig. S11. *tu374* retained wild-type chemotaxis to most shared attractants with *C. elegans*, but attraction to diacetyl was slightly reduced. **, $P < 0.01$ by Student's *t* test.

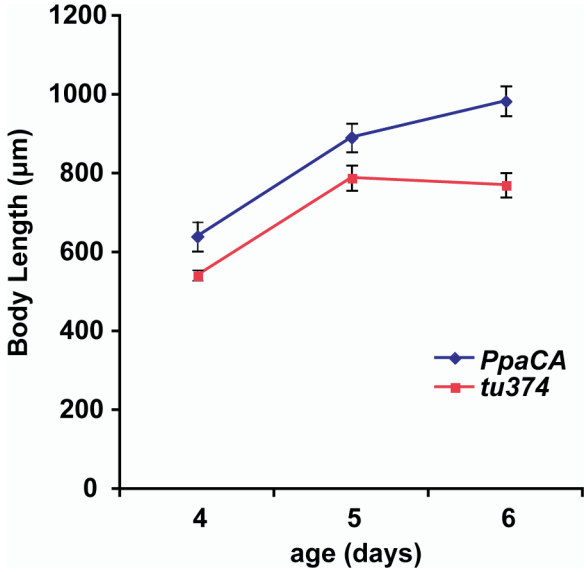

Fig. S12. Body length measurements of J4 and young adults at 24-h intervals showed *tu374* to be significantly shorter than wild-type California on all 3 days (≈78–88% of wild type) at 20°C ($n = 15$ for day 4 and $n = 20$ for days 5–6). Error bars denote 95% confidence intervals and all differences between the two strains are significant by Student's t test ($P < 0.001$).

Table S1. *Pristionchus pacificus* and *Pristionchus* sp. 11 strains

Strain	Geographical locality	Ecological origin
RS5228*	Japan (Mount Hiei)	soil
JU150†	Madagascar (Antananarivo)	soil
RS106†	Poland (Augustow)	soil
JU723†	China (Longsheng, Guangxi)	soil
PS312†	California (Pasadena)	soil
PS1843†	Washington (Port Angeles)	soil
JU138†	Hawaii (Captain Cook)	soil
SB5880†	New York	soil
RS5131†	Massachusetts (Carver)	*Exomala* sp.
RS5134†	Ohio (Wooster)	*Phyllophaga* sp.
RS5275	Bolivia (Santa Cruz)	scarab beetles
RS5270	Bolivia (Santa Cruz)	scarab beetles
RS5271	Bolivia (Santa Cruz)	scarab beetles
RS5264	Bolivia (Santa Cruz)	scarab beetles
JU482†	Japan (Hakone)	soil
RS5180†	Japan (Hakone)	*Exomala orientalis*
RS5187†	Japan (Hakone)	*Exomala orientalis*
RS5195†	Japan (Hakone)	*Exomala orientalis*
RS5210†	Japan (Hakone)	*Exomala orientalis*
RS5188†	Japan (Hakone)	*Exomala orientalis*

*All strains are *Pristionchus pacificus*, except for RS5228, which is a strain of *Pristionchus* sp. 11.

†These strains were described in refs. 1 and 2.previously . With the exception of RS106, all ''RS'' strains were isolated from field trips aimed to search for the natural habitat of *P. pacificus*. Bolivian strains came from multiple species of ″scarab beetles.″

1. Herrmann M, Mayer W, Hong RL, Kienle S, Minasaki R, Sommer RJ (2007) The nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) is associated with the Oriental beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan. *Zoological Science*, 24:883–889.
2. Zauner H, *et al.* (2007) Distinct patterns of genetic variation in *Pristionchus pacificus* and *Caenorhabditis elegans*, two partially selfing nematodes with cosmopolitan distribution. *Mol Ecol* 16:1267–1280.

Table S2. SSCP markers used for genetic mapping of 15 nl ETDA insensitivity in RILs, their genetic locations, and genotypes in near isogenic lines NILs.

Linkage group	Marker	Genetic location, cM	*NIL CA1,2* (*egl-4* WA)	*NIL CA3* (*egl-4* WA)	*NIL CA4* (*egl-4* WA)	*NIL WA1* (*egl-4* CA)
I	S449	23	CA	CA	CA	WA
233 cM	S563	79	CA	CA	CA	WA
	S450	90	—	—	—	—
	S254	132	CA	CA	CA	WA
	S209	223	CA	CA	CA	WA
II	S97	23	—	—	—	—
170 cM	S598	47	CA	CA	CA	WA
	S50	57	—	—	—	—
	S325	73	—	—	—	—
	S558	106	CA	CA	CA	WA
	S289	135	CA	CA	CA	WA
III	S108	11	—	—	—	—
118 cM	S299	45	CA	CA	CA	WA
	S263	78	—	—	—	—
	S238	96	CA	CA	CA	WA
IV	S210	0	—	—	—	—
203 cM	S49	5	CA	CA	CA	WA
	S273	51	CA	CA	CA	WA
	S132	76	CA	CA	CA	WA
	S221 (C09G4.2)	77	CA	CA	CA	WA
	S298	77	CA	CA	CA	WA
	S320	84	CA	CA	CA	WA
	S344	126	CA	CA	CA	WA
	S286	150	—	—	—	—
	S285	156	CA	CA	CA	WA
	S34	159	CA	CA	CA	WA
	S589	162	CA	CA	CA	CA*
	S591 (*egl-4*)	**165**	**WA***	**WA***	**WA***	**CA***
	S11	166	CA	CA	CA	CA*
	S587	166	CA	CA	CA	CA*
	S588	167	—	—	—	—
	S590	169	—	—	—	—
	S290	176	—	—	—	—
	S288	182	CA	CA	CA	WA
	S284	203	CA	CA	CA	WA
V	S247	20	—	—	—	—
167 cM	S223	53	CA	CA	CA	WA
	S327	101	—	—	—	—
	S200	129	—	—	—	—
	S503	144	CA	CA	CA	WA
X	S57	22	CA	CA	CA	WA
198 cM	S438	22	CA	CA	CA	WA
	S422	83	—	—	—	—
	S419	130	CA	CA	CA	WA
	S259	156	—	—	—	—

NIL CA lines share the same genotype. —, not genotyped for NILs. Underlined markers were genotyped in NILs. C09G4.2 is a putative paralog of *egl-4*. Boldface text indicates targeted donor *eg1-4* locus.
*Regions containing the donor genotypes.

Table S3. Predicted *P. pacificus* genes based on *E* values of $<e^{-10}$ (Wormpep160) in the region contig 85.25 to contig 85.32

Predicted gene (predicted function)	TBLASTX E value
cyp-14 (cytochrome P450)	e^{-12}
glt-3 (amino acid glutamate transporter)	e^{-23}
ATP pathway	e^{-29}
CE27192 (?)	e^{-19}
CE36059 (UTP-galactose transporter)	e^{-12}
CE27512 (nuclear transport)	e^{-16}
CE36898 (?)	e^{-11}
CE33241 (ATPase)	e^{-22}
CE36718 (?)	e^{-17}

?, unknown function in *C. elegans*.

Table S4. Number of eggs and brood size in California wild type and *tu374*

Strain	Eggs, no.*	Progeny, no.*	Hatching, %
Wild type	155 ± 5	149 ± 4	96
tu374	139 ± 5	107 ± 4	77

Average and SEM values for total eggs and live progeny produced over three days at 20° C ($n = 34$ each).

*Significant differences between the two genotypes for the number of eggs laid and progeny are $P = 0.017$ and $P < 0.0001$, respectively, using Wilcoxon one-way test. The *P. pacificus* CA wild type holds ≤2 eggs in uterus and may mask the egg-laying phenotype that is more apparent in the *C. elegans* N2 wild type, which holds more eggs.

Table S5. List of primers used (5′ → 3′)

Primer	Sequence	Purpose
RH11962	aacaatattttcgggctatg	*Ppa-egl-4* Deletion screen
RH11964	atgagaagtgatgatcgttg	*Ppa-egl-4* Deletion screen
RH11963	aatcatgtctgtaagcagtc	*Ppa-egl-4* Deletion screen
RH11965	actatctctcaactgacaac	*Ppa-egl-4* Deletion screen
RH12548	GCAGGAACATATATTCGCAG	*Ppa-egl-4* cDNA; qRT-PCR
RH11818	GAATCCCTCATCCCATCCAGAG	*Ppa-egl-4* cDNA
RH12589	GTTCCGTGACACCAAGTATG	*Ppa-egl-4* cDNA
RH11819	ATCTGGAGGCACATCGGGATC	*Ppa-egl-4* cDNA
RH11820	cagGTGCAAATCGGCACGAAG	*Ppa-egl-4* cDNA
RH12550	AGGATAATTTCCGGCGAGAC	*Ppa-egl-4* cDNA
RH11821	TGTACGAAACGCACGAACTGCAG	*Ppa-egl-4* cDNA
RH12162	GAAGACAATTCTCCGGCTTG	*Ppa-egl-4* cDNA
RH12549	TGTCACGGAACGTTTTGTAC	qRT-PCR *Ppa-egl-4*
AG11112	CTCGGAGGAGGAACTGGATC	qRT-PCR *Ppa-beta-tubulin*
AG11113	GACCGTGTCAGAGACCTTAG	qRT-PCR *Ppa-beta-tubulin*
RH14744	ATGAGCAACAACGGCTCTGC	*P. expectatus egl-4* cDNA
RH14745	CTCTGCGAGGTCCGCGAGCG	*P. expectatus egl-4* cDNA
RH14746	TAGTTAAGTAGTCGAACCAG	*P. expectatus egl-4* cDNA
RH14747	AAGAGGAGAACGACGATTAG	*P. expectatus egl-4* cDNA
RH12587	AAGCGCGATCAGCAAATTCG	*P. expectatus egl-4* cDNA
RH13990	TGCCACCTTAGTGGGACTGG	*P. expectatus egl-4* cDNA
RH15339	GCGAAATAGTGAGTGTGCTC	2 kb 5′ *Ppa-egl-4* sequence
RH15305	GTAGGCGATCCACTCTTAC	2 kb 5′ *Ppa-egl-4* sequence
RH15340	CTTGCGGACCTCGCTGAGGC	2 kb 5′ *Ppa-egl-4* sequence
RH15304	GCAACTCTCTCGGTGCTC	2 kb 5′ *Ppa-egl-4* sequence
RH15719	GCTGATCCCGATGTGCCTCC	0.7 kb 3′ *Ppa-egl-4* sequence
RH15720	GTAAAGGATAACGAACCAAAC	0.7 kb 3′ *Ppa-egl-4* sequence