Natural variation in *Pristionchus pacificus* insect pheromone attraction involves the protein kinase EGL-4

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The geographical mosaic theory of coevolution predicts that different local species interactions will shape population traits, but little is known about the molecular factors involved in mediating the specificity of these interactions. *Pristionchus* nematodes associate with different scarab beetles around the world, with *Pristionchus pacificus* isolated primarily from the oriental beetle in Japan. In particular, the constituent populations of *P. pacificus* represent a rare opportunity to study multiple specialized interactions and the mechanisms that influence population traits at the genetic level. We identified a component of the cGMP signaling pathway to be involved in the natural variation for sensing the insect pheromone ETDA, using targeted introgression lines, exogenous cGMP treatment, and a null *egl-4* allele. Our data strongly implicate *egl-4* as one of several loci involved in behavioral variation in *P. pacificus* populations. That EGL-4 homologs have been independently implicated for behavioral variations in other invertebrate models suggests that EGL-4 may act as a modulator for interspecies behavioral repertoires across large phylogenetic distances.

chemosensation | nematode | near-isogenic lines

The interactions among organisms are part of the adaptive forces shaping the evolution of species, but the molecular factors mediating the specificity of these interactions are largely elusive. The geographical mosaic theory of coevolution posits that most organisms specialize their species interactions locally. This is based on the assumption that species are groups of genetically differentiated populations and that most interacting species have nonidentical geographical ranges (1). One important aspect of these interactions involves communication between species, but it is not clear whether perception or modulation of signals can diverge at the population level or which signal transduction molecules are involved. Therefore, further insights into ecological genetics would require access to natural variation in species interactions and mature genetic tools to identify the molecular basis for population differences.

Nematodes occupy innumerable ecological niches in plants and animals. One particular type of association with invertebrates is known as necromeny, in which nematodes infest live insects and wait for their hosts to die before resuming their life cycle on the cadaver (2). It is believed that the infested insects are not harmed and provide means of dispersal and food source for the hitchhiking nematodes. *Pristionchus* nematodes associate with different scarab beetles worldwide, with *Pristionchus pacificus* isolated primarily from the oriental beetle (*Anomala/Exomala orientalis*) in Japan and in the northeastern United States (3–5). In particular, *P. pacificus* has been used as a tool to compare with the model organism *Caenorhabditis elegans* in developmental biology, genetics, and genomics (6). In addition to the oriental beetle, *P. pacificus* populations have also been isolated from soil and other scarab beetles. Thus, *P. pacificus* populations have geographically mosaic associations, representing a rare opportunity to study multiple specialized interactions and the mechanisms that influence population traits. Recent studies in the chemosensory behavior of *P. pacificus* suggest that specific beetle pheromones contribute to the unique chemoattraction profiles of closely related *Pristionchus* species (5, 7). Chemoattraction to insect and plant derived compounds also differs between *Pristionchus* species and even some *P. pacificus* strains (7). One example is the attraction to (*E*)-11-tetradecenyl acetate (ETDA), a well studied moth sex pheromone. Here, we identified a component of the cGMP signaling pathway to be involved in the natural variation for sensing ETDA, using targeted introgression lines, exogenous cGMP treatment, and a null *egl-4* allele. Interestingly, a homolog of EGL-4 in *Drosophila* was shown to play a role in natural variation of foraging behavior, indicating a conserved role of cGMP-dependent protein kinases as a modulator of interspecies behaviors.

Results

Natural Variation in Insect Pheromone Attraction. To determine the range of natural variation in *P. pacificus* chemoattraction to ETDA, we surveyed 19 *P. pacificus* strains that represent their known global distribution [Fig. 1*A* and supporting information (SI) Table S1]. In addition, we also measured attraction to (*Z*)-7-tetradecen-2-one (ZTDO), the sex pheromone of the oriental beetles recently identified as a host to *P. pacificus* populations in Japan (5). We found that attraction responses sorted by the geographical provenance of the strains showed strong attraction to ETDA in strains from the northeastern U.S., Bolivia, and Japan, most of which were derived from *Exomala* or other scarab beetles. Not surprisingly, all strains derived from *Exomala* also exhibited robust ZTDO attraction. At the same time, strains from the geographically diverse soil sources showed extremes: High attraction (Washington strain) or complete insensitivity to both pheromones (California and China strains). When compared with the California strain, the three strains from Poland, Madagascar, and Bolivia (5270) were attracted to only ETDA and not ZTDO, whereas the remaining strains display attraction to both insect pheromones. As a comparison, the strain from the closest sister species, *Pristionchus* sp. 11, showed weak attraction to both pheromones. Taken together, the natural variation in insect pheromone attraction observed in the different *P. pacificus* strains is consistent with the geographic mosaic theory of species interaction.

***egl-4* Is a Major Locus for Pheromone Sensing.** We next sought to identify the major factors mediating the natural variation in

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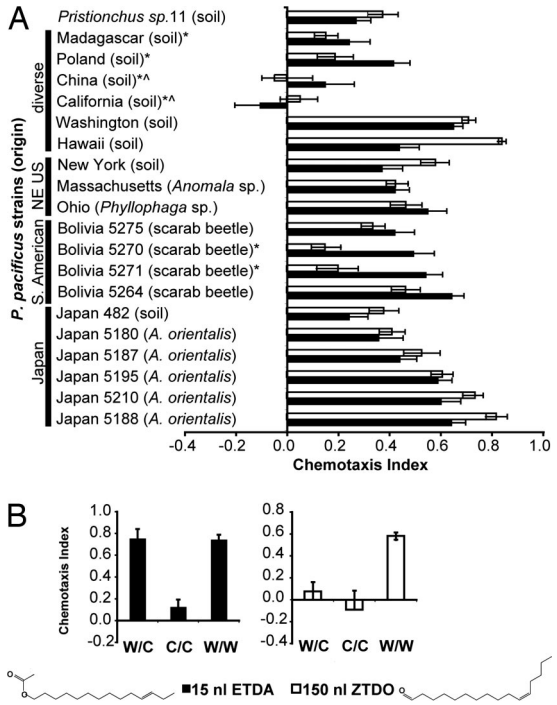

Fig. 1. Chemoattraction profiles of *P. pacificus* strains. (*A*) Natural variations in *P. pacificus* attraction toward insect pheromones *E*-11-tetradecenyl acetate (EDTA) and *Z*-7-tetradece-2-one (ZTDO). ^ and * denote no attraction toward 15 nl of EDTA and 150 nl of ZTDO and no significant difference to the control California strain (PS312), respectively (Dunnet's *posthoc* multiple comparisons test, $P < 0.05$). (*B*) Insensitivity to ETDA in the California strain is recessive with respect to Washington, whereas the insensitivity to ZTDO is a dominant trait. $n \geq 10$ replicates were performed for each condition. Error bars denote SEM. The ecological origins of each strain is indicated in parentheses. See Table S1 for details.

insect pheromone attraction for ETDA by determining the genetic loci associated with the difference between the ETDA-insensitive California and the ETDA-attractive Washington strains. We made recombinant inbred lines (RILs) starting with 250 F_2 lines, maintained them by single worm descent for 10 generations, and looked for markers associated with insensitivity to 15 nl of ETDA. Of the 206 lines that survived to F_{12} generation, we picked 22 most insensitive RILs and, after three rounds of assays, genotyped them by using SSCP markers across all chromosomes (Table S2). The region on the bottom of chromosome IV from S286 to S284 contained the highest proportion of California alleles, and genotyping at shorter intervals in this region revealed two likely regions, S591 and S284 to be associated with ETDA insensitivity. All lines contained at least a California allele at these loci. S591, a genetic marker for *Ppa-egl-4* (Fig. 2*A*; see below), was generated from routine genetic placement of putative *C. elegans* homologs of genes involved in chemosensation.

Because *Cel-egl-4* is known to regulate olfaction in *C. elegans* and natural variation in foraging behaviors in *Drosophila melanogaster* larvae and honey bees (8–10), we further investigated the extent of contribution of *Ppa-egl-4* in mediating *P. pacificus* ETDA attraction. We surmised that the Washington *egl-4* allele in the California background would enhance ETDA attraction; so we constructed near-isogenic lines (NILs) containing either the California or Washington *egl-4* locus in reciprocal genetic backgrounds by introgressing for 12 generations to obtain the lines *NIL CA1* with the *egl-4* WA allele and *NIL WA1* with the *egl-4* CA allele (see *Materials and Methods*). Using SNP markers on Supercontig 85, we ascertained that the maximum interval in the resulting California background containing the Washington donor *egl-4* locus was 110 kb (markers SRH1 to L154) (Fig. 2*A*). The ≈80-kb regions flanking the 30-kb *egl-4* locus contains nine other predicted genes with significant similarity to *C. elegans* genes (BLASTX; $e < e^{-10}$), but none was previously implicated to be involved in chemosensation (see *Materials and Methods* and Table S3). In *NIL CA1* containing the Washington *egl-4* allele, attraction to ETDA was significantly enhanced compared with the California wild-type parent (Fig. 3). Specifically, *NIL CA1* has a chemotaxis index (CI) of ≈0.4, whereas CA wild type has no response. This result suggests that the *Ppa-egl-4* locus strongly contributes to ETDA sensing. Two other independent *NILs (CA3*, *CA4*) with the *egl-4 WA* allele in California background also showed enhanced ETDA attraction compared with the California parent (Fig. S1). However, we cannot exclude the possibility that other genes within the introgressed 110-kb WA region can also contribute to variations in ETDA attraction.

To further confirm that the enhanced ETDA attraction was due to the Washington *egl-4* allele and not due to a particular interstrain NIL background, we measured the attraction of a sibling line (*NIL CA2*) that segregated for the California *egl-4* allele and found it to be less attracted to ETDA than *NIL CA1* (Fig. 3). Interestingly, *NIL CA2* still showed a small increase in ETDA attraction independent of the *egl-4* locus when compared with the California parent, suggesting that other remaining loci from the Washington donor genome may also contribute to ETDA attraction. For the *NIL WA1*, however, we were not able to completely introgress the Washington loci near the *egl-4* region spanning ≈8 cM of possible regions of incompatibility (S591–S587) (Table S2; see *Materials and Methods*). Because of this resulting donor drag (4–20 cM) from the California genome, the *egl-4* CA allele did not alter the chemotaxis response of *NIL WA1* compared with wild-type Washington (Fig. S2).

Unlike *Cel-egl-4*, which has seven known splice forms, we found only a single *Ppa-egl-4* splice form in four *P. pacificus* strains. *Ppa-egl-4* (CA) encodes a 777-aa protein with no predicted amino acid difference to *Ppa-egl-4* (WA) (Fig. 2*B*). Furthermore, both Poland and Hawaii strains with moderate ETDA attraction have predicted amino acid sequences identical to *Ppa*-EGL-4 (CA) (data not shown). Thus, there appears to be no predicted coding sequence difference in *egl-4* alleles that would account for the difference in chemoattraction among the four strains.

To ascertain the protein size of *Ppa*-EGL-4, we performed Western blot analysis, using the *Cel*-EGL-4 antibody on adult hermaphrodites. We found, consistent with the result of the RT-PCR experiments, only a single ≈95-kDa band in *P. pacificus* compared with at least two bands observed in *C. elegans* and *Pristionchus* sp. 11 samples (Figs. 2*C* and S3 *A* and *B*; *Pristionchus* sp. 11 data not shown). To determine the cellular expression pattern of *Ppa*-EGL-4, we performed immunostaining on whole *P. pacificus* adult hermaphrodites (California) and found that the EGL-4 protein is expressed in several unidentified head neurons similar to the pattern observed in *C. elegans*, consistent with its likely conserved chemosensory function (Fig. 2*D*) (11).

***Ppa-egl-4* Is Differently Expressed and Regulatory Sequences Differ Among Strains.** To determine whether regulatory sequence changes are associated with ETDA insensitivity, we compared 1.9-kb 5′ and 700-bp 3′ regions (including ≈220 bp of 3′ UTRs) of eight strains having either attractive or insensitive response to ETDA. In the 5′ region, we identified 7-bp and 9-bp deletions located 1.6 kb and 691 bp upstream of the start codon, respectively, and three separate single base pair polymorphisms shared between the two ETDA-insensitive strains California and China that are not present in the five attractive strains (Fig. S4). However, the same changes were also found in the ETDA attractive strain Poland. There were no California/China-specific polymorphisms in the 3′ downstream sequences. Thus, we could not identify shared polymorphisms in these potential regulatory

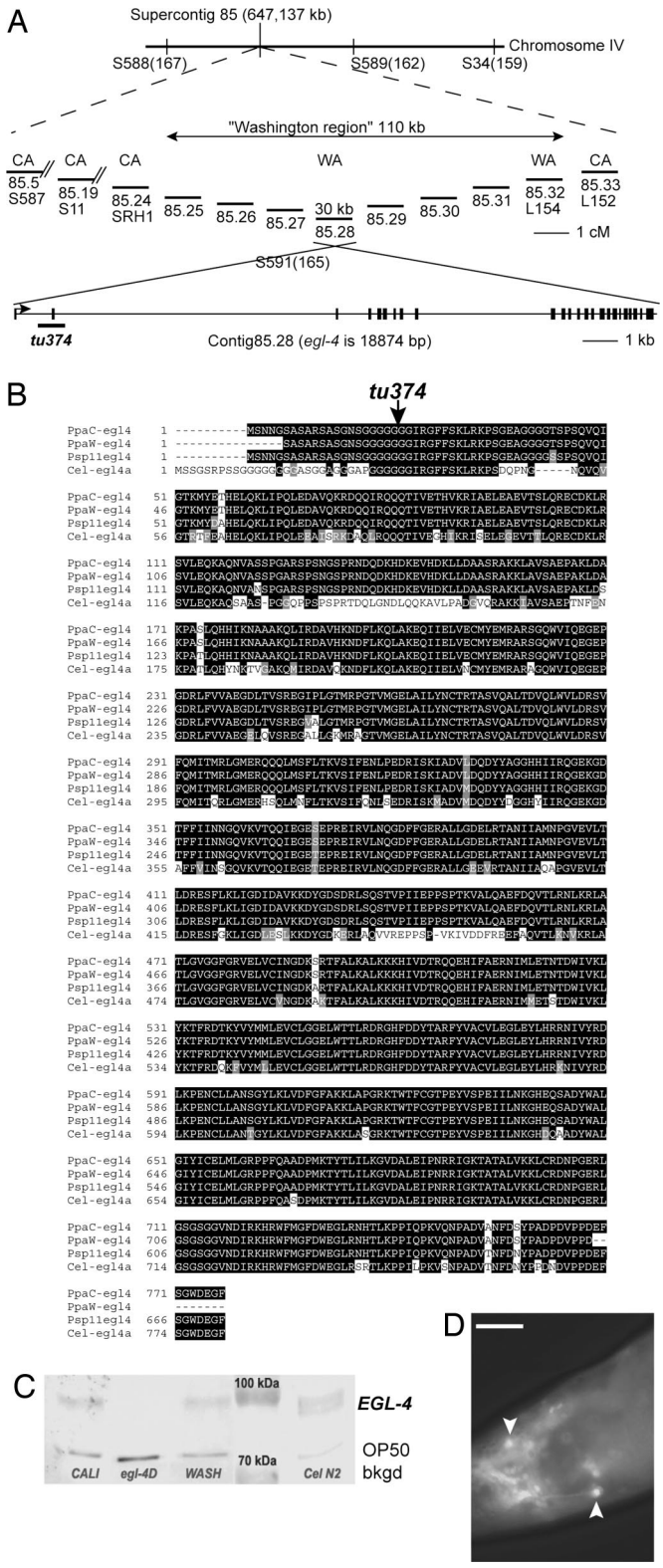

Fig. 2. *Ppa-egl-4* genome location, EGL-4 protein sequence alignment, and protein expression. (*A*) The position of *Ppa-egl-4* on integrated genetic linkage and physical maps. *Ppa-egl-4* is located at 167 cM on Chromosome IV on Supercontig 85. Nearby subcontigs 85.x flanking the contig85.28 containing *egl-4* are shown (≈30 kb, not to scale) with the genotyped strain background indicated above. The only region containing the Washington donor sequence is between contigs 85.25 and 85.32, spanning ≈110 kb. The 780-bp deletion containing the second exon (*tu374*), which results in frame shift and a premature stop codon, is shown below the diagram of the *egl-4* gene structure. Genetic distances are indicated in parentheses. (*B*) Amino acid sequence alignment of *egl-4* orthologs among *P. pacificus* strains California and Washington, *C. elegans* N2, and *Pristionchus* sp. 11. (*C*) Western blot of whole adult hermaphrodite protein extracts, using an anti-*Cel*-EGL-4 antibody. Additional loading and antibody controls are shown in Fig. S3 *A* and *B*. (*D*) *P. pacificus* California adult hermaphrodites immunostained with anti-*Cel*-EGL-4 antibody show staining in head neurons. (Scale bars, 20 μm.)

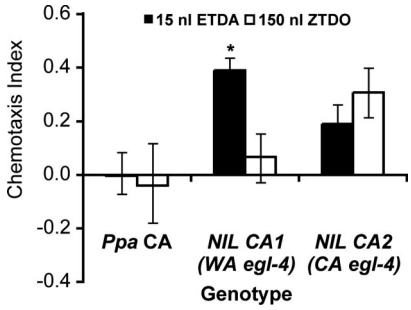

Fig. 3. Chemoattraction of near-isogenic lines (NILs). Chemoattraction of NILs of *Ppa-egl-4* in California (CA) background segregating for *egl-4* Washington (WA) (*NIL CA1*) or *egl-4* CA (*NIL CA2*) alleles show that the *egl-4* WA locus confer partial but significant enhancement of attraction toward 15 nl of ETDA but not 150 nl of ZTDO compared with both the parental California strain and a sibling line with a *egl-4* CA locus (*NIL CA2*). *, $P < 0.05$, Dunnett's *posthoc* multiple comparisons test and Tukey's HSD tests. More than 20 replicate assays were performed for each genotype on at least four separate days. Additional NILs are shown in Fig. S1. Error bars denote SEM.

regions that are exclusive to ETDA-attractive and insensitive strains. This suggests that either independent changes mediate the difference between the insensitive strains and the attractive strains and/or that additional regulatory regions or genes are required for this effect.

Given the sequence differences in the promoter region of *Ppa-egl-4*, we proceeded to determine whether *Ppa-egl-4* expression is different between the California and Washington strains. Quantitative real time reverse transcriptase-PCR (qRT-PCR) of the young adult stage showed slightly higher expression of *Ppa-egl-4* in Washington than in the California strain (Fig. 4*A*). Interestingly, J4 and adult hermaphrodites that show the small but significant difference in *egl-4* expression represents those stages in which the chemotaxis assays were performed. A pattern of low *egl-4* transcript levels associated with low or no ETDA attraction was also observed in the Madagascar and China strains (Fig. 1; see also Fig. S8).

Exogenous cGMP Up-Regulates *Ppa-egl-4* and Phenocopies the Pheromone-Attractive Strain. Exogenous cGMP has been shown to increase the activity of cGMP-dependent protein kinases (PKGs) and thereby affect development and behavior in *C. elegans* and the honey bee (10, 12). To test whether exogenous cGMP can also increase *egl-4* transcript level in the adult California worms, we treated worms briefly with a membrane permeable cyclic guanosine monophosphate, cGMP (8-bromo-cGMP). We found that exogenous cGMP treatment resulted in a 2-fold increase in the transcript level of *egl-4* in the California but not the Washington strain (Fig. 4*B*). To test whether this increase in *egl-4* level directly enhances chemoattraction to the two insect pheromones, we similarly treated young adult stage *P. pacificus* with cGMP for 1 h before chemotaxis assays. We found that cGMP treatment indeed increased the attraction toward ETDA in a cGMP concentration dependent manner in the California but not the Washington strain (Fig. 4*C*). We found a similar enhanced attraction to ZTDO in the California strain (data not shown). In contrast, cGMP treatment of the *NIL* lines

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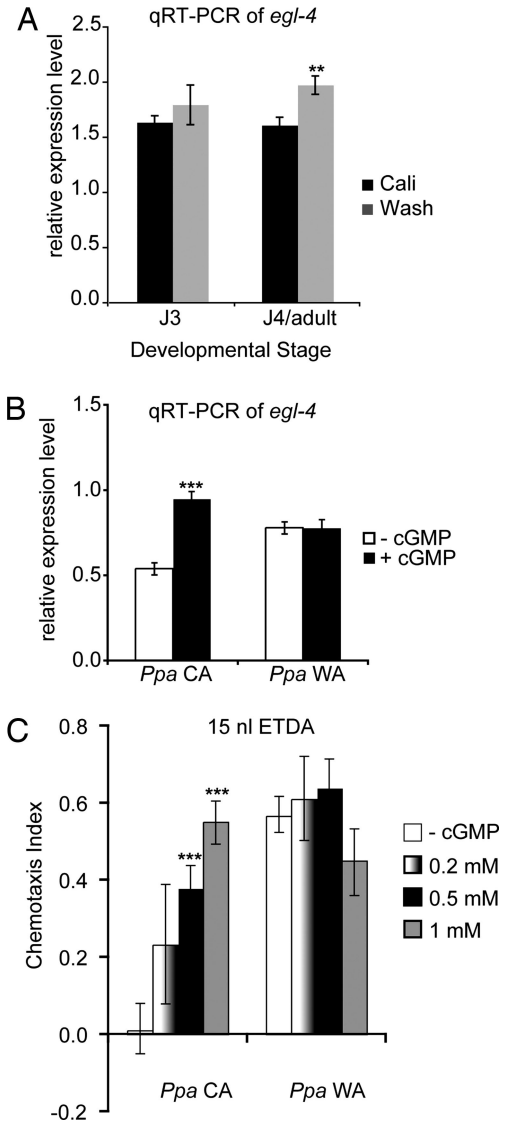

Fig. 4. *Ppa-egl-4* expression level and enhanced attraction with exogenous cGMP. One-hour incubation in 8-bromo-cGMP (+cGMP) is compared with mock treatment in buffer (−cGMP). (*A*) qRT-PCR measurement of whole worm *egl-4* expression in two postembryonic stages of *P. pacificus* California and Washington (N6 cDNA). Relative expression level is defined as: [(level of *Ppa-egl-4*/*Ppa-beta-tubulin*) × 100]. **, significant difference in *egl-4* expression levels between California and Washington, $P < 0.01$ by *t* test). (*B*) Exogenous cGMP [0.5 mM] significantly increased *egl-4* expression level in the California but not the Washington strain (Q_t cDNA). (*C*) Increasing concentrations of exogenous cGMP enhanced attraction to 15 nl of ETDA pheromone in the California but not the Washington strain. Ten to 15 replicate assays were performed for each condition over >3 separate days. ***, significant difference between mock and cGMP treated populations, $P < 0.001$ by two-sampled *t* test. Error bars denote SEM.

enhanced ETDA attraction only in the *NIL* carrying the *egl-4 CA* allele, suggesting that the response to exogenous cGMP is primarily due to regions *cis*- to the *egl-4 CA* locus. (Fig. S5). However, exogenous cAMP treatment had no effect on EDTA or ZTDO attraction (Fig. S6). To show that exogenous cGMP does not result in indiscriminate enhanced attraction to all odors, we investigated the effects of exogenous cGMP on the responses to strong *C. elegans* attractants known to be appreciably less attractive to *P. pacificus* Washington (7). We found that cGMP did not significantly alter the chemoattraction of *P. pacificus* California to 150 nl of butanone, benzaldehyde, or isoamyl alcohol or 15 nl of diacetyl or pentanedione (Fig. S7). Finally, to test whether exogenous cGMP can also enhance ETDA attraction in strains with no or low ETDA attraction and low endogenous *egl-4* transcript levels, we found that cGMP can enhance the ETDA attraction of *P. pacificus* strains Madagascar and China (Figs. S8 and S9). These results suggest that the following effects of exogenous cGMP in *P. pacificus*: (*i*) it is specific for certain odors, such as insect pheromones, and does not cause hyperattraction and (*ii*) interstrain difference in ETDA attraction is partly due to modulation of odor signals through *egl-4* expression levels rather than in pheromone receptors.

***Ppa-egl-4* Null Allele Abolished cGMP-Dependent ETDA Attraction.** To provide molecular evidence that the *Ppa-egl-4* locus in the California background is involved in the cGMP-dependent chemoattraction to insect pheromones, we analyzed an *egl-4* loss-of-function allele in *P. pacificus* California. We generated a mutant allele, *egl-4D* (*tu374*), which contains a 780-bp deletion of the second exon and parts its flanking introns (Fig. 2*A*). This deletion is predicted to result in a transcript of 111 bp, leading to an early stop, and was confirmed to not express any EGL-4 protein by Western blot analysis of whole worms (Figs. 2*C* and Fig. S3*B*). We found that, as in mock cGMP treatments, *tu374* remain unattracted to both ETDA and ZTDO showed no altered chemotaxis toward other *P. pacificus* attractants myristate and β-caryophyllene (Fig. S10). In addition, *tu374* led to a defect in odor adaptation in myristate attraction, reduction in diacetyl attraction, and in nonchemotaxis physiological phenotypes, such as fewer egg count, lower fecundity, and smaller body size (Fig. 5*A*, Figs. S11 and S12, and Table S4). Most importantly, the resulting *Ppa-egl-4* null mutant was no longer attracted to ETDA after cGMP treatment. *egl-4* is therefore required for exogenous cGMP dependent ETDA attraction (Fig. 5*B*). In contrast, *tu374* attraction to ZTDO after cGMP treatment remained unaltered, demonstrating that the enhanced ZTDO attraction by exogenous cGMP does not involve *egl-4* and ZTDO attraction depends on other loci (Fig. 5*C*).

Discussion

Our data strongly implicate the cGMP-dependent protein kinase EGL-4 as being involved in natural variation in *P. pacificus* host insect pheromone attraction. Although cGMP signaling is required for many physiological processes, we have shown that the effect of a brief exogenous cGMP treatment in *P. pacificus* is very specific to ETDA and dependent on *egl-4*. In *C. elegans*, the *egl-4* locus itself is known to have complex transcriptional and post-transcriptional regulations that can further modulate the cGMP signaling. RT-PCR and sequence analyses of the *P. pacificus* whole genome sequence confirmed the existence of several *C. elegans* orthologues in the cGMP signaling pathway in addition to *egl-4*, such as seven transmembrane G protein coupled receptors, G proteins (*odr-3*, *gpa-3*, and *goa-1* homologs), guanylase cyclases, and cyclic nucleotide-gated cation channels (*tax-2* and *tax-4* homologs). Because many functional homologs in the cGMP pathway are present in *P. pacificus* and some EGL-4 functions such as odor sensing and adaptation are conserved in *C. elegans*, EGL-4 is likely to be also regulated at various levels and have multiple functions in *P. pacificus* chemosensation.

In addition to chemosensory defects, *C. elegans egl-4* alleles have been isolated for a wide range of defects, from egg laying to body size, and for both enhancers and suppressors for constitutive dauer formation (8, 11, 13, 14). The *Ppa-egl-4* allele *tu374* has a noticeable reduction in diacetyl attraction, in adaptation to the odor myristate and in egg laying and body size, suggesting that there are certain conserved functions of EGL-4 between *P. pacificus* and *C. elegans*. Surprisingly, the loss of *egl-4* in *P. pacificus* caused a reduction in body size, in contrast to the large body phenotype of loss-of-function *egl-4* alleles in *C. elegans*. The small body phenotype was

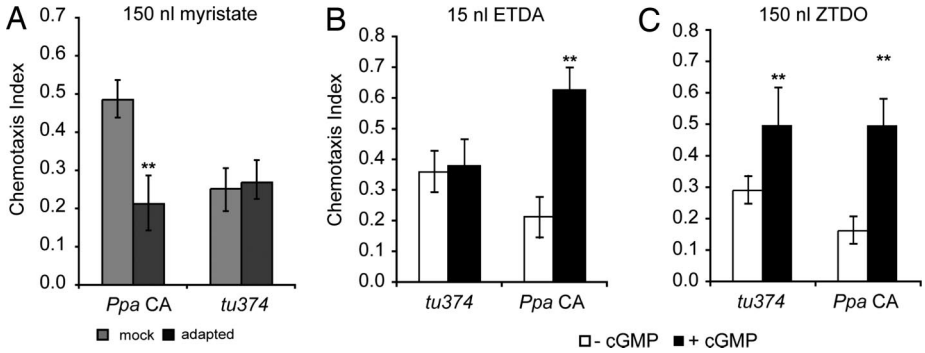

Fig. 5. Chemosensory behavior of *Ppa-egl-4* null mutant *tu374*. (*A*) *tu374* showed lack of adaptation to myristate after one hour incubation with 0.5% of myristate (vol/vol). **, significant difference between mock and myristate exposed populations, $P < 0.01$ by two-sampled *t* test. (*B* and *C*) *tu374* deletion also abolished cGMP-dependent attraction to ETDA (*B*) but not to ZTDO (*C*). **, significant difference between mock and cGMP treated populations, $P < 0.01$ by two-sampled *t* test.

also observed in the F_1 progeny from 45% of *PpaW-egl-4* morpholino injected Washington animals. In contrast, the (−)ETDA phenotype was not observed in these F_1 progeny, perhaps because of the refractory nature of neurons to morpholino. The precise contribution of *Ppa-egl-4* to variations in *P. pacificus* chemosensation, however, may be multifaceted and dependent on the genetic background. Such sheer diversity of *egl-4* alleles in *C. elegans* found for processes which can potentially affect fitness in the wild—chemoattraction, dauer formation, foraging behavior, body size—highlights the intrinsic capacity of EGL-4 to coordinately fine-tune existing behavioral and developmental programs.

We speculate that strains isolated from soil in which beetles and other insect larvae spend a considerable period of their life cycles contain both nonnecromenic and necromenic *P. pacificus* populations. More work will be needed to address the selective pressure for attraction to different insect pheromones, but we can already surmise that distinct genetic factors are responsible for attraction toward the two pheromones based on the following: (*i*) natural variation in the attraction of the two pheromones are uncoupled, because several strains attracted to ETDA lack attraction toward ZTDO (Poland and Bolivia 5270 and 5271). In particular, the Poland strain is genetically very similar to California; however, unlike the California strain, it shows significant attraction to ETDA. (ii) The Washington genotype is dominant with regard to chemoattraction toward ETDA but recessive in ZTDO attraction (Fig. 1*B*). (iii) Loss of *egl-4* function in *tu374* abolished only cGMP-dependent attraction to ETDA but not ZTDO. This separation of signaling pathways has allowed us to compare the role of *egl-4* in attraction to these two insect pheromones and to highlight the specificity of cGMP signaling pathways.

Given that *P. pacificus* is a selfing species that can allow a single individual to colonize and produce a population in a new habitat, more studies will be needed to understand the precise interaction of EGL-4 with other allelic variations in the cGMP signal transduction pathway. Modulation of primary signals by multifunctional proteins, such as protein kinases, may be a beneficial strategy for coordinating multiple phenotype changes rapidly. Thus, it is tempting to speculate that the modulation of odor signals by different *P. pacificus* populations may happen more often than the turnover of novel odor receptors in the context of mosaic species interactions. This is consistent with our finding that EGL-4 and its homologs in two nematodes, *P. pacificus* and *C. elegans*, and in *D. melanogaster* function as a conserved modulator of instinctive behaviors at the population level.

Materials and Methods

Nematode Maintenance and Genetics. All *P. pacificus* strains were maintained at 20–23°C as described in ref. 7. All *Pristionchus* strains have been inbred by single hermaphrodite descent for at least 10 generations and are preserved as frozen stock available upon request. We surveyed proportionally more strains isolated in Japan and Bolivia, because more than half of *P. pacificus* strains were derived from these locations. To ascertain whether the chemoattraction to ETDA and ZTDO was genetically dominant, we assayed the interstrain cross progeny of the pheromone-insensitive California strain (PS312) and pheromone-attracted Washington (PS1843) strain (Fig. 1*B*). Wild-type California males were crossed to the phenotypically marked Washington *dpy-like* lines, and wild-type Washington males were crossed to the marked California *unc-1*. We scored the resulting non-*unc* or non-*dpy* F_1 progeny in chemoattraction assays. As controls, we used both wild-type strains and the intrastrain cross progeny, i.e., Washington × Washington *dpy-like* and California × California *unc-1*.

Recombinant inbred lines (RILs) were produced by single worm descent from 250 F_2 progeny of crosses between Washington hermaprodites (attractive) and California males (insensitive). In *P. pacificus*, multiple recombinations occur per chromosome per generation. We constructed nearly isogenic lines (NILs) (15, 16) from initial crosses between Washington hermaphrodites and California males. F_1 progeny were repeatedly crossed to California (recurrent parent), followed by selfing to homozygosity and selecting those with the homozygous Washington *egl-4* allele (donor parent) and vise versa. The final genotypes of the NILs are shown in Table S2. (For details, see *SI Text*.)

Primer sequences are listed in Table S5.

Chemoattraction Assay. Population chemoattraction assays were performed on 8.5-cm NGM agar plates as described in ref. 7. Chemotaxis differs significantly between *P. pacificus* and *C. elegans*; *P. pacificus* chemotaxis is slower, partly because of slower locomotion, and the attractive concentration range for *P. pacificus* is also much narrower, ≈10-fold. The chemotaxis index (CI) for a given condition is a summary of at least 10 replicate assays with ≈100 J3 to adult nonstarved worms per assay measured over three days to minimize effects of batch variation. More than 99% pure (*E*)-11-tetradecenyl acetate (ETDA) and (*Z*)-7-tetradecen-2-one (ZTDO) pheromones were obtained from Sigma–Aldrich and Bedoukian Research, respectively. All attractants were diluted in pure ethanol. The CI values for ZTDO and ETDA assays were recorded after a 9- to 15-h incubation at 23°C. To treat *P. pacificus* with exogenous 8-bromo-cGMP or 8-bromo-cAMP (as HCl salt; Sigma), worms were washed once with M9 buffer, incubated with 500 μM cGMP or cAMP from a 20 mM stock diluted in dH_2O for 1 h at room temperature, washed again with 20x volume of M9 buffer, and then loaded onto assay plates.

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1. Thompson JN (2005) *The Geographical Mosaic of Coevolution* (Univ of Chicago Press, Chicago), p 443.
2. Kiontke K, Fitch D (2006) The Phylogenetic Relationships of *Caenorhabditis* and other rhabditids. *Wormbook*, www.wormbook.org, 10.1895/wormbook. 1.11.1. Accessed November 17, 2005.
3. Herrmann M, Mayer WE, Sommer RJ (2006) Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. *Zoology* 109:96–108.
4. Herrmann M, Mayer WE, Sommer RJ (2006) Sex, bugs and Haldane's rule: The nematode genus *Pristionchus* in the United States. *Frontiers Zool* 3(14).

5. 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.
6. Hong RL, Sommer RJ (2006) *Pristionchus pacificus*: A well-rounded nematode. *Bioessays* 28:651–659.
7. Hong RL, Sommer RJ (2006) Chemoattraction in Pristionchus nematodes and implications for insect recognition. *Curr Biol* 16:2359–2365.
8. L'Etoile ND, *et al.* (2002) The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron* 36:1079–1089.
9. Osborne Ka, *et al.* (1997) Natural behavior polymorphism due to a cGMP-dependent protein kinase of *Drosophila. Science* 277:834–836.
10. Ben-Shahar Y, Robichon A, Sokolowski MB, Robinson GE (2002) cGMP-dependent changes in phototaxis: A possible role for the foraging gene in honey bee division of labor. *Science* 296:741–744.
11. 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.
12. Nakano Y, Nagamatsu Y, Ohshima Y (2004) cGMP and a germ-line signal control body size in C-elegans through cGMP-dependent protein kinase EGL-4. *Genes Cell* 9:773–779.
13. Trent C, Tsung N, Horvitz HR (1983) Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. *Genetics* 104:619–647.
14. Daniels SA, Ailion M, Thomas JH, Sengupta P (2000) egl-4 acts through a transforming growth factor-beta/SMAD pathway in *Caenorhabditis elegans* to regulate multiple neuronal circuits in response to sensory cues. *Genetics* 156:123–141.
15. Muehlbauer GJ, Specht JE, Thomas-Compton MA, Staswick PE, Bernard RL (1988) Near-isogenic lines—a potential resource in the integration of conventional and molecular marker linkage maps. *Crop Science* 28:729–735.
16. Kaeppler SM, Phillips RL, Kim TS (1993) Use of near-isogenic lines derived by backcrossing or selfing to map qualitative traits. *Theor Appl Genet* 87:233–237.
17. Colbert HA, Bargmann CI (1997) Environmental signals modulate olfactory acuity, discrimination, and memory in *Caenorhabditis elegans*. *Learn Mem* 4:179–191.
18. 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.
19. Zauner H, Sommer RJ (2007) Evolution of robustness in the signaling network of Pristionchus vulva development. *Proc Natl Acad Sci USA* 104:10086–10091.
20. Srinivasan J, *et al.* (2002) A bacterial artificial chromosome-based genetic linkage map of the nematode *Pristionchus pacificus*. *Genetics* 162:129–134.
21. Lynch M, Walsh B (1998) *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Sunderland, MA).
22. Sommer RJ, Eizinger A, Lee K-Z, Jungblut B, Bubeck A, Schlak I (1998) The Pristionchus HOX gene Ppa-lin-39 inhibits programmed cell death to specify the vulva equivalence group and is not required during vulval induction. *Development* 125:3865–3873.
23. Hirose T, *et al.* (2003) Cyclic GMP-dependent protein kinase EGL-4 controls body size and lifespan in *C. elegans*. *Development* 130:1089–1099.
24. Finney M, Ruvkun G (1990) The unc-86 gene product couples cell lineage and cell identity in *C. elegans*. *Cell* 63:895–905.
25. Kolotuev I, Podbilewicz B (2004) *Pristionchus pacificus* vulva formation: Polarized division, cell migration, cell fusion, and evolution of invagination. *Dev Biol* 266:322–333.
26. 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.