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Report

Chemoattraction in *Pristionchus* Nematodes and Implications for Insect Recognition

Ray L. Hong[1,*] and Ralf J. Sommer[1,*]
[1] Max Planck Institute for Developmental Biology
Department for Evolutionary Biology
Spemannstrasse 37–39
72076 Tuebingen
Germany

Summary

Nematodes and insects are the two dominant animal taxa in species numbers, and nematode-insect interactions constitute a significant portion of interspecies associations in a diversity of ecosystems. It has been speculated that most insects represent mobile microhabitats in which nematodes can obtain food, mobility, and shelter. Nematode-insect associations can be classified as phoretic (insects used for transportation, not as food), necromenic (insect used for transportation, then carcass as food), and entomopathogenic (insect is killed and used as food). To determine how nematodes target their hosts, we analyzed the chemosensory response and behavioral parameters of closely related *Pristionchus* nematodes that form species-specific necromenic associations with scarab beetles and the Colorado potato beetle [1, 2]. We found that all four studied *Pristionchus* species displayed unique chemoattractive profiles toward insect pheromones and plant volatiles with links to *Pristionchus* habitats. Moreover, chemoattraction in *P. pacificus* differs from that of *C. elegans* not only in the types of attractants, but also in its tempo, mode, and concentration response range. We conclude that *Pristionchus* olfaction is highly diverse among closely related species and is likely to be involved in shaping nematode-host interactions.

Results and Discussion

Pristionchus pacificus is a nematode cultivated to provide comparisons to the *C. elegans* model in the area of development, genetics, and evolution [3]. Many genera in the Diplogastridae to which *Pristionchus* belongs are associated with insects: *Micoletzkya* species associate with bark beetles, and *Parasitodiplogaster* species are intimately associated with fig wasps (Agaonidae) [4–6]. Recent studies have shown that several species within the *Pristionchus* genus are necromenic and are closely associated with scarab beetles from the United States as well as western Europe [1, 2]. *P. maupasi* is a closely related species to *P. pacificus* and is found primarily on *Melolontha* species, the European cockchafer (Figure 1). *P. entomophagus* and *P. uniformis* are sister species that are found primarily on *Geotrupes* (dung beetles) and *Leptinotarsa decemlineata*, the Colorado potato beetle (CPB), respectively (Figure 1). Except for the CPB, which belongs to the Chrysomelidae, *Pristionchus* populations so far have only been isolated extensively from beetles belonging to the Scarabaeoidea superfamily. Because odors from organisms can convey abundant information on their location and identity, olfaction may be the favored modality for nematodes that lack vision and have to contend with a complex gustatory environment in the soil. Hence, the orientation and taxis of nematodes toward specific odors in chemotaxis assays might recapitulate their behavioral response in nature toward their preferred environments, including their beetle hosts.

To establish species-specific attraction profiles of four *Pristionchus* species, we surveyed 31 compounds for their attractiveness to *P. pacificus* and *C. elegans*, including previously known *C. elegans* attractants, as well as 11 known semiochemicals involved in insect and plant communication on two pairs of highly related *Pristionchus* species (Table 1 and Figure 1). Semiochemicals are communication compounds emitted by insects to attract mates (sex pheromone), to aggregate into groups (aggregation pheromone), and to ward of competitors (allomone) [7]. Semiochemicals also include volatile secondary metabolites from plants in response to insect herbivory, and such metabolites can either recruit the natural predators of the insects as a defense mechanism or enhance the sex pheromone of the insects (kairomone) [8]. The goal of our study was to identify attractants for *Pristionchus* species and to delineate their attraction parameters as well as those semiochemicals unique to each species.

We found that all four *Pristionchus* species, especially *P. maupasi*, were attracted to β-caryophyllene, a ubiquitous and inducible plant defense volatile known to be attractive to the entomopathogenic nematode *Heterorhabditis megidis* [9] (Figure 1). That β-caryophyllene is a common *Pristionchus* attractant may likely be because of the habitat and diet of their phytophagus beetle hosts. All *Pristionchus* species also showed some attraction toward the 16 carbon Z-HDA, a Lepidopteran pheromone, but Z-HDA was not the most attractive compound to any of these species. In the wild, *Pristionchus aerivorous* has been reportedly found on a Lepidopteran host, *Helicoverpa zea* [10].

More importantly, we found that closely related pairs of *Pristionchus* species have significantly diverged chemoattraction profiles (Figure 1). *P. pacificus*, whose insect host has yet to be identified, was most attracted to long-chain fatty-acid esters and acetates (E-TDA and myristate) compared to *P. maupasi*. By contrast, *P. maupasi* was much more attracted to other plant derived compounds such as linalool [11] and the green-leaf alcohol ((*Z*)-3-hexen-1-ol) [8]. Similarly, *P. entomophagus,* the most frequent *Pristionchus* species found on dung beetles in Europe [1], displayed the highest attraction to isopentylamine. Isopentylamine smells of

*Correspondence: ray.hong@tuebingen.mpg.de (R.L.H.), ralf.sommer@tuebingen.mpg.de (R.J.S.)

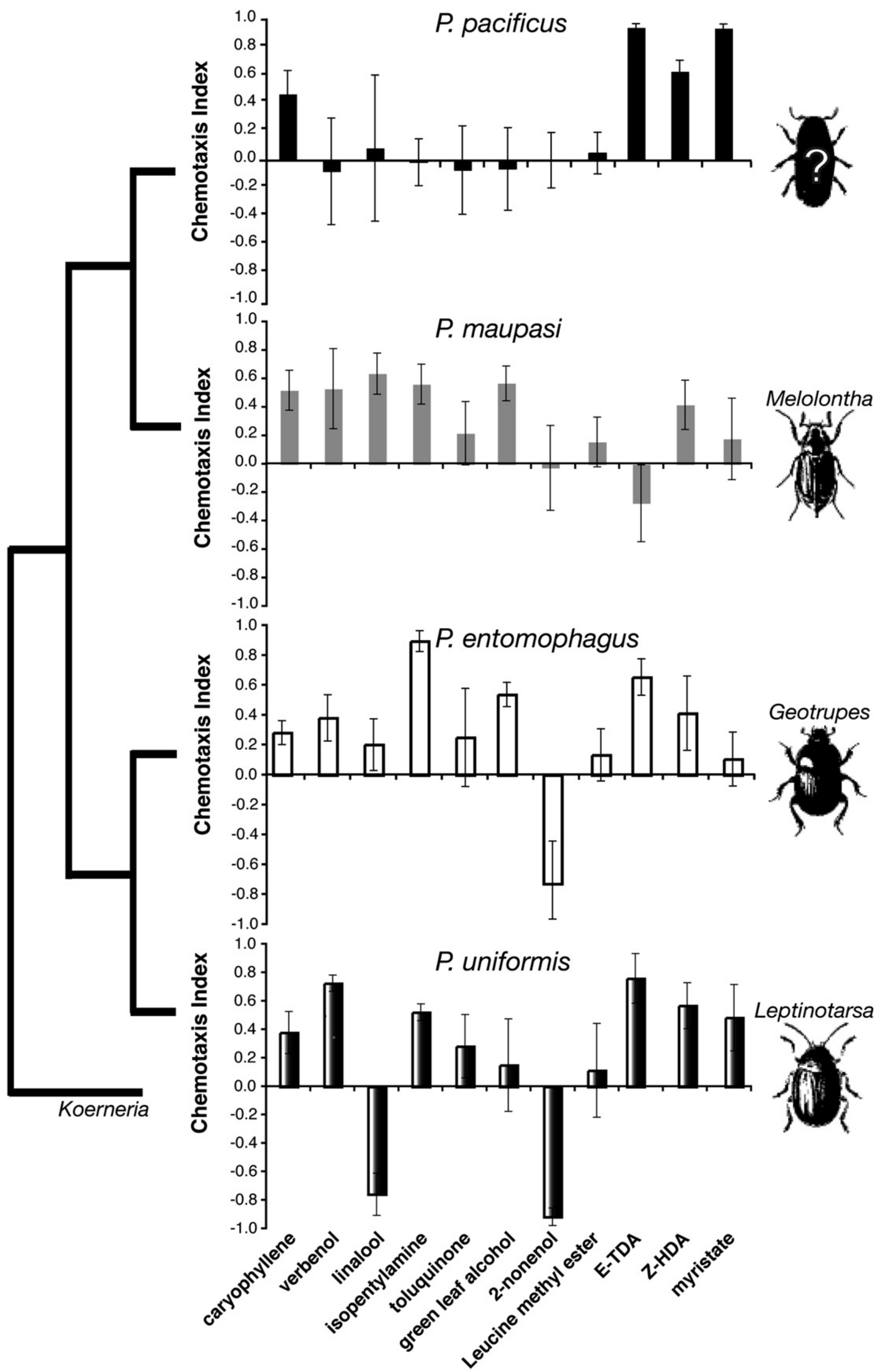


Figure 1. Semiochemical Attraction Profiles of *Pristionchus* Species

Pristionchus attraction profiles to semiochemicals at 10% concentration (w/v or v/v). Verbenol and leucine methyl ester were at 100 mM. 10-fold dilutions of these compounds resulted in similar profiles (data not shown). The left side shows the phylogenetic relationship of four *Pristionchus* species based on 18S ribosomal DNA sequences, with *Koerneria* as the most basal species, along with known beetle associations: *P. maupasi* decaying material and can be used to trap *Geotrupes* beetles. Interestingly, isopentylamine has a branched carbon group similar to the nonattractive leucine methyl ester, a sex pheromone of June beetles, *Phyllophaga lanceolata* [12]. *P. uniformis,* which has the highest fidelity to CPB, showed the strongest attraction to verbenol, a known aggregation pheromone of bark beetles (*Ips*) made from plant monoterpenoid precursors. Taken together, the attraction profiles to plant- and insect-derived compounds provide sufficient resolution for discerning four closely related, morphologically similar nematode species. These results suggest that species-specific olfaction profiles are likely to be involved in host recognition and attraction. However, the choice of stimuli we tested can be further refined according to their relevant ecological contexts, with the goal of understanding the precise involvement of each compound in specific insect-nematode interactions.

Because the analyzed *Pristionchus* species have species-specific chemoattraction profiles, we wondered whether closely related *Caenorhabditis* species also show such diversity in chemotaxis behavior. Wild *C. elegans* isolates are primarily found in decaying organic matter, whereas the sister species *C. briggsae* and *C. remanei* have been found to associate casually with snails and isopods, as well as to live freely in composts [13, 14]. We proceeded to test known *C. elegans* N2 attractants on *C. elegans* Hawaii, *C. briggsae*, and *C. remanei* (Figure 2A). When compared to *C. elegans* N2, we observed statistically significant quantitative differences in the chemoattraction of *Caenorhabditis* species toward isoamyl alcohol, benzaldehyde, pentanedione, diacetyl, and pyrazine but not for 2-butanone. However, these compounds were attractive to all *Caenorhabditis* species to some extent, with no qualitative differences in which chemoattraction was completely absent or repulsive. Similarly, we found that *Caenorhabditis* species were repulsed by 10% β-caryophyllene, and all but *C. elegans* Hawaii was repulsed by 1% myristate and E-TDA (see Figures S1A–S1C in the Supplemental Data available with this article online). Thus, not only is the difference in semiochemicals attraction conserved between other *Caenorhabditis* species and *P. pacificus*, the closely related *Pristionchus* species but not *Caenorhabditis* species have distinguishing chemotaxis profiles. Nevertheless, other semiochemicals not tested here might potentially distinguish related *Caenorhabditis* species once specific invertebrate associations can be identified.

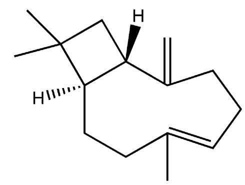
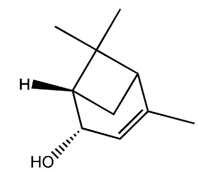
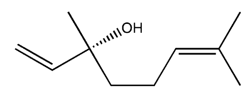
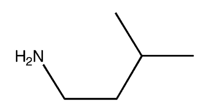
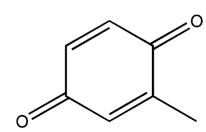
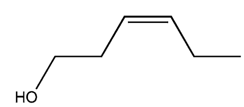
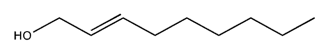
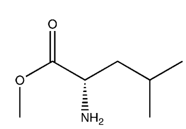
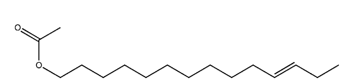
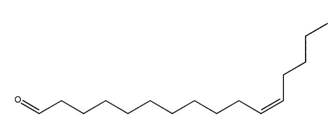
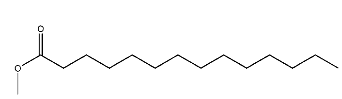
We wondered whether *P. pacificus*, in order to be able to proliferate under the same standard laboratory conditions as *C. elegans*, is also attracted to some of the simple organic compounds as *C. elegans* is. We tested seven of the most attractive compounds and found that *P. pacificus* was attracted to high concentrations of pentanedione, pyrazine, and diacetyl (Figure 2B). *P. pacificus* was weakly attracted to a low concentration of 2,4,5-trimethylthiazole TMT (1%) as well and repulsed by high concentrations of undiluted TMT and benzaldehyde. Furthermore, *P. pacificus* showed no remarkable attractions toward isoamyl alcohol, benzaldehyde, and 2-butanone, except an extremely weak attraction for 10% butanone (CI ~0.3). *C. elegans,* as expected, was highly attracted to the same compounds at the same 1–100× dilutions; thus, only the 1% profile is shown for comparison to *P. pacificus* (Figure 2B). These results suggest that the recognition of high concentrations of diacetyl, pentanedione, and pyrazine are only partially conserved between *P. pacificus* and *C. elegans* or that downstream signaling events are more tempered than they are in *C. elegans*.

In nature, organisms must be able to discriminate one odor from other, more abundant odors. We utilized the discrimination assay to demonstrate that separate signaling pathways in *P. pacificus* can perceive two simultaneous odors, one of which is saturating [15](Figure 2C). We tested eight attractants on plain, diacetyl-, and β-caryophyllene-saturated agar plates in a modified chemotaxis assay. Diacetyl represented the common attractant for all *Caenorhabditis* and *Pristionchus* species that we have tested; hence, there may be a conserved receptor and signaling pathway for diacetyl (Figure S1D). Meanwhile, β-caryophyllene represents a basic *Pristionchus* odor that is likely to be present in most *Pristionchus* habitats. We found that *P. pacificus* attraction to the four shared *P. pacificus*-*C. elegans* attractants—diacetyl, pyrazine, isobutanol, and pentanedione—were not reduced on saturated plates as compared to plain plates. Similarly, four *P. pacificus*-specific attractants—β-caryophyllene, mono-olein, E-TDA, and myristate—were also not compromised on saturated plates. We conclude that *P. pacificus,* like *C. elegans*, can discriminate simultaneous odors.

In general, *P. pacificus* has the slowest population chemoattraction response among the four *Pristionchus* species tested, with the fastest response to diacetyl and β-caryophyllene peaking in 2–3 hr and the slowest response to myristate and E-TDA peaking after 9 hr. *C. elegans* populations, by contrast, attained peak attraction to all attractive odors within 1 hr. Although observations of single worms indicated that *P. pacificus* can reach attractant sources within 2 hr, attraction at the population level was only apparent after much longer periods. To describe this population behavior in more detail, we conducted time-course assays for attraction toward myristate and E-TDA over a period of 22 hr (Figure 3). CI for both myristate and E-TDA (10% and 1%) increased steadily over this time span, reaching a climax in 9–22 hr. The long incubation time required to attain peak CI is a *Pristionchus*-specific chemoattraction phenomenon not observed in *C. elegans* because all *C. elegans* attractants tested require 30–45 min to attain peak CI (this study and [16]) and greater than 9 hr was also required to achieve peak CI for myristate and E-TDA in other *Pristionchus* species tested (Figure 1). The slower response of *P. pacificus* compared to *C. elegans* at the individual level is primarily due to the relatively slower locomotion of *P. pacificus*, as well as their more frequent turning and reversal behavior both in the presence and absence of food (J. Srinivasan

(*Melolontha* sp., European Cockchafers), *P. entomophagus* (*Geotrupes* sp., dung beetles), and *P. uniformis* (*Leptinotarsa decemlineata*, Colorado potato beetle) (modified from [1]). The natural insect host for *P. pacificus* remains unidentified. All data points were derived from at least two separate experiments with 3–6 replicates each. Error bars denote 95% confidence intervals.

Table 1. A List of Semiochemical Compounds Tested in Four *Pristionchus* Species

Compound	Known Insect or Plant Source	Known Function	Structure
(E)-β-caryophyllene	Maize and other plants	Plant defense volatile [9]	
(S)-verbenol	*Ips paraconfusus*	Aggregation pheromone [26]	
(R)-(-)-linalool	Many plants	Plant volatile which enhances sex pheromone [11]	
isopentylamine	*Phyllophaga lanceolata*	Phyllophaga and Geotrupes attractant [12]	
Toluquinone	*Melolontha melolontha*, *M. hippocastani*	Sex pheromone [27, 28]	
(Z)-3-hexen-1-ol(Green-leaf alcohol)	Many plants	Plant volatile that enhances sex pheromone [8]	
2-*(E)*-nonenol	*Melolontha melolontha*, *M. hippocastani*	Sex pheromone [29]	
L-Leucine methyl ester	*Phyllophaga lanceolata*	Sex pheromone [12]	
(E)-11-tetradecenyl acetate (E-TDA)	*Lepidoptera*; i.e., *Spodoptera* and *Heliothis* species	Sex pheromone [30, 31]	
(Z)-11-hexadecenal(Z-HDA)	*Lepidoptera*; i.e., *Helicoverpa zea*	Sex pheromone [32, 33]	
methyl tetradecanoate (myristate)	*Philanthus* and *Musca* species	Allomone [34–36]	

Reviewed in [7, 25].

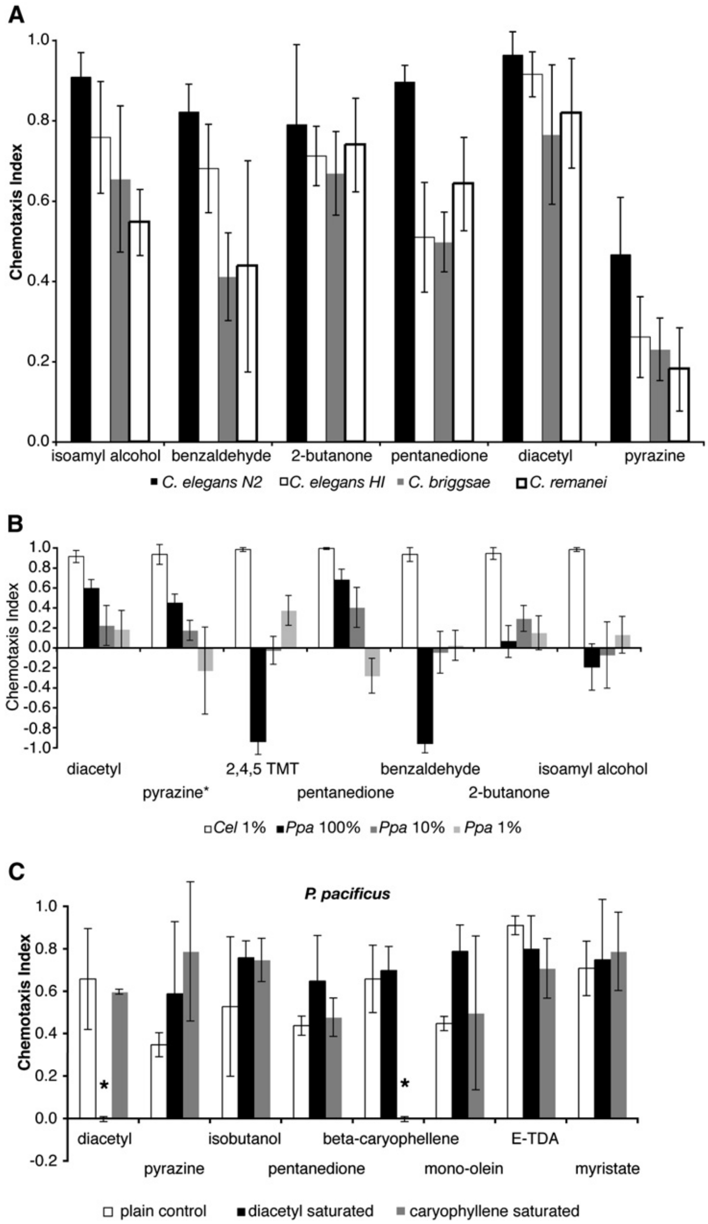


Figure 2. Differences between *C. elegans* and *P. pacificus* Chemoattraction

Error bars denote 95% confidence intervals. All assays for different species were conducted concurrently during multiple sessions with each value representing at least six experiments.

(A) The strongest *C. elegans* N2 attractants elicited quantitatively but not qualitatively different attraction for *C. elegans* Hawaii, *C. briggsae*, and *C. remanei*.

(B) *P. pacificus* share a set of attractants with *C. elegans*. *C. elegans* attraction profiles at 1% concentration are shown as positive controls (white bars). *P. pacificus* attraction profiles 100%–1% are shown for all compounds except for pyrazine (*), for which the 10%–0.1% profile is shown (filled and shaded bars). *P. pacificus* was attracted to 100% diacetyl and pentanedione as well as weakly attracted to 10% pyrazine and 1% 2,4,5-trimethylthiazole (2,4,5-TMT). *P. pacificus* was repulsed by high concentrations of benzaldehyde and TMT and showed no attraction to isoamyl alcohol, benzaldehyde, and 2-butanone. The response to 10% 2-butanone is considered borderline attraction (CI ~0.3).

(C) Discrimination assays show that *P. pacificus* discriminated conserved attractants to *C. elegans* (100% diacetyl, 10% pyrazine, 10% isobutanol, and 100% 2,3-pentanedione) from 1:10,000 dilution of β-caryophyllene in NGM agar. *P. pacificus* also discriminated *P. pacificus*-specific attractants (10% β-caryophyllene, 10mM 1-mono-olein, 1% myristate, and 10% E-TDA) from 1:10,000 dilution of diacetyl in NGM agar. As controls (asterisks), diacetyl and β-caryophyllene chemoattraction were abolished in the presence of the same scent in the agar.

and P. Sternberg, personal communication). More precise tracking of *P. pacificus* velocity as well as orientation would be required for determining whether *P. pacificus* modulates its locomotion behavior according to the type or strength of attractants. We have tentatively referred to these attraction modes requiring greater than 9 hr as "long-term attraction" based solely on the incremental increase in CI as a roaming population and not on the behavioral mechanisms involved. Taken together, chemoattraction in *Pristionchus* species represent more complex olfactory responses than previously described for *C. elegans*, and these differences may be important for *Pristionchus*' necromenic lifestyle.

Although *P. pacificus* and *C. elegans* share a common set of attractants, *P. pacificus* was attracted to diacetyl, pentanedione, pyrazine, and isobutanol only at the 10%–100% concentration range (Figure 2B). Even strong *P. pacificus* attractants, such as E-TDA and myristate, elicited attraction only within a 100-fold concentration range (1%–100%). Such limited sensitivity range may be conserved in all *Pristionchus* species in light of the fact that the semiochemicals profiles and the response to diacetyl showed attraction only within a 10-fold dilution range (Figures 1 and 2). This is in stark contrast to the seven strong *C. elegans* attractants we tested; several of these attractants can elicit *C. elegans* attraction in the range of 10,000-fold, with diacetyl having the greatest attraction range of a million fold (0.0001%–100%) [16]. Although it is possible that some of the *Pristionchus* attractants are not the optimal ligands, it is most likely that certain blends or sequence medley of odors are required for eliciting more sensitive responses, as may be the situations encountered in nature.

Finally, we asked whether chemoattraction behavior also differs between isolates of the same *P. pacificus*

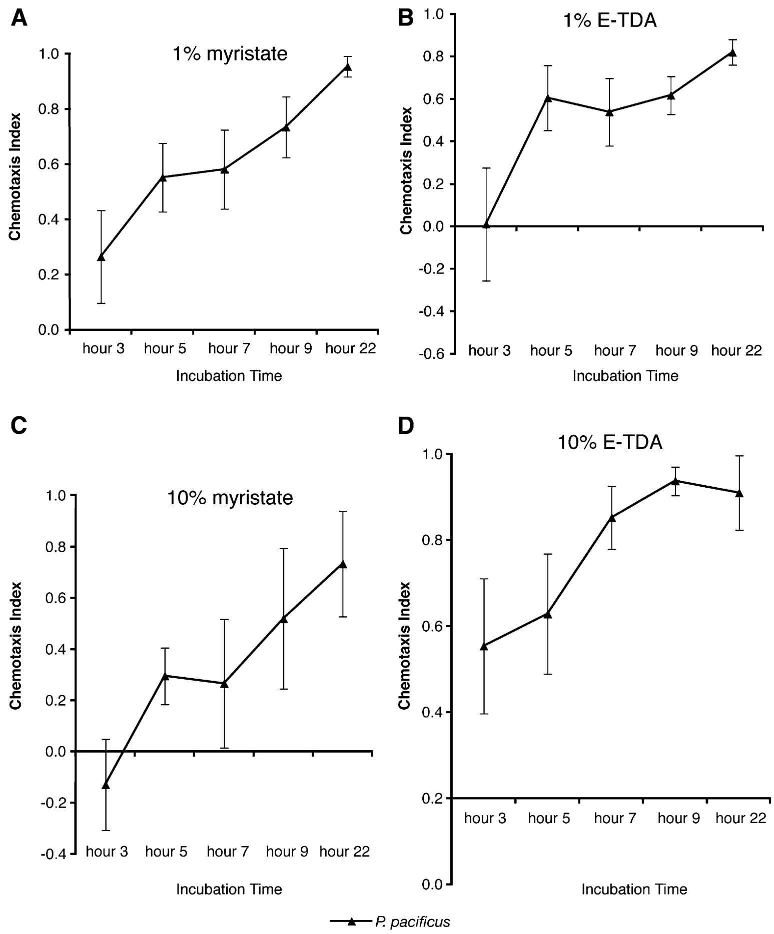


Figure 3. Time-Course Comparisons of *P. pacificus* Attractions to 1% Myristate and E-TDA

The chemoattraction indices were taken at multiple time intervals for attraction to myristate at 1% (A) and 10% (C) as well as E-TDA at 1% (B) and 10% (D). Except for 10% myristate (three replicates each), each data point is an average of 6–12 replicates from three experiments.

species. To our surprise, we observed significant chemoattraction differences between the two standard laboratory strains [17]. Specifically, the California strain was not attracted to E-TDA at all, compared to the Washington strain, although both strains showed comparable attraction toward β-caryophyllene and diacetyl (Figure S1E and data not shown). To determine the extent of this behavioral polymorphism in *P. pacificus*, we tested two additional strains. Previous studies by AFLP analysis (Amplified Fragment Length Polymorphism) indicated that the Hawaii strain is more similar to the Washington than the California strain, whereas the California and Poland strains share almost identical band patterns [18]. In our olfaction studies, the Hawaii strain was similarly attracted to 1% E-TDA as the Washington strain, but with slightly lower attraction to 10% E-TDA. More surprisingly, the Poland strain was also attracted to E-TDA. Thus, a small number of genetic differences below the threshold detectable by AFLP may be responsible for divergence in *P. pacificus* chemosensory behavior, although it is not yet clear whether this difference was adapted or accumulated during the cultivation of *P. pacificus*.

In conclusion, we found that all four studied *Pristionchus* species displayed unique chemoattractive profiles toward insect pheromones and plant volatiles with connections to *Pristionchus* habitats. Moreover, chemoattraction in *P. pacificus* differs from that of *C. elegans* not only in the types of attractants but also in its tempo, mode, and concentration response range at the population level. Although past studies on entomopathogenic nematodes have shown that plant defense volatiles and odors from insect hosts are important for attraction to insect hosts [9, 19–23], the species-specificity of these attractions were not addressed nor were the contributions of individual compounds identified. Owing to the enormous contributions by researchers in the *C. elegans* community, we already hold some key concepts on nematode olfactory behavior, such as discrimination, adaptation, and associative learning [15, 16, 24]. Given that *P. pacificus* does not have strong attraction toward any of the key seven *C. elegans* attractants (Figure 2B), it is likely that the functions of the homologous AWA and AWC neurons differs significantly from those in *C. elegans.* Interestingly, the current predicted number of seven transmembrane receptor genes in the *P. pacificus* genome is substantially less than those in the *C. elegans* genome (www.pristionchus.org). Our long-term goal will include investigations into the neuroanatomy and receptor-neuron mapping of *P. pacificus* olfaction. Identifying the molecular changes underlying the difference between *P. pacificus* and *C. elegans* will be the first step toward understanding how dramatically new chemosensations can arise, how these changes alter downstream intracellular signaling pathways, and how these molecular changes feed back to the organismal interactions in their ecological habitats.

Supplemental Data

Supplemental Data include Experimental Procedures, one figure, and one table and can be found with this article online at http://www.current-biology.com/cgi/content/full/16/23/2359/DC1/.

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