The Nematode Pristionchus pacificus (Nematoda: Diplogastridae) Is Associated with the Oriental Beetle Exomala orientalis (Coleoptera: Scarabaeidae) in Japan

Authors: Herrmann, Matthias, Mayer, Werner E., Hong, Ray L., Kienle, Simone, Minasaki, Ryuji, et al.

Source: Zoological Science, 24(9) : 883-889

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.24.883

ZOOLOGICAL SCIENCE **24**: 883–889 (2007)

The Nematode *Pristionchus pacificus* (Nematoda: Diplogastridae) Is Associated with the Oriental Beetle *Exomala orientalis* (Coleoptera: Scarabaeidae) in Japan

Matthias Herrmann, Werner E. Mayer, Ray L. Hong, Simone Kienle, Ryuji Minasaki and Ralf J. Sommer*

Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Tübingen, Germany

***Pristionchus pacificus* has been developed as a nematode satellite organism in evolutionary developmental biology. Detailed studies of vulva development revealed multiple differences in genetic and molecular control in *P. pacificus* compared to the model organism *Caenorhabditis elegans*. To place evolutionary developmental biology in a comprehensive evolutionary context, such studies have to be complemented with ecology. In recent field studies in western Europe and eastern North America we found 11 *Pristionchus* species that are closely associated with scarab beetles and the Colorado potato beetle. However, *P. pacificus* was not commonly found in association with scarab beetles in these studies. Here, we describe the results of a similar survey of scarab beetles in Japan. *Pristionchus pacificus* was the most common *Pristionchus* species on scarab beetles in Japan, with 40 out of 43 (93%) isolates. The other *Pristionchus* isolates represent three novel species, which we refer to as *Pristionchus* sp. 11, *Pristionchus* sp. 14, and *Pristionchus* sp. 15. Thirty-seven of the established *P. pacificus* strains were found on the oriental beetle *Exomala orientalis*. Laboratory studies with the sex pheromone *(Z)*-7-tetradecen-2-one of the oriental beetle revealed that *P. pacificus* shows strong olfactory attraction to the beetle's sex pheromone, which provides a potential mechanism for the recognition and interaction of *P. pacificus* and *E. orientalis*. Together, this study identifies *P. pacificus* as the most common *Pristionchus* nematode in field studies in Japan, identifies *E. orientalis* as an important host species, and provides the basis for the ecology of *P. pacificus*.**

Key words: *Pristionchus pacificus*, scarab beetle, oriental beetle, *Exomala orientalis*, *Anomala orientalis*, sex pheromone

INTRODUCTION

The hermaphroditic nematode *Pristionchus pacificus* Sommer, Carta, Kim and Sternberg, 1996 has been developed as a satellite organism in evolutionary developmental biology (Hong and Sommer, 2006a). Previous studies of *P. pacificus* concentrated on the developmental, genetic and molecular analysis of sex determination and vulva and gonad formation (Pires-daSilva and Sommer, 2004; Rudel *et al.*, 2005; Zheng *et al.*, 2005). More recently, a genomic initiative including the generation of a genetic linkage map and a physical map and an ongoing whole-genome sequencing project have complemented the developmental and genetic studies (Dieterich *et al.*, 2007).

The ecology of nematodes such as *P. pacificus* and *C. elegans* that are used as laboratory organisms is only poorly understood. We have recently shown that nematodes of the genus *Pristionchus* live in close association with scarab beetles and the Colorado potato beetle (CPB) in western Europe and eastern North America (Herrmann *et al.*, 2006a, b). However, the satellite organism *P. pacificus* was observed neither on scarab beetles nor on the CPB in western Europe, and only three out of 289 *Pristionchus* isolates obtained from field studies in the United States were *P. pacificus* (Herrmann *et al.*, 2006b). Here, we describe 40 isolates of *P. pacificus* from scarab beetles in Japan, in particular from the oriental beetle *Exomala (Anomala) orientalis*.

Pristionchus pacificus was the most common *Pristionchus* species in our scarab beetle survey in Japan. Laboratory studies with the sex pheromone *(Z)*-7-tetradecen-2-one of the oriental beetle revealed that *P. pacificus* shows strong olfactory attraction. Only four of the 44 *Pristionchus* isolates from Japan (43 isolates from beetles, one from a soil sample) represent species other than *P. pacificus*. Interestingly, these isolates represent three distinct species, all of which are novel to us.

* Corresponding author. Phone: +49-7071-601371;
Fax : +49-7071-601498;
E-mail: ralf.sommer@tuebingen.mpg.de

Note: Nucleotide sequence data reported are available in the GenBank database under the accession numbers EF216858, EF216859 and EF216860.

doi:10.2108/zsj.24.883

MATERIALS AND METHODS

Isolation of nematodes

We collected different beetles at the adult stage using pheromones, sweeping nets, blacklight traps and pitfall traps baited with dung. The beetles were transferred to the lab alive, sacrificed by cutting them in half transversely, and put on NGM agar plates (6 cm diameter) seeded with 300 μl of the slowly growing *E. coli* strain OP50. The plates were checked daily using a Zeiss Stemi 2000 dissecting scope over a period of one to three weeks for emerging and reproducing nematodes. From the emerging nematodes we produced isogenic lines by transferring single gravid females or hermaphrodites to new plates. To determine whether the isolated nematodes belonged to gonochoristic or hermaphroditic species, virgin larvae were singled out onto plates. The presence of offspring indicated that they represented a hermaphroditic species.

Emerging nematodes were determined to family level with a Zeiss Stemi 2000 dissecting scope and to genus level with a Zeiss Axioplan 2 microscope using the key by Sudhaus and Fürst von Lieven (2003). For determination several worms were transferred onto microscopic slides covered with a 0.5 mm thick layer of 5% agar and either immobilized by heating the slide over an open flame to about 60°C for a few seconds or anesthesized with sodium azide. Within the genus *Pristionchus* many species can not be identified by morphological methods. We therefore chose to use molecular tools and mating experiments with reference strains to distinguish the different species (Herrmann *et al*., 2006a, b).

Collection by pheromone traps

Pheromone baits (Fuji Flavour Co., Tokyo, Japan) for the Oriental beetle were provided by Dr. Paul Robbins, Geneva NY. These baits were either put into funnel traps made out of empty 1.5-L green tea PET bottles or directly put on the ground and observed for attracted beetles which were then caught manually.

Molecular species identification

Molecular species identification was done using the small subunit rRNA gene (*SSU*). In brief, genomic DNA from single nematodes was prepared using the NaOH lysis procedure described by Floyd *et al.* (2002) and as in Herrmann *et al.* (2006a). A 1-kb fragment of the *SSU* was amplified by PCR using the primers SSU18A (5'-AAAGATTAAGCCATGCATG-3') and SSU26R (5'-CATTCTTGGCAAATGCTTTCG-3') (Blaxter, 1998). The reactions were performed in 25 μl of 1× PCR buffer (Amersham Biosciences, Freiburg, Germany) containing 2.5 mM $MgCl_2$, 0.16 mM each deoxynucleoside triphosphate, 0.5 μM each primer, 1 μl of lysate, and 1 unit of *Taq* DNA polymerase (Amersham Biosciences). After an initial denaturation step at 95°C for 2 min in a T gradient thermocyler (Biometra, Göttingen, Germany), the amplification was performed by 35 to 40 cycles of 95°C for 15 sec, 50°C for 15 sec, and 72°C for 2 min, followed by 72°C for 7 min. The PCR mix was diluted 20x and 1 μl was used for sequencing of approximately 500 bp of the 5'-terminal end using the primer SSU9R (5'-AGCTGGAATTACCGCGGCTG-3') and the Big Dye terminator protocol (Applied Biosystems, Darmstadt, Germany). Sequences were aligned manually using Seqpup 0.6f software for Macintosh (Gilbert, 1996).

Mating experiments

To confirm the species identification by the molecular sequence of a new strain, we performed mating experiments with the reference strain of the respective species (see below for definitions). Five virgin females were put on a plate with a small spot of *E. coli* OP50 together with five males of the reference strain of a certain species. On a second plate we picked the opposite sexes of the two strains to test for reciprocity. If there were no offspring after one week, the experiments were repeated two more times. If fertile offspring occurred, we considered the two strains to belong to the same species.

Isolate and strain definitions

We use the following definitions to distinguish "isolates" and "strains". An isolate is an isogenic female line derived from a beetle sample and subjected to molecular and experimental analysis. After species identification, we established one distinct isolate per species and location as a strain. The strains permanently cultured in the lab have strain numbers and are also kept as frozen stocks. For each new species designated by molecular sequence analysis and mating experiments, one strain was defined as a reference strain. The number of strains can be higher than the number of beetles sampled, *i.e*., multiple strains from a given beetle.

Olfaction assay

Population chemotaxis assays were performed on 8.5 cm wide NGM agar plates, as previously described for *Pristionchus* species (Hong and Sommer, 2006b). Briefly, mixed stage nematodes containing mostly adults were washed three times in M9 buffer and then loaded onto the agar plates prepared with two point sources of odors (attractant and solvent control) and sodium azide on opposite sides of each plate. The chemotaxis index is defined as [the number of worms at the attractant site – worms at control site]/total number of worms scored. At least two separate experiments were conducted for each strain and pheromone concentration to control for worm-batch variation, and each experiment contained 3–5 replicates. On average, each replicate represented the outcome for 10–50 worms. Only the highest chemotaxis index was recorded, which peaked between 9–15 hours at 23°C. *Pristionchus pacificus* PS1843 is our standard strain for chemotaxis assays. *(Z)*-7-tetradecen-2-one (97% pure) and *(E)*-2-nonen-1-ol were purchased from Bedoukian Research, Inc. (Danbury, CT, USA). *(E)*-2-nonenal was obtained from Sigma-Aldrich (MO, USA).

RESULTS AND DISCUSSION

Pristionchus pacificus was the most common *Pristionchus* species found on scarab beetles in Japan

To determine whether *P. pacificus* and other species of the genus *Pristionchus* are associated with scarab beetles in Japan, we analyzed beetles from more than 60 sampling sites on the main island of Honshu (Fig. 1). The scarab beetle fauna of Japan differs from those in Europe and North America. In total, more than 450 species of scarab beetles are known from Japan (Kawai *et al.*, 2005). We investigated 1,650 beetles of more than 10 genera and obtained 40 individual beetles infested with *Pristionchus* (Fig. 1, Table 1), which represents an infestation rate of 2.4%. This number is substantially lower than infestation rates of scarab beetles in western Europe and the eastern United States, where 10.2% and 23.4% of the beetles were infested, respectively (Herrmann *et al.*, 2006a, b). Interestingly, another nematode that was commonly found on Japanese Melolonthine was a member of the genus *Diplogasteroides* related to *Pristionchus*. This observation is similar to findings in Europe, where *D. magnus* and *D. nasuensis* can be found on scarabs of the genus *Melolontha* (Manegold and Kiontke, 2001; Herrmann *et al.*, 2006a).

From the 40 individual beetles infested with *Pristionchus*, we generated a total of 43 distinct isolates. Of these 43 *Pristionchus* isolates only three represent gonochoristic strains, whereas the majority of the 40 isolates is hermaphroditic. *SSU* sequence analysis and mating experiments of the 43 *Pristionchus* isolates revealed that they fall into three distinct species. All hermaphroditic strains had *SSU* sequ-

Fig. 1. Map of Japan showing the distribution of sampled *Pristionchus* species. Locations; 1: Hiroshima, Kawajiri-cho, Norosan, 2: Hiroshima, Kumano-cho Hagiwara, 3: Hiroshima, Mihara-shi, Kui, 4: Hyogo, Himeji-shi, Shiratori, 5: Hyogo, Miki-shi, Kasa, 6: Hyogo, Miki-shi, Yoshikawacho, Kamiarakawa, 7: Hyogo, Sasayama-shi, Jyonan, 8: Hyogo, Amagasaki-shi, Mokawa, 9: Osaka, Takatsuki-shi, Karasaki, 10: Kyoto, Hachiman-shi, Nishiyama, 11: Kyoto, Jyoyo-shi, 12: Shiga, Otsu-shi, Hieidaira, 13: Shiga, Otsu-shi, Hieidaira, 14: Hukui, Obama-shi, Anou, 15: Hukui, Minami-echizencho, Nanjyo, 16: Gifu, Gunjyou-shi, Takawashi-cho, 17: Toyama, Oyabe-shi, 18: Gunma, Shibukawa-shi, Ishihara, 19: Osaka, Takatsuki-shi, Tsutsumicho, 20: Osaka, Takatsuki-shi, Tsutsumicho, 21: Nara, Kasuganocho, Kasugataisha.

Table 1. Frequencies of *Pristionchus* isolates on beetles.

Beetle species	No. of individuals	*Pristionchus species*			
		P. pacificus	*P*. sp 14	*P*. sp 15	*P*. sp 11
Exomala orientalis	842	37(4.4%)	1(0.1%)	–	–
Phleotrupes auratus	47	–	–	2(4.3%)	–
Holotrichia parallela	170	2(1.2%)	–	–	–

Numbers in parentheses indicate percent of individual beetles infested.

ences that were identical to *P. pacificus*. Mating experiments confirmed that all new isolates from Japan belong to this species. Of the 40 *P. pacificus* isolates, 37 were obtained from the oriental beetle *Exomala orientalis* (Table 1). Taken together, *P. pacificus* represents the most common *Pristionchus* species of our scarab beetle survey and the oriental beetle *E. orientalis* represents an important insect host species in Japan.

In addition to the beetle samples, we also took soil samples and looked for the occurrence of *Pristionchus* nematodes. We obtained one additional gonochoristic isolate from soil samples, which was analyzed together with the beetle-derived gonochoristic isolates (see below).

E. orientalis in the United States of America is also a host to *P. pacificus*

The oriental beetle was introduced to the East Coast of the United States around 1920 (Friend, 1929; Hallock, 1933) and is a known pest in particular in the northeastern part of the US (Vittum *et al.*, 1999). To determine if *P. pacificus* is also associated with *E. orientalis* in the US, we collected oriental beetles at Cold Spring Harbor Laboratory (Long Island, New York). From 20 oriental beetles, four were infested with *P. pacificus* (data not shown). Also, one of the three *P. pacificus* strains obtained in a field study in the United States in 2005 has been isolated from *E. orientalis* (Herrmann *et al.*, 2006b). Therefore, we conclude that *E. orientalis* represents an important host species for *P. pacificus* both in Japan and in the US.

The gonochoristic isolates belong to three novel species

In addition to the 40 isolates of *P. pacificus,* we obtained four isolates that belong to gonochoristic species, three from scarab beetles and one from a soil sample (Table 1). *SSU* sequence analysis and mating experiments of these isolates revealed that they fall into three species, all of which were not observed in our surveys in Europe and the US (Herrmann *et al.*, 2006a, b). Interestingly, the strain obtained from the soil sample is identical in sequence to a strain provided by Dr. Walter Sudhaus (FU Berlin, Germany) from another soil sample in Japan. Mating experiments confirmed that both soil sample-derived strains belong to the same species.

In their catalogue of the Diplogastridae, Sudhaus and Fürst von Lieven (2003) reported 27 valid species descriptions in the genus *Pristionchus*. However, none of these species was reported from Japan. The three species discovered in our survey in Japan have distinct *SSU* sequences, do not mate succesfully with any of the established European and American species, but are morphologically similar to other *Pristionchus* species that we culture in the laboratory. We consider them to be novel species based on the molecular *SSU* sequence data and the results of mating experiments (Fig. 2, Tables 1 and 2). We designate them as *Pristionchus* sp. 11*, Pristionchus* sp. 14 and *Pristionchus* sp. 15. We will provide more detailed morphological descriptions elsewhere. The *SSU* sequence data show complete identity of the 471-bp fragment analyzed within all members of a given species (Fig. 2). The *SSU* of *P.* sp. 11 differs from that of *P. pacificus* by three diagnostic substitutions (54G, 213C, 243C). *Pristionchus* sp. 14 is characterized by the combination of the unique substitution 78C, substitution 128T shared with *P.* sp. 6, and the substitutions 211T and 226A shared with *P. pacificus* and *P.* sp. 11. The diagnostic substitutions for *P.* sp. 15 are 47G, 67A, 82T, 118T, 120T, 122G, 123T, 148T, 165A, 198C, 201A, 233T, 238C, 268T, 276A, 282A, 419T, 459A, and 469G. The reference strains of all novel species are available as living cultures and frozen stocks in our laboratory and can be provided to other researchers upon request.

The *E. orientalis* sex pheromone *(Z)*-7-tetradecen-2-one is specifically attractive to *P. pacificus*

Previous studies indicated that four closely related *Pristionchus* species associated with beetles have distinct chemotaxis profiles toward plant and insect compounds (Hong and Sommer, 2006b). To test if compounds from *E. orientalis* could be used by *P. pacificus* nematodes to locate its natural host, we performed chemotaxis assays using *(Z)*-7-tetradecen-2-one, the known *E. orientalis* sex pheromone, as an attractant (Leal *et al*., 1994; Zhang *et al*., 1994) (Fig. 3). We found that *(Z)*-7-tetradecen-2-one is attractive to only *P. pacificus* at 10- to100-fold dilutions. Closely related *P. maupasi, P. entomophagus* and *P. uniformis* were either not affected or were repulsed by this pheromone (Fig. 3). Furthermore, the model nematode *C. elegans*, which has not been found to be associated with beetles, was also repulsed by *(Z)*-7-tetradecen-2-one (Fig. 3). Finally, we tested two other known pheromones from closely related *Anomala* species, *(E)*-2-nonenal (*A. schoenfeldti*) and *(E)*-2-nonen-1-ol (*A. vitis*), but found no attraction of *P. pacificus* (Leal *et al*., 1992; Hasegawa *et al*., 1993; Toth *et al.,* 1994). Therefore, consistent with our field findings, *P. pacificus* is specifically attracted to the sex pheromone of its preferred natural host, and may possibly be able to distinguish closely related *Exomala*/*Anomala* species using beetle species-specific pheromones.

We identified *P. pacificus* as the most frequent *Pristionchus* nematode in this survey and consider *E. orientalis* as an important host species of *P. pacificus*. Previous sampling throughout the world revealed that *P. pacificus* is a widespread but rare species (Srinivasan *et al.*, 2001; Zauner *et al.*, 2007). *Pristionchus pacificus* was found in lower frequencies than other *Pristionchus* species in pre-

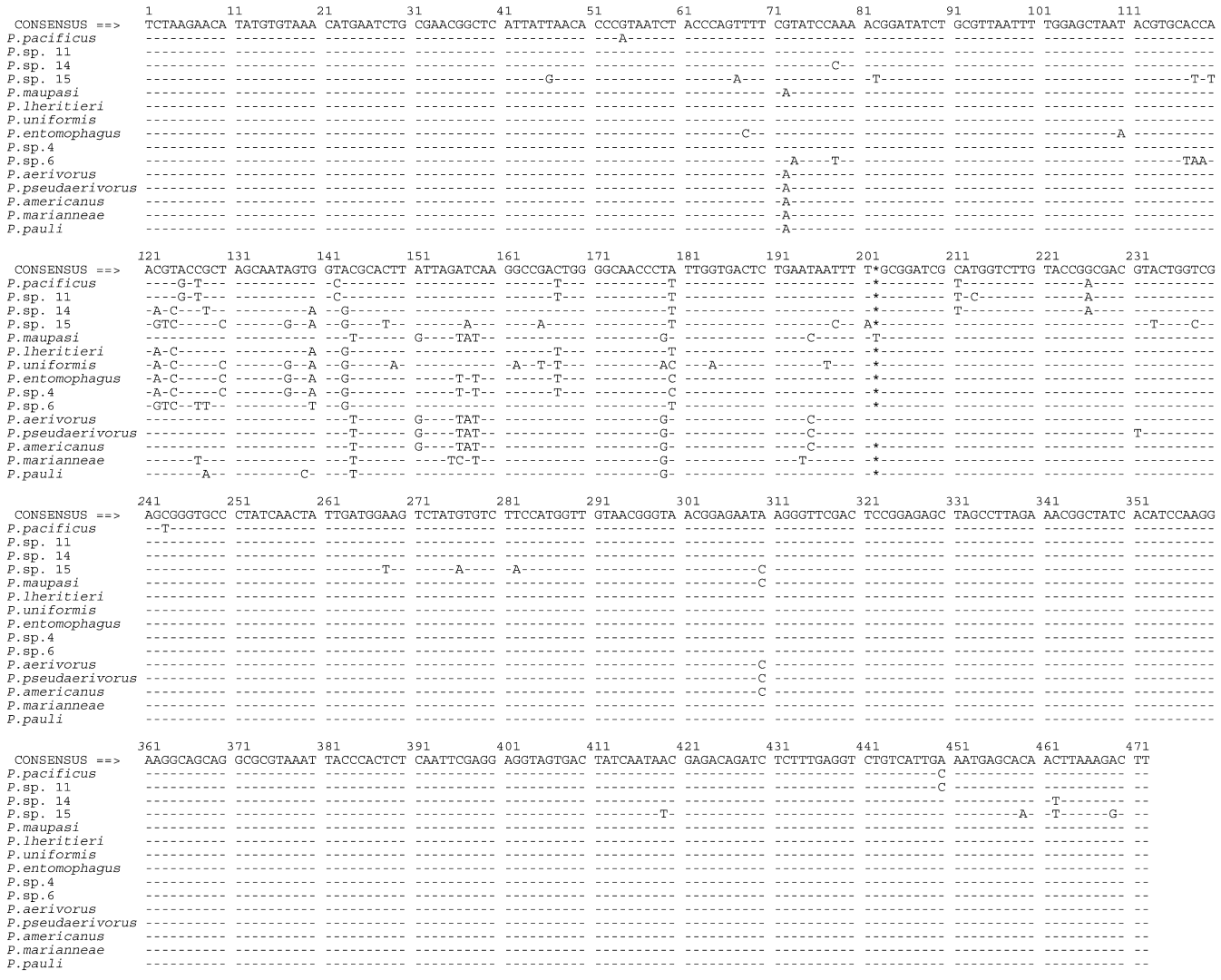

Fig. 2. *SSU* sequence comparison of *Pristionchus* species. The alignment shows the part of the *SSU* sequences used for species identification. The sequences of the species found in Japan are compared to those from Western Europe and the Eastern United States (Herrmann *et al.*, 2006a, b). Dashes (–) indicate identity to the majority consensus sequence at the top; asterisks (*) indicate alignment gaps. Numbering refers to the *SSU* segment obtained in this study. The sequences have been submitted to the GenBank database and are available under accession codes DQ270018–DQ270025, DQ419900–DQ419904, and EF216858–EF216860.

Table 2. Description of three novel *Pristionchus* species

Species designation	Reproductive mode	Strain number	Type host and locality
Pristionchus sp. 11	gonochoristic	RS5228	soil sample taken around Mount Hiei (35°2'12.3" N; 135°50'11.0'E)
Pristionchus sp. 14	gonochoristic	RS5230	on *Exomala orientalis* (Coleoptera: Scarabaeidae) at the Mokawa river in Amagasaki-shi
Pristionchus sp. 15	gonochoristic	RS5229	on *Phleotrupes auratus* (Coleoptera: Scarabaeidae) in a forest near Kutsuki, Takashima-shi, Shiga Prefecture (35°22'9.5" N;135°54'15.5'E)

vious samplings, either in soil or in scarab beetle surveys in Europe, the US and South Africa. By contrast, our field studies in Japan yielded 40 new *P. pacificus* isolates and represent 66% of all available *P. pacificus* strains. Also, for the first time, our collection came from several independent isolates from the same type locality and/or beetle. Therefore, this collection will be very helpful for future studies of the population structure of *P. pacificus*.

The second major result of this study is the identification of a beetle host for *P. pacificus*. The oriental beetle *E. orientalis* was the origin of 93% of all Japanese *P. pacificus* isolates described here. These findings from Japan are consistent with the observation that one of three *P. pacificus* strains obtained from similar samplings trips in the US in 2005 was also from *E. orientalis* (Herrmann *et al.*, 2006b). Furthermore, a small survey at Cold Spring Harbor Labora-

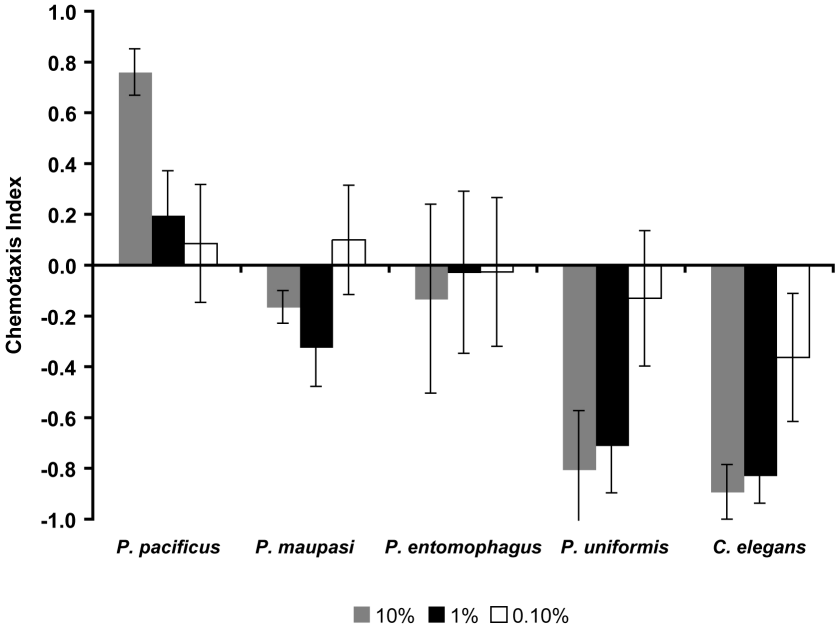

Fig. 3. Chemoattraction assay of closely related *Pristionchus* species and the distantly related model nematode *C. elegans* using the *Exomala orientalis* female sex pheromone (*Z*)7-tetradecen-2-one (Leal *et al*., 1994; Zhang *et al*., 1994). (*Z*)-7-tetradecen-2-one is highly attractive only to *Pristionchus pacificus*, whereas it is highly repulsive to *P. uniformis* and *C. elegans*, and neutral for *P. maupasi* and *P. entomophagus*. Error bars denote 95% confidence intervals. *Pristionchus pacificus* attraction at 10% and 1% dilutions of (*Z*)-7-tetradecen-2-one is significantly different to all other species except for *P. uniformis* at 1% attractant concentration (Student's T-test).

tory on Long Island (New York) identified four additional *P. pacificus* strains among 20 oriental beetle specimens sampled. However, it should be noted that the very high number of isolates from Japan might, in part, be the result of a sampling bias: after the first identifications of *P. pacificus* on *E. orientalis*, the use of sex pheromone traps significantly increased the numbers of *E. orientalis* beetles in this study. The use of pheromone traps also results in a strong sex bias, as only male beetles can be collected. However, in previous surveys in Europe (*Melolontha* and *Geotrupes*) (Herrmann *et al.*, 2006a) we never observed any sex bias in the infestation rate. Given this reservation, our findings can identify *E. orientalis* as a major host of *P. pacificus*. The observed infestation rates, however, cannot be directly compared to infestation rates observed in our previous samplings because of different climate and soil ecology in the sampled areas. Nonetheless, we feel that the use of pheromone traps also provides a unique advantage for future studies because it provides access to beetle material around the world.

The identification of a beetle host for *P. pacificus* encouraged us to investigate for possible host cues involved in *P. pacificus* attraction toward *E. orientalis*. We found that only *P. pacificus* is attracted to the *E. orientalis* sex pheromone *(Z)*-7-tetradecen-2-one, whereas its close relatives were not attracted. Although the 10% concentration needed for robust *P. pacificus* attraction is high compared to the 1–10% effective range for other *P. pacificus* attractants with similar molecular masses, we speculate that unknown minor beetle attractants may synergize with *(Z)*-7-tetradecen-2-one to become a more powerful bouquet for *P. pacificus* in nature. Alternatively, conventional population chemotaxis assays may not be sensitive enough to determine the necessary pheromone levels for successful *P. pacificus* infestations, since the developmental stage for locating the host and the ability to colonize as single hermaphrodites have not yet been investigated. We hope to address these open questions in more controlled settings in the future. Nevertheless, *P. pacificus'* unique attraction towards the sex pheromone of its natural host underscores an emerging theme for nematode-beetle interactions showing that insect pheromones play a crucial role in host selectivity (Hong and Sommer, 2006b).

Exomala orientalis has a very broad distribution in the Japanese region and it has been suggested that the species was introduced to Hawaii before 1908 and to New Haven, Connecticut in the 1920s (Vittum *et al.*, 1999). It has not been isolated in Europe, but several closely related species of the genus *Anomala* are known from various locations around the world. For example, *A. solida* is a vineyard and orchard pest in southeastern Europe (Toth *et al.*, 1994). This observation is of particular importance, as one of the two strains of *P. pacificus* from Europe (RS5171) is from a vineyard in Montenegro. Future studies will investigate if there is any association between other *Anomala* species and *P. pacificus* and if the molecularly divergent *P. pacificus* laboratory strain PS312 from California (which is untypical and does not correlate to most other American isolates of *P. pacificus*) might represent a vine-related introduction that is independent of the other American strains.

The identification of a beetle host for *P. pacificus* is most useful for studies in evolutionary developmental biology and allows a progression of developmental studies from laboratory conditions towards the natural environment.

Evolutionary developmental biology ultimately requires the incorporation of ecological factors (Zauner *et al.*, 2007). This study presents the basis for the incorporation of ecological and behavioral aspects into nematode evolutionary developmental biology. Following the identification of *E. orientalis* as a host of *P. pacificus*, we will set out to establish laboratory cultures of *E. orientalis* with the hope to better understand the nematode life cycle in this more complex ecological context. Ultimately, such studies might provide evidence for how developmental processes that are usually studied under extremely artifical laboratory conditions are influenced by environmental factors. They might indicate how selection can act on these processes to result in well-adapted developmental patterns. Given the surprising amount of developmental flexibility seen in homologous developmental processes, such as vulva formation in *P. pacificus* and *C. elegans*, the incorporation of environmental factors might be very useful.

ACKNOWLEDGMENTS

We thank Shigeru Kuratani and his team as well as Asako Sugimoto and her team (all at RIKEN Institute in Kobe) for their kind support and hospitality. We thank Masakazu Kawahara, Masahiro Kon, and all other beetle friends and colleagues for their help in collecting. We thank P. Robbins for the sex pheromone traps, H. Haussmann for maintaining of *Pristionchus* strains, and M. Riebesell for critically reading this manuscript.

REFERENCES

Blaxter ML, de Ley P, Garey JR, Liu LX, Scheldeman P, Vierstraete A, Vanfleteren JR, Mackey LY, Dorris M, Frisse LM, Vida JT, Thomas K (1998) A molecular evolutionary framework for the phylum Nematoda. Nature 392: 71–75

Dieterich C, Roeseler, W, Sobetzko P, Sommer RJ (2007) Pristionchus.org: a genome-centric database of the nematode satellite species *Pristionchus pacificus*. Nucleic Acids Res 35: 498–502. http://dx.doi.org/10.1093/nar/gkl804

Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soil nematode identification. Mol Ecol 11: 839–850

Friend RB (1929) The Asiatic beetle in Connecticut. Conn Agr Exp Stn Bull 304: 585–664

Gilbert DG (1996) SeqPup version 0.6f: a biosequence editor and analysis application. http://iubio.bio.indiana.edu/soft/molbio

Hallock HC (1933) Present status of two Asiatic beetles (*Anomala orientalis* and *Autoserica castanea*) in the United States. J Econ Entomol 26: 80–85

Hasegawa M, Leal WS, Sawada M (1993) Field evaluation of *Anomala schoenfeldti* Ohaus (Coleoptera, Scarabaeidae) synthetic sex pheromone. J Chem Ecol 19: 1453–1459

Herrmann M, Mayer WE, Sommer RJ (2006a) Nematodes of the genus *Pristionchus* are closely associated with scarab beetles and the Colorado potato beetle in Western Europe. Zoology 109: 96–108

Herrmann M, Mayer WE, Sommer RJ (2006b) Sex, bugs and Haldane's rule: the nematode genus *Pristionchus* in the United States. http://www.frontiersinzoology.com/content/3/1/14

Hong RL, Sommer RJ (2006a) *Pristionchus pacificus*: a well-rounded nematode. Bioessays 28: 651–659

Hong RL, Sommer RJ (2006b) Chemoattraction in *Pristionchus* nematodes and implications for insect recognition. Curr Biol 16: 2359–2365

Kawai S, Hori S, Kawahara M, Inagaki M (2005) Atlas of Japanese Scarabaeoidea. Vol. 1. Coprophagous Group. Roppon-Ashi

Leal WS, Hasegawa M, Sawada M (1992) Identification of *Anomala schoenfeldti* sex pheromone by high-resolution GC-behavior bioassay. Naturwissenschaften 79: 518–519

Leal WS, Hasegawa M, Sawada M, Ono M (1994) Sex pheromone of oriental beetle, *Exomala orientalis*: identification and field evaluation. J Chem Ecol 20: 1705–1718

Manegold A, Kiontke K (2001) The association of two *Diplogasteroides* species (Secernentea: Diplogastrina) and cockchafers (*Melolontha* spp., Scarabaeidae). Nematology 3: 603–606

Pires-daSilva A, Sommer RJ (2004) Conservation of the global sex determination gene tra-1 in distantly related nematodes. Genes Dev 18: 1198–1208

Rudel D, Riebesell M, Sommer RJ (2005) Gonadogenesis in *Pristionchus pacificus* and organ evolution: development, adult morphology and cell/cell interactions in the hermaphrodite gonad. Dev Biol 277: 200–221

Sommer RJ, Carta LK, Kim SY, Sternberg PW (1996) Morphological, genetic and molecular description of *Pristionchus pacificus* sp. n. (Nematoda, Diplogastridae). Fund Appl Nematol 19: 511–521

Srinivasan J, Pires-daSilva A, Guitierrez A, Zheng M, Jungblut B, Witte H, Schlak I, Sommer RJ (2001) Microevolutionary analysis of the nematode genus *Pristionchus* suggests a recent evolution of redundant developmental mechanisms during vulva formation. Evol Dev 3: 229–240

Sudhaus W, Fürst von Lieven A (2003) A phylogenetic classification and catalogue of the Diplogastridae (Secernentea, Nematoda). J Nem Morph Syst 6: 43–90

Tóth M, Leal WS, Szarukán I, Lesznyák M, Szöcs G (1994) 2-(E)-Nonen-1-ol: male attractant for chafers *Anomala vitis* Fabr. and *A. dubia* Scop. (Coleoptera: Scarabaeidae). J Chem Ecol 20: 2481–2487

Vittum PJ, Villani MG, Tashiro H (1999) Turfgrass Insects of the United States and Canada. Cornell University Press, Ithaca, NY

Zauner H, Mayer WE, Herrmann M, Weller A, Erwig M, Sommer RJ (2007) Distinct patterns of genetic diversity in *Pristionchus pacificus* and *Caenorhabditis elegans*, two partially selfing nematodes with cosmopolitan distribution. Mol Ecol 16: 1267–1280

Zhang A, Facundo HT, Robbins PS, Linn Jr CE, Hanula JL, Villani MG, Roelofs WL (1994) Identification and synthesis of female sex pheromone of oriental beetle, *Anomala orientalis* (Coleoptera: Scarabaeidae). J Chem Ecol 20: 2415–2427

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

(Received January 26, 2007 / Accepted April 6, 2007)