



Haplotype diversity of the nematode *Pristionchus pacificus* on Réunion in the Indian Ocean suggests multiple independent invasions

MATTHIAS HERRMANN^{1†}, SIMONE KIENLE^{1†}, JACQUES ROCHAT²,
WERNER E. MAYER¹ and RALF J. SOMMER^{1*}

¹Max Planck Institute for Developmental Biology, Department of Evolutionary Biology, Spemannstr. 37, 72076 Tübingen, Germany

²Insectarium de la Réunion, Pépinière Communale, rue Simon Pernic, 97420 Le Port, Réunion, France

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Pristionchus pacificus has been established as a nematode model system in evolutionary developmental biology and evolutionary ecology. Field studies in North and South America, Asia, Africa and Europe indicated that nematodes of the genus *Pristionchus* live in association with scarab beetles. Here, we describe the first account of soil- and beetle-associated nematodes on an island setting by investigating the island of Réunion in the Indian Ocean. Réunion has high numbers of endemic insects and is one among several attractive islands for biodiversity studies. Being of volcanic origin, Réunion is 2–3 million years old, making it the youngest of the Mascareigne islands. We show that beetle- and soil-derived nematodes on Réunion are nearly exclusively hermaphroditic, suggesting that selfing is favoured over gonochorism (outcrossing) during island colonization. Among members of four nematode genera observed on Réunion, *Pristionchus pacificus* was the most prevalent species. A total of 76 isolates, in association with five different scarab beetles, has been obtained for this cosmopolitan nematode. A detailed mitochondrial haplotype analysis indicates that the Réunion isolates of *P. pacificus* cover all four worldwide clades of the species. This extraordinary haplotype diversity suggests multiple independent invasions, most likely in association with different scarab beetles. Together, we establish Réunion as a case study for nematode island biogeography, in which the analysis of nematode population genetics and population dynamics can provide insight into evolutionary and ecological processes. © 2010 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2010, **100**, 170–179.

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INTRODUCTION

There is probably no portion of the globe that contains within itself so many and such varied features of interest connected with geographical distribution, or which so well illustrates the mode of solving the problems it presents, as the comparatively small insular region which comprises the great island of Madagascar and the smaller islands and island groups which immediately surround it. (Alfred Russel Wallace, 1880 in: *Island Life*)

*Corresponding author.

E-mail: ralf.sommer@tuebingen.mpg.de

†These authors contributed equally to this work.

The nematode *Pristionchus pacificus* Sommer, Carta, Kim & Sternberg, 1996 has been established as a model system in evolutionary developmental biology (evo-devo), evolutionary ecology and for comparison with *Caenorhabditis elegans* Maupas, 1900 (Sommer, 2009). *Pristionchus pacificus* and other species of the genus *Pristionchus* were shown to have a necromenic association with scarab beetles, i.e. nematodes invade the insect in the arrested dauer stage and wait for the insect's death to reassume development by feeding on the growing microbes (Herrmann, Mayer & Sommer, 2006a). Systematic field studies in North and South America, Asia, Africa

and Europe resulted in the identification of 24 *Pristionchus* species (Herrmann *et al.*, 2006a, 2007; Herrmann, Mayer & Sommer, 2006b). *Pristionchus* species are either hermaphroditic or gonochoristic and hermaphroditism has evolved several times independently (Mayer, Herrmann & Sommer, 2007). *Pristionchus pacificus*, the species with well-established forward and reverse genetics, genomic and transgenic tools and an available genome sequence, is the most widely distributed species of the genus with isolates from four continents (Zauner *et al.*, 2007; Dieterich *et al.*, 2008). However, previous samplings did not consider the distribution of this nematode in the context of island biogeography.

While many biogeographic studies have focused on insects, vertebrates and plants, little is known about small terrestrial animals. This includes the nematodes, one of the most abundant and largest animal phylum that is basically known from all ecosystems on earth (Blaxter, 1998). Nematodes are an excellent system to study animal distribution, dispersal and colonization, because many species cannot only be studied in the wild, but can also be cultured in the laboratory for further analyses. Also, nematodes can be cryo-preserved for future investigations in liquid nitrogen so that population genetics and population dynamics of island systems can be monitored and the dynamics of natural variation investigated. The small size of nematode genomes and the availability of several completed and ongoing genome-sequencing projects provide a unique molecular perspective of nematodes in the era of next generation sequencing.

In the Indian Ocean, the small island of Réunion, with its high number of endemic beetles and other insects, is one among several attractive islands for biodiversity studies. Réunion is part of the Mascareignes, an island group east of Madagascar, comprising Réunion (2512 km²), Mauritius (1865 km²) and Rodrigues (104 km²) (Thebaud *et al.*, 2009). Madagascar and the adjacent Indian Ocean islands have been classified as one of the major biodiversity hotspots on earth (Myers *et al.*, 2000). Mauritius is 8–10 million years (Myr) old and is well known for the flightless Dodo, an enigmatic bird that is one of the most famous examples of extinction in animals by human beings (Cheke & Hume, 2008). Réunion, like the other two islands, is of volcanic origin and 2–3 Myr old, thus making it the youngest of the Mascareigne islands. Several beetles and other insects were introduced to Réunion recently (Gomy, 2000) and the time and ways of introduction are known in many cases (Vercambre *et al.*, 1991, 2007; Ahrens, 2003). Altogether, the young age, the endemic and invaded insects of known origin, the high diversity of ecozones (Martiré & Rochat, 2008)

plus the excellent accessibility, make Réunion a perfect choice for investigating nematode island biogeography.

Here, we provide the first account of free-living nematodes of any island setting and we establish Réunion as a case study for nematode island biogeography. We show that beetle- and soil-derived nematodes on Réunion represent a unique platform for studying population genetics and population dynamics of nematodes and their influence on evolutionary and ecological patterns.

MATERIAL AND METHODS

SAMPLE COLLECTION

Beetles were collected either with blacklights or using sweeping nets. They were then brought to the laboratory and processed as described previously (Herrmann *et al.*, 2006a). Briefly, beetles were killed and put on Nematode Growth Medium (NGM) agar-filled Petri dishes, awaiting the emergence of nematodes. If at least one nematode of a species was found on a single beetle, this beetle was considered positive for the given nematode species. Full names of the positive beetles are as follows: *Alissonotum piceum besucheti* Endrödi, 1977; *Aphodius sublividus* Balthasar, 1941; *Adoretus* sp.; *Hoplia retusa* Klug, 1832; *Hybosorus illigeri* Reiche 1853; *Maladera affinis* (Blanchard, 1850); *Oryctes borbonicus* Dechambre, 1982; *Oxyctonia versicolor* (Fabricius, 1775). Infestation rate was calculated by dividing the number of nematode-positive by the number of nematode-negative beetle specimens. Gravid females of emerging nematodes were counted and transferred to new plates to start isogenic lines for molecular analysis. Up to 20 individual nematode lines per insect were analysed to test for genetic variability on single insect specimens. At least one nematode strain per beetle species and sampling location was kept in culture in the laboratory.

GENETIC ANALYSES

For species identification, DNA was prepared from single individual nematodes and the small subunit ribosomal RNA (SSU) sequence was determined as described elsewhere (Herrmann *et al.*, 2006a). To assess the phylogenetic relationships and the genetic variability between the *P. pacificus* isolates, DNA was prepared from single worms and a 760-bp-fragment encompassing the mitochondrial *ND6* and *ND4L* genes was amplified by PCR using the primers HZ11896 (5'-TCCAGGCAAATTTATTTACAA-3') and HZ11897 (5'-CCATTTTAAACCATCTTAAACCA-3'). The sequences have been deposited in GenBank and can be retrieved by their accession

codes GU199045–GU199188. As best-fit substitution model for the aligned sequences, the K81uf+G model with six substitution types (A-C = 1.0000, A-G = 15.3631, A-T = 0.2570, C-G = 0.2570, C-T = 15.3631, G-T = 1.0000) and empirical nucleotide frequencies were determined by the hierarchical likelihood ratio tests implemented in the Modeltest 3.7 software (Posada & Crandall, 1998). The gamma distribution shape parameter was set to 0.1428. A phylogenetic tree with these settings was reconstructed using the PAUP*4.0b10 software (Swofford, 2002) by heuristic search under the maximum likelihood criterion. The resulting tree was displayed using the FigTree 1.2.2 software (Rambaut, 2009). Bootstrap support values from 1000 replications were obtained by the neighbour-joining algorithm using the maximum likelihood distance settings. Nucleotide diversity, haplotype diversity, number of segregating sites and other population genetic values were assessed using the DnaSP v.5 software (Librado & Rozas, 2009).

RESULTS

BEETLES-ASSOCIATED NEMATODES ON RÉUNION

In February 2008 and January 2009, we sampled 15 locations on Réunion and collected a total of 375 beetles from seven different scarab beetle genera (Table 1). Carriers of nematodes were *Oryctes borbonicus* Dechambre, 1982, *Adoretus* sp., *Maladera affinis* (Blanchard, 1850), *Hoplia retusa* Klug, 1832, *Hoplochelus marginalis* Fairmaire, *Alissonotum piceum besucheti* Endrödi, 1977 and *Aphodius sublividus* Balthasar, 1941 (Table 1; Supporting Information Table S1). Infestation rates differed between beetle species ranging from 92% (*Oryctes borbonicus*) to 3% (*Aphodius* sp.). In addition, nematodes were found in soil samples taken at each beetle collection site as well as in samples from other locations across the island (Table 1 and Supporting Information Table S1). Based on morphological and molecular analyses, we identified seven nematode species, which belong to the genera *Oscheius*, *Rhabditis*, *Panagrolaimus* and *Pristionchus* (Table 1). The species of the three former genera have SSU ribosomal RNA sequences that are most similar but not identical to known members of these taxa, suggesting that they might represent molecularly uncharacterized species. Nematodes of the genus *Pristionchus* were by far the most prominent group in our samplings on Réunion. In total, we obtained 96 isolates of four species in this genus representing 91% of all nematode isolates obtained on the island (Table 1). When compared with previous samplings in Europe, North America and South America, the Réunion *Pristionchus* data

Table 1. Distribution of different nematode taxa on beetle species at several sampling sites on Réunion

	Trois Bassins 2008, 2009	Trois Bassins Garden 2009	Étang Salée 2008, 2009	Basse Vallée 2008, 2009	Saint-Benoît 2009	Grand Etang 2008, 2009	Saint-Denis 2008	Colorado 2009	La Saline 2009
<i>Oryctes</i>	24/92/0/12/8	–	–	–	–	–	–	–	–
<i>Maladera</i>	–	2/50/0/0/0	4/25/0/0/0	–	48/22/0/0/0	–	–	–	–
<i>Adoretus</i>	1/0/0/0/0	–	10/0/0/0/0	29/14/0/3/0	14/14/0/0/0	5/100/0/0/0	–	–	–
<i>Hoplia</i>	19/21/0/4/0	13/0/0/0/0	14/7/0/7/0	–	–	–	–	1/0/0/0/0	–
<i>Hoplochelus</i>	–	56/9/0/0/0	48/0/0/0/0	–	–	–	–	–	–
<i>Aphodius</i>	1/0/0/0/0	30/3/0/0/0	5/0/0/0/0	–	–	–	–	1/0/0/0/0	–
<i>Alissonotum</i>	–	–	–	–	3/0/0/0/0	–	5/20/0/0/0	–	–
<i>Hybosorus</i>	–	–	35/0/0/0/0	–	–	–	–	–	–
<i>Oxyctonia</i>	–	2/0/0/0/0	5/0/0/0/0	–	–	–	–	–	–
Soil	0/+/+/0	NA	0/0/0/+/+	0/0/0/0	0/0/0/0	0/0/0/0	NA	0/+/0/0	+/0/0/0

First digits indicating the number of beetles caught, then the number of *Pristionchus/Rhabditis/Oscheius/Panagrolaimus*, respectively (percentage of beetles infested).

Collection years are given with the locations.

Presence of nematodes in soil samples is indicated by +.

NA, not applicable.

indicate a similar infestation rate of beetles (Herrmann *et al.*, 2006a, b, 2007).

HERMAPHRODITIC NEMATODES ARE OVERREPRESENTED ON RÉUNION

Three modes of reproduction can be distinguished in nematodes: gonochorism (male/female), hermaphroditism and parthenogenesis, respectively. While gonochorism represents the most prevalent reproduction mode in nematodes, hermaphroditism and parthenogenesis have evolved repeatedly. For example, of the 24 previously known *Pristionchus* species that we have obtained in worldwide samplings, six are hermaphroditic and phylogenetic analysis indicates that all six evolved hermaphroditism independently from gonochoristic ancestors (Mayer *et al.*, 2007).

Of the seven nematode species represented by 106 isolates from Réunion, six are hermaphroditic, whereas *Panagrolaimus* sp. (RS5490) is parthenogenetic, a known mode of reproduction in this clade (Félix *et al.*, 2000; Nadler *et al.*, 2006). All *Pristionchus* isolates show a hermaphroditic mode of reproduction and *SSU* sequence analysis and mating experiments proved that they belong to four distinct species (Fig. 1). Three of the species found on Réunion are known, *P. pacificus*, *Pristionchus entomophagus* (Steiner, 1929) and *Pristionchus* sp. 10, respectively, while the fourth species is unknown and is represented by six strains. We consider these isolates to be a novel species based on the *SSU* sequence data and the result of mating experiments. We designate this species as *Pristionchus* sp. 25 and will provide a more detailed morphological and phylogenetic description elsewhere. Taken together, the 106 nematode isolates from Réunion represent seven

species, all of which represent hermaphroditic or parthenogenetic species.

P. PACIFICUS IS THE MOST PREVALENT BEETLE-ASSOCIATED NEMATODE ON RÉUNION

Pristionchus is the dominant genus of beetle-associated nematodes on Réunion and *P. pacificus* represents the most prevalent species. Specifically, *SSU* sequence analysis and mating experiments revealed that 76 of the 96 *Pristionchus* isolates (79%) obtained in 2008 and 2009 belong to *P. pacificus* (Fig. 2, Supporting Information Tables S2, S3). *Pristionchus pacificus* was found repeatedly on five different scarab beetles, *O. borbonicus*, *Adoretus* sp., *Maladera affinis*, *Hoplia retusa* and *Hoplochelus marginalis* (Fig. 2). While there was some variation between the 2008 and 2009 samplings, the highest infestation rate was consistently observed for *O. borbonicus* with 92% of the analysed beetle individuals. This represents the highest *P. pacificus* infestation rate ever observed on any beetle and is also reflected by unusually high numbers of nematode individuals per single beetle. These observations correlate with the host association of *P. pacificus* and the nematode load on beetles in other parts of the world. While *P. pacificus* was exclusively observed on the Oriental beetle *E. orientalis* in Japan and at the east coast of the USA (Herrmann *et al.*, 2007), it is associated with other beetles elsewhere. For example, samplings at the west coast of the USA identified *Cyclocephala pasadenae* (Casey, 1915) as one scarab beetle hosting *P. pacificus* and studies in Bolivia identified several dynastine scarab beetles as hosts. Together, these data suggest a higher plasticity of *P. pacificus* in its beetle association when compared with other *Pristionchus* species, which have more restricted beetle

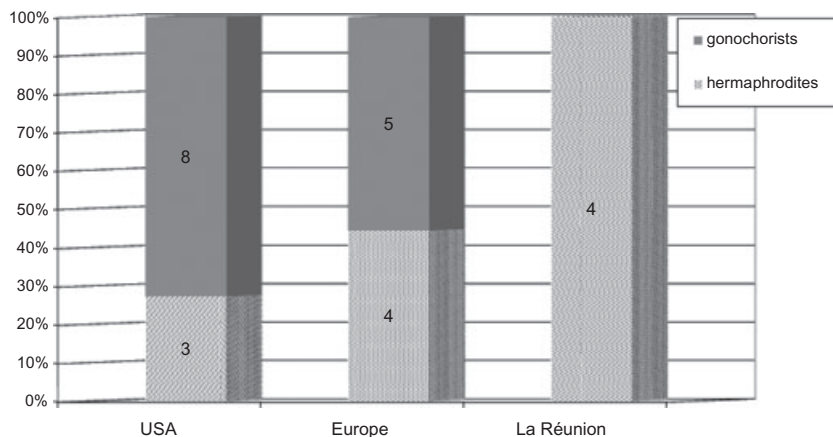


Figure 1. Frequencies of hermaphroditic and gonochoristic *Pristionchus* species in different parts of the world.

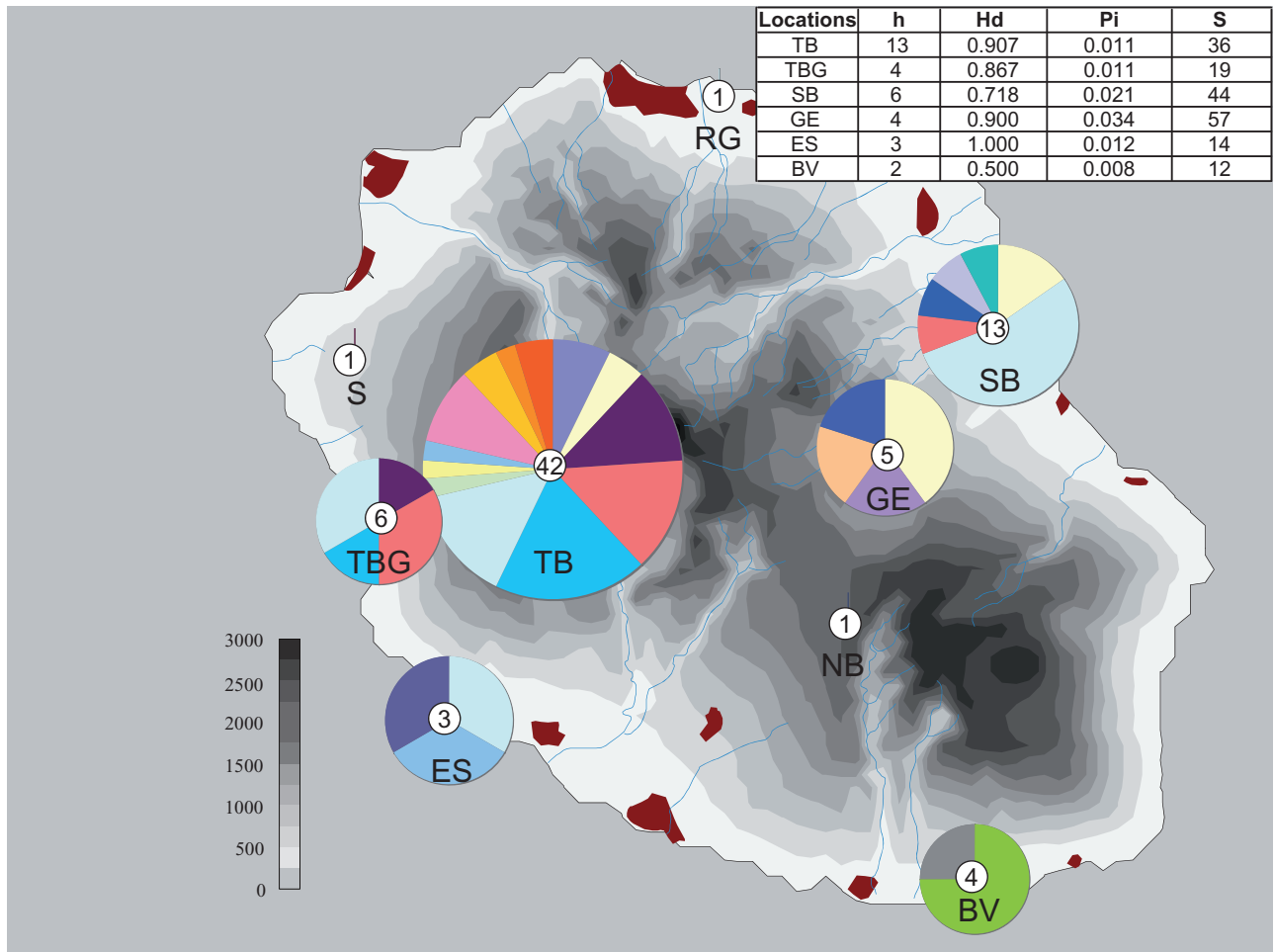


Figure 2. Map showing distribution and haplotype diversity of *Pristionchus pacificus* on Réunion. Diameter of the circles and number in the centre indicate the number of collected strains per location. Different colours indicate different haplotypes. Inserted box gives data on number of haplotypes (h), haplotype diversity (hd), nucleotide diversity (Pi) and number of segregating sites (S) per sampling location. Locations are Trois Bassins (TB), Trois Bassins Garden (TBG), Saint-Benoit (SB), Grand Étang (GE), Étang-Salé (ES), Basse Valée (BV), airport Roland Garros (RG), la Saline (S) and Nez de Boeuf (NB).

associations. At the same time, *P. pacificus* shows the widest distribution of any known *Pristionchus* species in the world. It remains unknown, however, if the observed *P. pacificus* plasticity in beetle association is the cause or the consequence of its cosmopolitan distribution.

P. PACIFICUS SHOWS A HAPLOTYPE DIVERSITY ON RÉUNION THAT REPRESENTS MUCH OF ITS KNOWN WORLDWIDE DIVERSITY

The *P. pacificus*-beetle association on Réunion described above might represent an ideal system to study biogeography, dispersal, but also population genetics of nematodes in the context of island biology. How often did *P. pacificus* invade the island? How

much genetic variation is observed among the isolates on Réunion? Recent studies in molluscs indicated that invasive species could show high levels of genetic variance, suggesting multiple introductions (Facon *et al.*, 2008). Our previous analysis of 18 strains of *P. pacificus* also revealed a high level of genetic variation (Zauner *et al.*, 2007).

To determine the genetic variance of the Réunion *P. pacificus* isolates, we analysed all available strains and compared them with all other isolates from around the world. We used the mitochondrial markers *ND6* and *ND4L* that were previously shown to be highly polymorphic between isolates (Zauner *et al.*, 2007). A total of 760 bp were compared among the 162 *P. pacificus* strains (Figs 2, 3; Supporting Information Table S3). The 76 isolates from Réunion represent

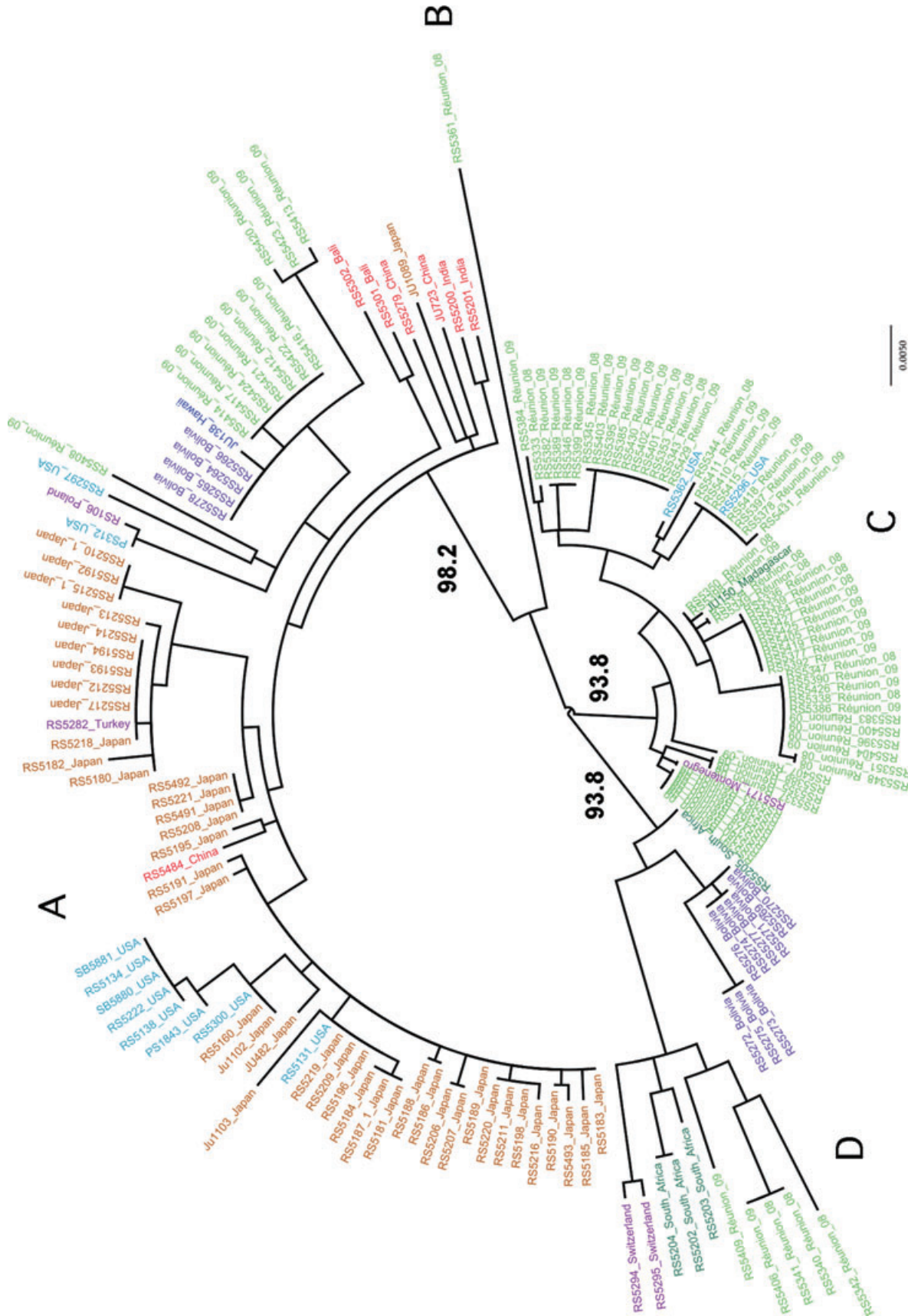


Figure 3. Phylogenetic relationships of *Pristionchus pacificus* strains. An unrooted maximum likelihood tree was reconstructed from aligned mitochondrial *ND6-ND4L* sequences. The tree topology was determined with the help of the PAUP*4.0b10 software by heuristic search under the maximum likelihood criterion using parameters for the K81uf+G substitution model as selected by the Modeltest 3.7 software. The graphic shows a radial representation of the tree. Taxons are labelled by strain number, geographic origin and sampling year. Taxon colours indicate the geographic origins of the *Pristionchus* strains. Neighbour-joining bootstrap support values for the major clades A, C and D are shown. The support value for clade B could not be obtained as a result of long-branch attraction effects.

47% of all available *P. pacificus* isolates. Their *ND6-ND4L* segment consists of 25 haplotypes, i.e. about 35% of the 72 haplotypes found in our global collection of 162 isolates. The haplotypes on Réunion alone differ by 1–44 nucleotide substitutions, which is as high as the genetic distance of up to 45 substitutions found in the worldwide dataset. The total number of mutations in the latter dataset is 174, the number of segregating sites (S) is 152, of which 102 are found in the Réunion isolates. The nucleotide diversity π in the Réunion isolates is 0.023, as compared with π of 0.029 in the remaining strains worldwide. The only other collection site with high abundance of *P. pacificus* is the Honshu island of Japan, which is by far larger than Réunion island (c. 228 000 km² vs. 2512 km²). Collection of *P. pacificus* on Honshu island showed 23 haplotypes among 43 isolates and a π of only 0.010 (Herrmann *et al.*, 2007). A substantial number of worldwide haplotypes and diversity could thus be found on the island of Réunion.

To estimate the haplotype phylogeny and to cluster haplotypes we used the mitochondrial *ND6-ND4L* segment for phylogenetic reconstruction. The optimal substitution parameters were set to the K81uf+G model as determined by the Modeltest 3.7 software (Posada & Crandall, 1998). A maximum likelihood tree was reconstructed by the PAUP*4.0b10 software (Swofford, 2002). Four distinct clades are apparent based on their separation by relatively large branch lengths and high support values (93.8–98.2) (Fig. 3). Clade A encompasses most of the strains originating from Asia, USA, Bolivia and a subset from Réunion. Clade B consists of a single isolate from soil of Réunion. Clade C contains mainly haplotypes from Réunion, including all isolates obtained from *O. borbonicus*, and four isolates from USA, Montenegro, and Madagascar. Clade D encompasses haplotypes from South Africa, Bolivia, Réunion and two from Switzerland. Whereas a few individual isolates from Europe, Japan and the USA do not cluster within the clade of their geographic origin, because of putative introduction to new locations, the isolates from Réunion are the only ones that are present in all four clades. The separation of the clades by rather long branches indicates an ancient split and points to multiple colonization events of this young island by *P. pacificus*.

DISCUSSION

We have provided the first account of free-living nematodes on Réunion and have established this island setting as a case study for nematode island biogeography. We show that beetle- and soil-derived nematodes on Réunion are nearly exclusively hermaphroditic and provide a detailed haplotype analy-

sis of the 76 *P. pacificus* isolates from Réunion in comparison with isolates from around the world.

ADVANTAGE OF HERMAPHRODITES DURING ISLAND COLONIZATION

The absence of gonochoristic species, the otherwise prevalent mode of nematode reproduction in the beetle-associated nematode fauna, argues for a strong advantage of selfing with respect to the invasion of the island (Fig. 1). Nematodes are most likely to have occupied Réunion together with invading beetles or other insects. One assumption would be that beetle-associated nematodes with a hermaphroditic mode of reproduction are more likely to be successful in island invasion, because a load of only one individual nematode on an insect can already establish colonization. In contrast, gonochoristic species require a higher nematode load per insect. Although our observations are currently based on a small number of beetle-associated nematode species on Réunion, these findings are consistent with the young age of the island. Further tests of this hypothesis will require the analysis of other nematode–invertebrate associations and surrounding island systems.

P. PACIFICUS HAPLOTYPE DIVERSITY

Unlike many other *Pristionchus* species that show limited geographic distribution, *P. pacificus* isolates have been found worldwide in close association with their beetle hosts. At present 162 strains are established in the laboratory. Although some of the scattered occurrences might be traced back to dispersal by human impact, it is evident that *P. pacificus* is able to accommodate to a variety of geographic ranges and ecological conditions. Réunion provides an excellent opportunity to study the population genetics and population dynamics of nematodes at the limited geographical scale of a young island. Mitochondrial *ND6-ND4L* sequence data of *P. pacificus* isolates revealed an exceptional high genetic diversity. The 76 isolates from Réunion show a nucleotide diversity π of 0.023, which compares with a π of 0.029 in *P. pacificus* strains from around the world and a π of only 0.010 in *P. pacificus* from Japan. On Réunion 25 *ND6-ND4L* haplotypes can be distinguished from the total of 72 haplotypes found worldwide. A similar number of 23 haplotypes was found in Japan. The resulting haplotype diversities (Hd) are 0.936 for Réunion, 0.980 worldwide and 0.959 for Japan. Thus, the nucleotide diversity on Réunion and the genetic distance between the haplotypes is considerably higher than on the Japanese island of Honshu, although similar numbers of haplotypes have been identified in both locations. This is also reflected by the number of

segregating sites (S), which is 102 for Réunion, but only 49 for Japan. Given the relatively young age of Réunion of 2–3 Myr, this high molecular diversity and the presence of all four worldwide clades on Réunion can best be explained by multiple recent colonizations of the island with at least four or more genetically distinct *P. pacificus* nematodes and subsequent diversification as supported by the phylogenetic tree (Fig. 3 and Supporting Information Fig. S1). In addition, the observed genetic variability might also result from a higher mutation rate or the occurrence of outcrossing. Indeed, most of the *P. pacificus* isolates generate spontaneous males under laboratory conditions (Click *et al.*, 2009). However, the exact contribution of mutation rate and outcrossing to the generation of the *P. pacificus* genetic variability on Réunion will require future studies involving additional molecular tools, such as, for example, microsatellite markers.

BEETLE ASSOCIATED SPECIES INVASIONS

The association of *P. pacificus* with five different scarab beetles on Réunion represents a paradigm for the analysis of species invasion and island evolution. While there is a certain amount of plasticity in the *P. pacificus*–beetle association, four main conclusions can be drawn from our observations. First, all *Oryctes*-associated *P. pacificus* haplotypes fall into clade C, the clade that predominantly consists of material from Réunion. At the same time, clade C contains haplotypes from all scarab beetles of Réunion that were found to harbour *P. pacificus*. Interestingly *O. borbonicus*, which is the beetle with the highest *P. pacificus* infestation rate and individual load, is endemic to Réunion. Therefore, one likely hypothesis would be that the founder of clade C invaded the island together with the *Oryctes* ancestor that ultimately gave rise to *O. borbonicus*. Subsequent diversification and the ability to associate with multiple scarab beetles resulted in the observed patterns. Because there was so far only one location on the island (Trois Bassins) where *O. borbonicus* could easily be obtained, future studies are necessary to rule out a micro-geographic influence. Similarly, the exact ancestor of *O. borbonicus* remains currently unknown because other related *Oryctes* species are known from Asia and from Africa.

Second, five Réunion haplotypes fall into clade A, which largely contains *P. pacificus* isolates from Asia (the haplotypes from the USA are thought to result from the invasion of *E. orientalis* into North America, see Herrmann *et al.*, 2006b). These five haplotypes were obtained from beetles of the genera *Maladera* and *Adoretus*. Interestingly, *Maladera affinis* invaded the island from Asia and it has been suggested that this occurred between the years 800 and 1800

(Ahrens, 2003). This could explain why so few Réunion haplotypes are found in clade A. However, some *M. affinis* associated haplotypes locate to clade C, suggesting a certain amount of plasticity in beetle association. Such plasticity is common in ecological interactions, in particular in nematode–insect interactions.

Third, at one of two locations *Hoplochelus marginalis* carried *P. pacificus* and *P. sp.* 25. This finding is of interest because *H. marginalis* invaded Réunion from Madagascar in the 1970s (Vercambre *et al.*, 1991). This beetle is a major pest of sugar cane and therefore a target of biocontrol programmes. Nevertheless, *H. marginalis* might represent a unique case to study host switching onto a newly invaded insect. Finally, Réunion haplotypes of clade D are all from *Adoretus*. Unfortunately, multiple species of *Adoretus* are known to exist on Réunion – endemics and introduced ones – and detailed morphological studies are necessary for species determination.

In summary, the analysis of the genetic variance of *P. pacificus* isolates from Réunion in comparison with the rest of the world identifies several parameters that influence the population structure of this nematode. First, the ancient split of *P. pacificus* haplotypes indicates multiple independent, most likely beetle-associated invasions. Second, more recent human impact influences the population structure of *P. pacificus* on Réunion in a way similar to that observed at a global scale. Finally, the single soil sample-derived *P. pacificus* population represents a unique clade in the current phylogenetic tree, indicating additional factors and potential associations, which require further investigations.

As with all studies on biogeography, our studies in 2008 and 2009 might have been influenced by a sampling bias and by micro-geographic and temporal peculiarities. We plan to overcome such limitations by long-term studies and yearly visits to Réunion, which at the same time would allow a detailed population genetic and population dynamic analysis. The fact that *P. pacificus* can be cultured in the laboratory for phenotypic analysis, can be cryo-preserved in liquid nitrogen and that it has well-developed genetic and genomic tools represents unique preconditions for a detailed analysis of the population genetics and population dynamics of an organism in the context of island biogeography.

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AUTHOR CONTRIBUTIONS

MH and RJS designed research; MH, SK, WEM and JR performed research; MH, SK and WEM analysed data; and MH, WEM and RJS wrote the paper. The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Phylogenetic neighbour-joining tree of *Pristionchus pacificus* strains from Réunion. A neighbour-joining tree was reconstructed from aligned mitochondrial *ND6-ND4L* sequences with the help of the PAUP*4.0b10 software using maximum likelihood distances corresponding to the K81uf+G substitution model. Taxons are labelled by strain number, geographic origin and sampling year. The four clades are depicted. The tree was rooted at midpoint. The number of haplotypes (h) and the number of segregating sites (S) in the clades is shown.

Table S1. Nematode–beetle association by sampling sites on Réunion.

Table S2. Sampling sites, origin and haplotypes of different *Pristionchus pacificus* isolates found on Réunion.

Table S3. Sampling sites, origins and collectors of 86 worldwide strains of *Pristionchus pacificus* excluding those from Réunion.

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