The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2014, **112**, 1–15. With 4 figures

Landscape and oceanic barriers shape dispersal and population structure in the island nematode *Pristionchus pacificus*

KATY MORGAN[1], ANGELA McGAUGHRAN[1], SEELAVARN GANESHAN[2], MATTHIAS HERRMANN[1] and RALF J. SOMMER[1]*

[1]*Department of Evolutionary Biology, Max Planck Institute for Developmental Biology, Spemannstr. 37, Tübingen, Germany*
[2]*Mauritius Sugarcane Industry Research Institute, Réduit, Mauritius*

Received 9 October 2013; revised 12 December 2013; accepted for publication 12 December 2013

Despite the biological importance and diversity of nematodes, little is known of the factors influencing their dispersal and shaping their evolutionary history. Populations of the cosmopolitan species *Pristionchus pacificus* are characterized by high genetic diversity and strong spatial structure, which contrasts with patterns detected in nematode species such as *Caenorhabditis elegans*. The environmentally heterogeneous volcanic Mascarene Islands provide an ideal setting for investigating fine-scale patterns of nematode migration and gene flow. Based on the analysis of data from 19 nuclear microsatellites and one mitochondrial marker, we infer support for the colonization of both La Réunion Island and Mauritius from similar multiple geographical sources. Although the long-term persistence of populations on both islands is well supported, the historical colonization of one island from the other cannot be discounted. In fact, periodic, bi-directional migration between the islands following their initial colonization is strongly supported in isolation with migration analyses, supporting the occurrence of rare trans-oceanic dispersal events in *P. pacificus*. Through a combination of population and landscape genetic analyses we also infer non-uniform dispersal across the landscape on the island of La Réunion, probably mediated by the movements of beetle hosts. Collectively, we show that gene flow in *P. pacificus* is limited by environmental and oceanic barriers, and shaped by the intricacies of the nematode–beetle host interaction. *Biological Journal of the Linnean Society*, 2014, **112**, 1–15.

ADDITIONAL KEYWORDS: island biogeography – population genetics.

INTRODUCTION

Nematodes are among the most diverse of all animal phyla, occupying a vast area of the earth's surface and exhibiting considerable diversity in terms of reproductive strategy and life history (Boucher & Lambshead, 1995; De Ley, 2006). The development of nematode species such as *Pristionchus pacificus* and *Caenorhabditis elegans* as model systems in evolutionary biology has greatly advanced understanding of developmental processes (e.g. Hong & Sommer, 2006), and an integration of population genetics to the *P. pacificus* system now enables investigation of how phenotypic and developmental changes are initiated at the micro-evolutionary level (Sommer, 2009). However, despite their biological importance nematodes have been relatively neglected in population genetic and biogeographical studies, and the factors shaping their evolutionary history remain a substantial gap in our understanding of global biodiversity. In particular, the capacity for nematode dispersal combined with the role of landscape barriers in limiting gene flow and shaping population structure and distribution are key aspects of nematode ecology about which little is known.

Most of the nematode species that have been studied in terms of population genetics are parasitic

*Corresponding author:
E-mail: ralf.sommer@tuebingen.mpg.de
The first two authors contributed equally to this work and are listed as joint first authors.

species of agricultural or medical importance. High migration and the consequent homogenization of spatial genetic structure is often detected, and attributed to the anthropogenically mediated movement of host individuals and populations (e.g. Blouin *et al*., 1995; Grillo *et al*., 2007; Wielgoss *et al*., 2008). These patterns are not shared by all nematodes, however; restricted gene flow and strong spatial structure have been reported in both the Antarctic nematode *Scottnema lindsayae* (Courtright *et al*., 2000) and several species of marine nematodes (Derycke *et al*., 2005, 2008) – species that lack clear anthropogenic associations. The model species *P. pacificus* and *C. elegans* also lack an association with livestock hosts, although *C. elegans* is commonly found in compost heaps and rotting vegetation and fruit – habitats that tend to be associated with humans (Andersen *et al*., 2012). Although *P. pacificus* and *C. elegans* share an androdioecious lifestyle and the ability to live freely within the soil column, they differ considerably in terms of population structure, with low genetic variation and weak population structure in global populations of *C. elegans* contrasting with high diversity and strong population structure detected in *P. pacificus* (Zauner *et al*., 2007; Andersen *et al*., 2012; Morgan *et al*., 2012). In *C. elegans*, long-distance dispersal in association with human and agricultural transport is thought to have been followed by global selective sweeps, homogenizing spatial genetic structure (Andersen *et al*., 2012).

Dispersal in *P. pacificus* is likely to be largely mediated by the movement of scarab beetle hosts, with which it has a necromenic association (Herrmann *et al*., 2006, 2010; Morgan *et al*., 2012). This involves the nematodes infesting a host and entering a state of arrested development until host death, upon which the nematodes emerge and begin feeding on the microbes that grow on the beetle carcass. Unlike the majority of *Pristionchus* species, which are host-specific, *P. pacificus* is found in association with a number of scarab beetle species and is generally found with different host species across the four continents that encompass its distribution (Asia, Europe, Africa, America; see Herrmann *et al*., 2007, 2010). Although the widespread distribution of *P. pacificus* suggests a high capacity for long-distance dispersal, strong population structure detected on a fine scale, using mitochondrial and nuclear microsatellite markers, suggests limited gene flow between geographically proximate populations (Morgan *et al*., 2012). Landscape barriers are likely to play an important role in restricting gene flow, and thus maintaining the strong population structure within *P. pacificus*. Volcanic islands are ideal settings for investigating such scenarios, as their landscape is often characterized by dramatic altitudinal and environmental changes. Additionally, as they have never been connected to one another or to the mainland, colonization of and dispersal between the islands necessitates trans-oceanic dispersal events.

The Mascarenes, although less intensively studied than the Galapagos and Hawaiian Islands, represent an important volcanic island system (Fig. 1). The chain consists of three islands, Rodriguez, Mauritius and La Réunion, which arose from the Indian Ocean between 15 Mya (Rodriguez) and 2 Mya (La Réunion). As the youngest of the islands, La Réunion is the largest and most environmentally heterogeneous, with steep altitudinal gradients and a maximum altitude of 3070 m above sea level (a.s.l.) (Strasberg *et al*., 2005). La Réunion is also the only Mascarene Island with a currently active volcano. The island harbours a high genetic diversity of *P. pacificus*, which has been found in association with several of the species comprising La Réunion's scarab beetle fauna (Herrmann *et al*., 2010; Morgan *et al*., 2012).

Population genetic analyses of *P. pacificus* on La Réunion Island have detected four highly divergent mitochondrial and nuclear (microsatellite) lineages (Herrmann *et al*., 2010; Morgan *et al*., 2012), which modelling analyses have shown to be the result of at least four independent colonizations (McGaughran, Morgan & Sommer, 2013). Morgan *et al*. (2012) detected differences in the distributions of these lineages on La Réunion, which are differentially restricted to the humid, eastern region of the island, to arid western areas, or to high-altitude sites of more than 2000 m a.s.l. This suggests that barriers caused by environmental and altitudinal variation may be playing a role in restricting dispersal and migration between populations. In addition, approximately 200 km separate La Réunion from the neighbouring island of Mauritius, and both the expanse of ocean and environmental differences between the islands are potential barriers to inter-island dispersal and may play a role in defining population structure in *P. pacificus*. Indeed, a prolonged period of independent evolution, together with different selection pressures driven by environmental differences between the islands, is thought to have led to the considerable genetic and phenotypic differentiation of La Réunion and Mauritius populations of the Mascarene grey white-eye *Zosterops borbonicus*, a small passerine bird endemic to the islands of La Réunion and Mauritius (Mila *et al*., 2010).

Here, we sample the beetle fauna of the remaining Mascarene Islands, Mauritius and Rodriguez, as well as two other islands within the Indian Ocean – the Seychelles and Mayotte – with the aim of more precisely defining the distribution and dispersal patterns of *P. pacificus* within the Mascarene region (Fig. 1). Despite extensive sampling, we find *P. pacificus* only

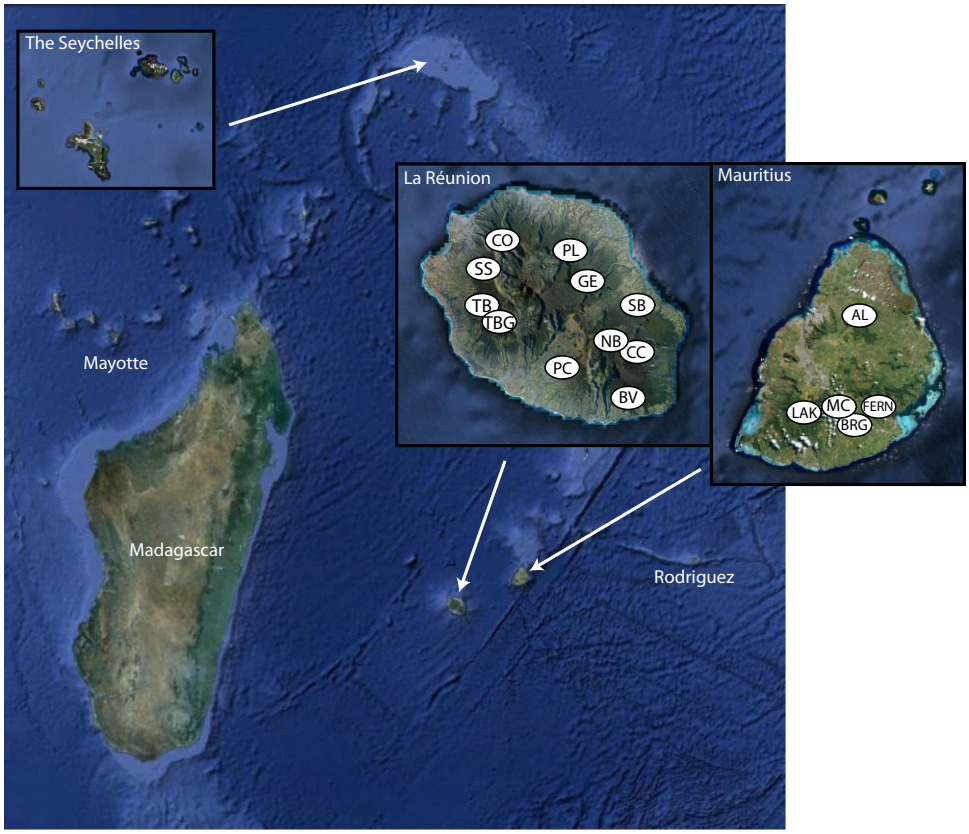

Figure 1. The geographical localities from which *P. pacificus* was sampled. The abbreviations for successful sampling sites on La Réunion and Mauritius are indicated at their approximate geographical position. A variety of scarab beetle species were also sampled on the islands of Rodriguez, the Seychelles and Mayotte, but *P. pacificus* was not detected on these islands.

on La Réunion Island's geographically closest island, Mauritius. We subsequently compare the new Mauritius samples with our existing La Réunion dataset, performing landscape genetic analyses to investigate patterns of migration between these two islands, as well as between populations on La Réunion Island to identify specific barriers to dispersal. Using 19 microsatellite ('single tandem repeat'; STR) markers, designed and utilized in Morgan *et al*. (2012), we generate an STR dataset for the new Mauritius samples. In addition, we use STR and mitochondrial (mt) data from Herrmann *et al*. (2010) and Morgan *et al*. (2012) to address the following specific questions: (1) Do Mauritius and La Réunion Island harbour a similar diversity of *P. pacificus*? (2) Following colonization, have geographical and oceanic barriers effectively reduced genetic contact between islands, or is there evidence for historical and/or current gene flow? (3) How much gene flow currently occurs between geographical regions within La Réunion Island, and what role do landscape barriers play in facilitating or preventing this?

METHODS

Sample collection

A variety of beetle species were sampled from several locations across Mauritius, Rodriguez, Mayotte and the Seychelles (Fig. 1; Table 1; Table S1). All islands were sampled by collecting beetles from a number of locations and variety of habitat types across the landscape over a 2-year period (Table S1). Specific localities and abbreviations used throughout the text, as well as the date of sample collection, are given in Table 1. In most cases sampling used light traps, although on Mauritius several samples were also obtained from sugarcane fields across the island by employees of the Mauritius Sugarcane Industry Research Institute (Table 1). Samples were processed according to the protocols of Herrmann *et al*. (2006). Briefly, the freshly killed carcasses of beetles were placed on individual nematode growth medium (NGM) plates (Brenner, 1974), seeded with the *Escherichia coli* strain OP50 and monitored daily over a period of 3 weeks for the emergence of adult nematodes. The nematodes were

Table 1. The sample localities and host beetle species from which strains used in this study were isolated; the diversity statistics for STR and mt markers are indicated for each of the sampling localities

Country of sampling locality	Sampling locality name and abbreviation	Beetle species from which samples were isolated	No. of beetle specimens from which strains were isolated	No. of strains isolated and analysed	STR gene diversity (H_E) for the sampling locality	STR rarefied allelic richness (Ar4) for the sampling locality	mt diversity ($\theta\pi$) for the sampling locality	No. of mt haplotypes detected at the sampling locality	No. of mt haplotypes unique to the sampling locality
La Réunion	Trois Bassins (TB)	*Oryctes borbonicus*	16	43	0.509	2.169	6.604	19	13
		Hoplia retusa	7	7					
		Hoplochelus sp.	8	13					
	Trois Bassins Garden (TBG)	*Oryctes borbonicus*	1	1	0.478	2.145	6.833	6	3
		Hoplia retusa	1	1					
		Hoplochelus	4	4					
		Maladera affinis	1	1					
	Sans Souci (SS)	*Oryctes borbonicus*	8	10	0.451	2.090	8.895	7	4
	Colorado (CO)	*Adoretus* sp.	1	5	0.414	2.672	3.682	2	0
		Hoplochelus sp.	3	7					
	Plaine des Cafres (PC)	*Hoplia retusa*	15	25	0.495	2.608	10.110	8	5
	Plaine des Lianes (PL)	*Adoretus* sp.	2	8	0.202	1.388	14.476	5	3
	Grand Etang (GE)	*Adoretus* sp.	13	14	0.682	2.776	22.046	12	8
		Chrysomelidae sp.	1	1					
	Saint Benoit (SB)	*Adoretus* sp.	1	1	0.536	2.183	13.379	7	5
		Maladera affinis	12	16					
		Aphodius sp.	3	13					
	Basse Vallée (BV)	*Adoretus* sp.	10	12	0.480	2.267	15.538	2	0
	Le Cratère de Commerson (CC)	*Amneidus godefroyi*	4	16	0.232	1.552	2.983	3	3
	Nez de Boeuf (NB)	*Amneidus godefroyi*	2	7	0.376	2.512	3.727	6	6
		Marronus borbonicus	1	5					
		Isolated from soil	NA	1					
Mauritius	Alma (ALMA)*	*Heteronychus licas*	4	8	0.624	2.831	5.778	3	1
	Riche en Eau (REE)*	*Clemora smithi*	1	1				1	0
	Ferney* (FERN)	*Alissonotum piceum*	5	8	0.626	2.733	14.889	5	3
	Black River Gorges (BRG)	*Hyposerica* sp.	1	1	0.429	2.158	7.333		
		Isolated from soil	NA	3					
	Mont Cocotte (MT)	Isolated from soil	NA	4	0.526	2.211	3.571	1	1
	Lakaz Chamarel (LAK)	*Hyposerica* sp.	7	10			8.156	5	3
		Clemora smithi	1	1	0.369	2.094			

*Samples were obtained from these localities by staff at the Mauritius Sugarcane Industry Research Institute.

picked individually onto freshly seeded NGM plates on emergence. As in Morgan *et al*. (2012), isogenic lines were generated from each adult nematode by allowing them to reproduce and maintaining their offspring in culture. Throughout this manuscript, the term 'strain' will refer to these laboratory-maintained, isogenic lines. These isogenic lines were maintained through at least ten generations of selfing, after which any heterozygosity present in the original P0 generation will have been eroded. This was necessary for the present study, despite the associated caveat of lost heterozygosity, as a single nematode yields insufficient DNA for sequencing and STR genotyping.

DNA sequencing and genotyping

STR genotypes were available at 19 loci for 223 strains, sampled from 11 localities across La Réunion in a previous study (Morgan *et al*., 2012; see Table 1). All beetles collected from Rodriguez, Mayotte and the Seychelles were negative for *P. pacificus* (Table S1). Thirty-five *P. pacificus* strains were isolated from Mauritius beetles collected at a number of sampling localities, after sampling over a 2-year period. These strains (Table 1) were genotyped at the same 19 loci (which included 14 di-nucleotide, four tri-nucleotide, one tetra-nucleotide and one penta-nucleotide repeat) as the available La Réunion strains, using the primer pairs, protocol and cycling conditions detailed in Morgan *et al*. (2012). Although it is possible that mutations could occur during the formation of isogenic lines, given the low mutation rate estimated for STR mutations using mutation accumulation lines (31 mutations identified in 41 STR markers, after 142 generations in 82 lines; Molnar *et al*., 2012), this is unlikely to have had a significant impact on our dataset. Our final STR dataset includes 258 strains from 14 and five sampling localities on La Réunion and Mauritius, respectively. Sequence data from the *ND4* and *ND6L* mt genes were available for 272 strains sampled from 17 localities on La Réunion Island (Morgan *et al*., 2012). These loci, which together form a 760-bp fragment, were also sequenced for the 35 new Mauritius strains using the primers, protocol and cycling conditions detailed in Herrmann *et al*. (2010). All newly generated mt sequences were deposited in GenBank, with accession numbers KJ577661–KJ577695. STR data generated in this study (for the Mauritius strains) are available from Dryad, with accession numbers, doi:10.5061/dryad.7194t.

Population genetic analysis

Characterizing the diversity of P. pacificus *on Mauritius and La Réunion*

Our first aim was to determine whether the same genetic lineages are present on Mauritius and La Réunion islands, and how Mauritius populations differ from those on La Réunion. A neighbour-net network was constructed from the STR dataset using Splitstree ver. 4.11.3 (Huson & Bryant, 2006). Network ver. 4.500 (Bandelt, Forster & Röhl, 1999) was used to construct a median-joining haplotype network with the mt data, using the weight parameters at their default of 10, and a connection cost of $\varepsilon = 10$. Population structure within the STR dataset was further examined using InStruct (Gao, Williamson & Bustamante, 2007), an extension of the commonly used program STRUCTURE (Pritchard, Stephens & Donnelly, 2000) designed especially for self-fertilizing or highly inbred species or populations. The software uses a Bayesian algorithm to infer the number of distinct populations present within a sample, and to probabilistically assign individuals to populations without any prior sampling information. Rather than assuming random mating and Hardy–Weinberg equilibrium, assumptions of STRUCTURE that are likely to be violated in selfing or inbred populations, InStruct accounts for selfing and/or inbreeding and allows their rates to vary between inferred clusters (Gao *et al*., 2007). InStruct has previously been used alongside STRUCTURE ver. 2.2 and was found to return consistent results for *P. pacificus* (Morgan *et al*., 2012). The InStruct analysis was used to infer the most likely number of genetic clusters (K) in the dataset, and to probabilistically assign individual strains to each of these clusters. Five replicate runs were performed for values of K between 1 and 20, each with a burn-in of 10 000 generations and 100 000 iterations, and results were checked for concordance.

To statistically evaluate levels of genetic differentiation between Mauritius and La Réunion populations, we estimated pair-wise genetic differentiation between populations with four samples or more using R_{ST} (significance determined over 1000 permutations) and Jost's D statistics, as estimated using Arlequin ver. 3.0 (Excoffier & Schneider, 2005) and SMOGD ver. 1.2.5 (Crawford, 2010), respectively. Previous results indicated that geography has a stronger influence on population structure than host beetle species, and that strains collected from different beetle species within a sampling locality show minimal differentiation from one another (Morgan *et al*., 2012). Thus, we define populations according to sampling locality. The standard diversity statistics, allelic richness and gene diversity (H_E), were estimated from the STR data for each sampling locality using Arlequin. Rarefied allelic richness (Ar_4), which controls for the effect of sample size on estimates of allelic richness, was also calculated using ADZE ver. 1.0 (Szpiech, Jakobsson & Rosenberg, 2008). These analyses were repeated after pooling samples more broadly into island-specific

populations (i.e. La Réunion: 14 localities vs. Mauritius: five localities).

Gene flow between La Réunion and Mauritius

Next, to investigate divergence and gene flow between Mauritius and La Réunion populations, an isolation with migration (IM) model was fitted to the combined STR and mt dataset using IMA2 (Hey & Nielsen, 2007). This approach is designed to detect any historical migration that has occurred between populations since their initial separation. The IMA2 approach involves the simultaneous estimation of six parameters using a Markov chain Monte Carlo (MCMC) sampling strategy. These parameters include: three q parameters, corresponding to θ, one for each of the ancestral and two daughter populations (where $\theta = 4N_e\mu$, N_e is the effective population size and μ is the geometric mean of the mutation rates of all markers analysed); t, the time since the initial divergence of the daughter populations; and $m1$ and $m2$, the migration from population 2 into population 1 and vice versa, respectively. Analyses were performed using pooled Mauritius and La Réunion populations as the two daughter populations. As the La Réunion dataset is substantially larger than the Mauritius dataset, to reduce computational complexity 35 individuals were sampled from the La Réunion population at random for analysis. Analyses were repeated using five independent sub-samples of the La Réunion population, to ensure no bias was introduced by the sub-sampling procedure.

Several preliminary runs were carried out for each dataset to optimize heating schemes and define the upper boundaries for the parameter priors. The performance of these runs was assessed through examination of the swapping rates between chains, the effective sample sizes (ESSs) and the trend plots. A geometric heating scheme was used with 40 chains, and the parameters ha and hb set to 0.975 and 0.75, respectively (-hfg -hn40 -ha0.975 -hb0.75). Runs were continued until at least 1 000 000 genealogies were sampled through the MCMC procedure (at least 100 000 000 MCMC steps with 100 steps between saved genealogies). Three independent runs were conducted for each dataset and convergence confirmed. An mt mutation rate of 7.6×10^{-8} mutations per bp per generation, as estimated for *P. pacificus* using mutation accumulation lines (Molnar *et al*., 2011), was used to calibrate divergence estimates.

Gene flow and barriers to recent migration within La Réunion populations

Here, we aimed to detect recent migration between populations and across the landscape, restricting our analysis to the more densely sampled La Réunion Island and using the STR dataset for all analyses. We used BAYESASS ver. 3.0 (Wilson & Rannala, 2003) to perform Bayesian inference of recent migration (i.e. within the last one to three generations) in a relatively assumption-free manner. Recent immigrants display temporary disequilibrium in their multi-locus genotypes relative to the population where they were captured; because this approach relates only to the past one to three generations, there are no assumptions needed regarding Hardy–Weinberg equilibrium, mutation or effective population size. For a given run, BAYESASS provides an estimate of the mean posterior distribution of m (migration), for all population pairs, which is the proportion of individuals in population i that have population j as their ancestral (past one to three generations) location. This provides both the proportion of residents and the proportion of immigrants in each population. To generate mixing parameters for migration (-m), allele frequency (-a) and inbreeding coefficients (-f) between the recommended 20 and 60%, BAYEASS was run with these parameters initially all set to 0.99. Burn-in was set to 1 000 000 and 10 000 000 iterations were performed, with a sampling interval of 100. A trace file was generated at the end of each run, and this was checked for convergence in TRACER ver. 1.5 (Rambaut & Drummond, 2007).

Several methods were used to detect the presence of barriers to dispersal within the Réunion landscape. ALLELES IN SPACE ver. 1.0 ('AIS'; Miller, 2005) is a program for the joint analysis of inter-individual spatial and genetic information using genotypic (STR) data and spatial/coordinate (utm) information. Allelic Aggregation Index analysis (AAIA) was performed to test the null hypothesis that each allele at a locus is distributed at random across a landscape (i.e. without aggregation/genetic structure). An index, Rj, is calculated, where $Rj = 1$ indicates a random distribution and $Rj < 1$ indicates clumping or aggregation. This analysis was run with 1000 permutations, with the area encompassed by each sample defined by both maximum and minimum utm coordinates, and by an empirical estimate derived from the PlotSampleLocations function in AIS. Next, landscape shape interpolation analysis was performed. This procedure produces a 3D surface plot, where x- and y-axes correspond to geographical locations and surface heights (z-axes) represent genetic distances. The plot contains peaks in areas where there are large genetic differences and in this way can be used to identify landscape barriers to gene flow. This analysis used Delaunay's triangulation method (Watson, 1992; Brouns, De Wulf & Constales, 2003) to derive a connectivity network and residual genetic distances. After these calculations, the plot was opened in the Genetic Landscape Interpolation Editor/Viewer of AIS using a distance weighting parameter (a) of 1, and grid settings of 80 × 80.

Another multivariate method, the spatial principal components analysis (sPCA) of Jombart, Devillard & Balloux (2010), was also performed using the adegenet package in R to identify spatial genetic patterns. This technique is often used in conjunction with traditional PCA approaches because it is more powerful at retrieving non-trivial spatial genetic patterns. In particular, the sPCA method can be used to identify genetic clines. Here, genetic data and utm coordinates were used to produce a Delaunay triangulation network and sPCA.

To complete the Boundary analyses, WOMBSOFT (Crida & Manel, 2007), which is a collection of Wombling-based R functions that analyse individually geo-referenced multilocus genotypes for the inference of genetic boundaries, was used. WOMBSOFT requires geo-coordinates to be different for each individual, and thus a 'jitter' function was applied to utm coordinates in R to vary population-specific coordinates by a null factor. The output from WOMBSOFT includes two maps, the first of which shows variations in the systemic function and in the mean direction of the barrier/gradient in the studied area. The second map shows candidate boundary elements that are significant at the chosen level. Before producing maps, the Mirror function in WOMBSOFT (m = 1.2) was used to add points just outside the convex hull that are similar to the points just inside the convex hull, thus reducing potential border effects due to a low density of individuals close to the border. The Wombling function was then applied using a bandwidth (h) of 1.4. Finally, the CandidateBoundaries function was employed using h = 1.4 and a 30 percentile (i.e. pB = 0.3) at each point of the grid.

RESULTS

Despite sampling of additional Indian Ocean Islands, Mauritius, Rodriguez, Mayotte and the Seychelles (Fig. 1; Table S1), *P. pacificus* isolates were found only on Mauritius and La Réunion. A variety of scarab beetle species were captured on all islands (Table S1). On both Mauritius and La Réunion Island, the beetle-associated nematode fauna was dominated by *P. pacificus*, whereas the majority of nematode species detected on Mayotte were gonochoristic (in contrast to the Mascarene Islands, on which gonochoristic nematodes have never been detected). Beetle-associated nematode fauna was rare on the Seychelles and Rodriguez (Table S1). On Mauritius, sample sizes were regrettably smaller than those obtained from La Réunion, although *P. pacificus* was isolated from four species of scarab beetle (*Heteronychus licas*, *Hyposerica* sp., *Clemora smithi* and *Alissonotum piceum*). All four beetle species that were positive for *P. pacificus* are sugarcane pests that were hand-collected from sugarcane fields. Light trap-collected scarab beetles on Mauritius, including several *Adoretus* species, were negative for *P. pacificus* although related *Adoretus* species on La Réunion are known to be infested with *P. pacificus*. Further sampling will be required to determine whether differences in infestation rates between the islands are significant. With the exception of *Alissonotum piceum*, the host species detected on Mauritius differ from those with which *P. pacificus* is associated on La Réunion (Table 1; Table S1).

Diversity of *P. pacificus* populations on La Réunion and Mauritius

Analyses were performed with the aim of characterizing the diversity of *P. pacificus* on La Réunion and Mauritius, as well as levels of differentiation and divergence between populations on the different islands. Strong population structure and high genetic diversity within both Mauritius and La Réunion populations is evident from the STR network (Fig. 2A). The Mauritius genotypes mainly cluster within the divergent La Réunion lineages, although there are several instances of individual Mauritius genotypes occurring in clusters throughout the STR network. Although the complexity of the STR network makes designating individual lineages problematic, correspondence with the mt haplotype network can be detected (Fig. 2).

Of the four mt lineages identified in previous work and designated as 'A', 'B', 'C' and 'D' (Herrmann *et al*., 2010; Morgan *et al*., 2012), lineages 'A', 'C' and 'D' were found to be common to both islands, and only lineage 'B' was apparently restricted to La Réunion (Fig. 2). Lineage B consists exclusively of strains isolated from the high-altitude La Réunion localities CC and NB, and from the beetle species *Amneidus godefroyi*. Neither this beetle species, nor similar altitudes are present on Mauritius. Matching previous patterns on La Réunion Island (see Morgan *et al*., 2012), a correspondence between mt lineage and sampling locality was detected in the new Mauritius samples, as can be seen in both the STR and the mt networks (Fig. 2). Specifically, lineage 'A' was detected in greatest frequency at the southern location LAC, lineage 'D' is limited to the southern and more easterly location BRG, and the more central sampling localities FERN and ALMA consist mainly of lineage 'C' individuals (Fig. 2b). A total of 54 and 12 unique mt haplotypes were detected on La Réunion and Mauritius, respectively, and four haplotypes were common to both islands. Hence both islands contain a similar assemblage of highly divergent STR and mt lineages, and although some degree of haplotype sharing is evident, both islands also harbour a collection of unique haplotypes.

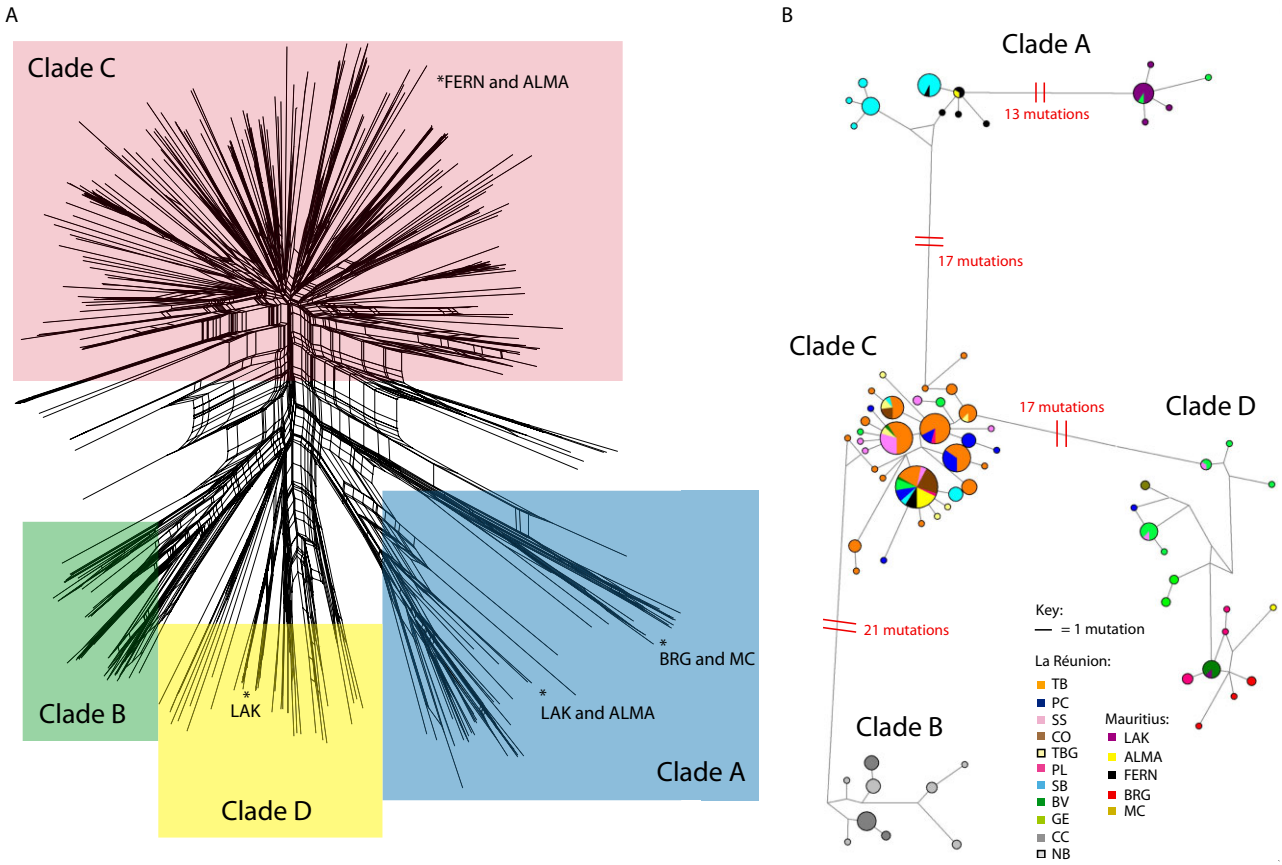

Figure 2. A, neighbour-net network constructed using STR data. The approximate correspondence with the mt clades is indicated. Clusters of haplotypes containing Mauritius strains are indicated with a star. B, median-joining haplotype network constructed using mt sequence data from *ND4* and *ND6L* loci. Each circle represents a haplotype and the size of the circle is proportional to the number of strains with that haplotype. The length of the branch between circles is approximately proportional to the number of mutational steps between haplotypes, except where indicated. The scale bar illustrates the branch length for one mutation.

InStruct infers the optimal number of clusters in a dataset using the deviance information criterion (DIC). The DIC values obtained here for each value of K are displayed in Supplementary Figure S1. The optimal value of K was inferred to be 15, although examination of the DIC values shows a clear decrease between $K = 1$ and $K = 8$, followed by a plateau from values of $K = 11$ and above (Fig. S1). In agreement with the results of Morgan *et al*. (2012), $K = 2$ split La Réunion populations into clear eastern (blue) and western (orange) clusters, with both clusters including strains from Mauritius (Fig. 3). Some distinction between Mauritius and La Réunion populations became apparent at $K = 6$. The black cluster, corresponding to strains isolated from the high-altitude La Réunion localities CC and NB (mt lineage 'B'), was the only unique cluster (present only on Réunion). All clusters detected on Mauritius were detected in high frequency on La Réunion, with the exception of the pink cluster, which was represented by only two La Réunion strains from the locality GE. There is clear correspondence between genetic clusters on both islands, with the orange cluster representing the Mauritius locality BRG + MT and the north-eastern La Réunion localities GE, BV and PL; the green and blue clusters representing the Mauritius clusters ALMA and FERN and the south-western La Réunion localities CO, PC, SS and TB; the yellow cluster representing the Mauritius localities LAK and FERN as well as the La Réunion locality SB; and the pink cluster primarily representing the Mauritius locality LAK, but also containing two individuals from the La Réunion locality GE. Increasing K further generally reveals additional structure within the La Réunion sample, although at $K = 11$ and above the Mauritius locality BRG + MT forms a distinct and exclusive cluster. Hence although there is often greater genetic distinction between localities within an island than

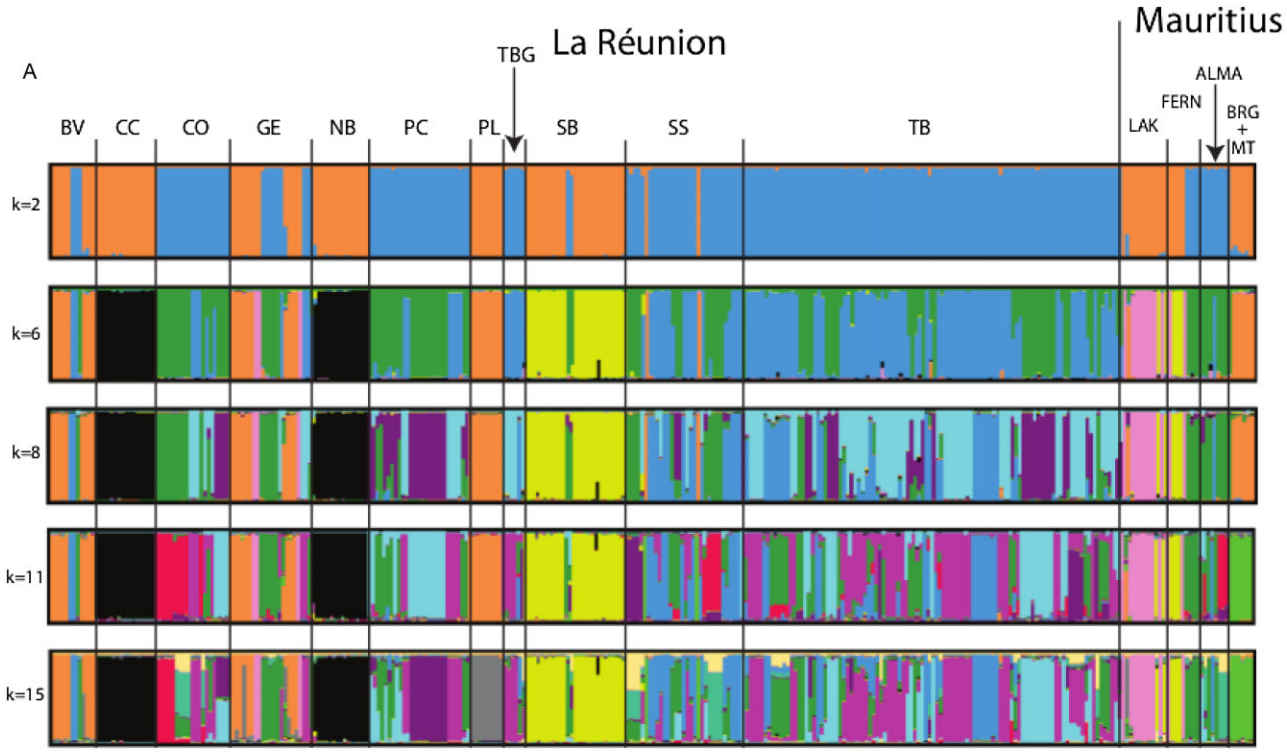

Figure 3. The inferred InStruct clusters at $K = 2$, 6, 8, 11 and 15. The optimal value of K was inferred to be 15, although examination of the DIC values shows a clear decrease between $K = 1$ and $K = 8$, followed by a plateau from values of $K = 11$ and above (Fig. S1).

between localities on different islands, some distinction between Mauritius and La Réunion does become apparent at higher K values.

Similar patterns of diversity and differentiation were detected using STR and mt loci, and using R_{ST} and Jost's D for the STR loci, so only the R_{ST} results from the STR loci are presented. Genetic diversity at STR loci within La Réunion populations ranged from $H_E = 0.202$ and $Ar_4 = 1.388$ (PL population) to $H_E = 0.682$ and $Ar_4 = 2.776$ (at GE), with a mean H_E of 0.441 and Ar_4 of 2.215 (Table 2; Fig. 1). Diversity statistics for the Mauritius populations fall within a similar range to those estimated for La Réunion (H_E range 0.369–0.626, mean = 0.515; Ar_4 range 2.094–2.831, mean = 2.405).

The R_{ST} values reveal generally high levels of differentiation between populations on both islands, which in most cases remain significant after Bonferroni's correction for multiple tests (Table 2). The mean differentiation between populations on different islands ($R_{ST} = 0.462$) is not substantially different from that between populations on the same island ($R_{ST} = 0.460$ and 0.355 for populations on La Réunion and Mauritius, respectively). Indeed, several pairs of populations that are isolated on different islands show R_{ST} statistics that are considerably lower than the average for the within-island comparisons, for example the La Réunion population GE and both the Mauritius populations ALMA ($R_{ST} = 0.105$) and FERN (0.034), and the La Réunion population BRG and the Mauritius population SB ($R_{ST} = 0.136$).

Historical migration between Mauritius and La Réunion

The posterior distributions of the majority of parameters within the IMA2 analyses, including *t0*, *m1* > 0 and *q1* and *m0* > 1, had clear peaks and bounds within the prior distributions, indicating reliable inference of these parameters. However, the *qa* and *q2* parameters had flat posterior distributions, indicating a lack of information for these parameters. The results were consistent across all five independent draws from the La Réunion population, and remained consistent across replicates.

Using the mitochondrial mutation rate of Molnar *et al*. (2011), the Mauritius and La Réunion populations were estimated to have diverged 129 421 generations ago with 95% HPD intervals (which represent the interval in which 95% of the distribution lies, and are analogous to 95% confidence intervals) supporting a broader divergence range of

Table 2. Population differentiation between all pairs of sampled populations, estimated using R_{ST} statistics

	BV	CC	CO	GE	NB	PC	PL	SB	SS	TB	TBG	LAK	ALMA	FERN	BRG
CC	**0.791**														
CO	**0.582**	**0.903**													
GE	0.141	**0.600**	**0.533**												
NB	**0.747**	0.097	**0.868**	**0.600**											
PC	**0.427**	**0.700**	**0.317**	**0.231**	**0.683**										
PL	**0.354**	**0.872**	**0.772**	**0.317**	**0.790**	**0.597**									
SB	**0.222**	**0.495**	**0.606**	**0.200**	**0.547**	**0.372**	0.110								
SS	0.311	**0.811**	**0.361**	**0.236**	**0.732**	0.172	**0.639**	**0.346**							
TB	**0.399**	**0.736**	0.125	**0.371**	**0.731**	0.092	**0.596**	**0.506**	0.077						
TBG	**0.356**	**0.799**	**0.326**	**0.202**	**0.756**	0.021	**0.569**	**0.374**	0.125	0.072					
LAK	**0.634**	**0.834**	**0.809**	**0.374**	**0.728**	**0.620**	**0.756**	**0.426**	**0.713**	**0.635**	**0.677**				
ALMA	0.293	**0.711**	**0.388**	0.105	**0.677**	0.057	0.484	0.242	0.213	0.209	0.075	**0.526**			
FERN	0.212	**0.721**	**0.401**	0.034	**0.669**	0.025	**0.406**	**0.257**	0.177	**0.217**	0.067	**0.509**	0.062		
BRG	0.250	**0.859**	0.732	0.151	**0.725**	**0.506**	0.376	0.136	**0.551**	**0.558**	0.547	0.602	0.310	0.313	
MT	0.448	**0.864**	**0.770**	0.206	**0.679**	**0.545**	**0.602**	0.227	**0.637**	**0.597**	**0.608**	0.426	0.347	0.367	0.214

The population codes correspond to the sampling localities shown in Figure 1. Statistically significant R_{ST} values, with Bonferroni's correction reducing the significance threshold from 0.05 to 0.0005, are shown in bold.

between 70 997 and 322 144 generations. Non-zero, bi-directional migration was inferred between Mauritius and La Réunion populations since their initial divergence, and this was higher from Mauritius to La Réunion than in the opposite direction (m = 5.25, 95% HPD 0.38–25.23 from Mauritius to La Réunion, and m = 1.337, 95% HPD 0.03–13.28 from La Réunion to Mauritius).

As no reliable effective population size estimates were obtained for the Mauritius populations, the migration rate estimates can only be converted into migrant per generation estimates using the estimated effective population size estimate for the La Réunion populations ($q1 = 4N_e\mu$), and assuming similar effective population sizes for Mauritius and La Réunion. Using this estimate, migration rates were inferred to be 2.97 (95% HPD 0.18–14.51) and 0.76 (95% HPD 0.01–7.64) migrants per generation to La Réunion and Mauritius, respectively.

Recent migration on La Réunion Island

Given the high sampling density on La Réunion, we next aimed to detect recent migrations across the landscape on La Réunion Island. Patterns of recent immigration were shown to often be asymmetrical (i.e. greater in one direction than the other) between pairs of populations. The estimated percentage of immigrants varied from 0.3 to 14.0% across all pair-wise comparisons using BAYESASS, with the total percentage of immigrants per population ranging from 10.7 to 32.2% (Table 3). There were four instances where pair-wise migration accounted for over 5% of population membership; this corresponded to migration from GE to BV (10.7%) and PL (14%), and from TB to SS (11.8%) and TBG (7%) (Table 3).

AAIA indicated an average Rj value of 0.328, indicating significant ($P < 0.001$) genetic structure among *P. pacificus* populations. Landscape shape interpolation analysis produced a 3D surface plot, showing peaks in areas where there are large genetic differences (i.e. landscape barriers to gene flow). In this plot, differentiation increased from largely smooth (low barriers to gene flow) genetic surfaces at the plot edges towards regions of elevated genetic distance in central areas. This roughly corresponded to barriers separating the north/south-east part of the island (locations: NB, CC, GE) from south/north-west areas (CO, SS, TB, TBG, PC, BV) (Fig. 4). To identify genetic clines, the sPCA method was used. This analysis identified gradual gradients of genetic diversity over two main geographical areas: in Figure S2, light areas (corresponding to higher genetic differentiation) encompassed north-eastern locations (NB, CC, GE, PL, SB) and darker areas (lower differentiation) encompassed south-western areas.

Table 3. Migration rates in recent generations among *Pristionchus pacificus* populations as estimated in the program BAYEASS

	Population j											
Population i	*BV*	*CC*	*CO*	*GE*	*NB*	*PC*	*PL*	*SB*	*SS*	*TB*	*TBG*	Imm
BV	0.679	0.012	0.012	0.107	0.012	0.022	0.012	0.012	0.012	0.025	0.012	0.321
CC	0.008	0.859	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.141
CO	0.006	0.007	0.864	0.007	0.007	0.006	0.006	0.006	0.006	0.032	0.009	0.136
GE	0.004	0.004	0.033	0.874	0.004	0.025	0.004	0.004	0.004	0.011	0.004	0.126
NB	0.009	0.009	0.009	0.009	0.844	0.009	0.009	0.009	0.009	0.009	0.009	0.156
PC	0.008	0.008	0.008	0.008	0.008	0.834	0.008	0.008	0.008	0.038	0.010	0.166
PL	0.011	0.011	0.022	0.140	0.011	0.011	0.678	0.011	0.011	0.011	0.011	0.322
SB	0.007	0.007	0.007	0.007	0.007	0.041	0.007	0.838	0.007	0.007	0.019	0.162
SS	0.011	0.011	0.011	0.021	0.011	0.011	0.011	0.011	0.678	0.118	0.032	0.322
TB	0.003	0.003	0.004	0.010	0.003	0.007	0.003	0.003	0.003	0.893	0.042	0.107
TBG	0.008	0.008	0.013	0.008	0.008	0.008	0.008	0.008	0.008	0.070	0.804	0.196

Values in rows represent the expected proportions of individuals in population **i** (bold) that were derived/have migrated from population *j* (italics). Values underlined along the diagonal are the proportions of individuals derived from their population of capture (i.e. residents) in each generation, while values in the far right column are the total proportion of immigrants per population (Imm).

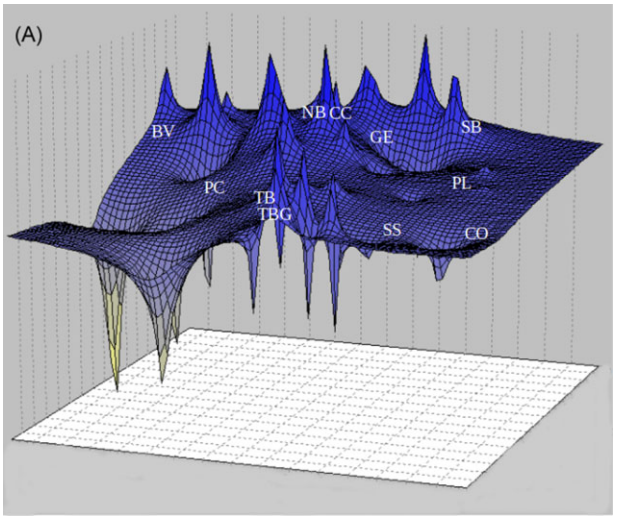

Figure 4. Results of landscape shape interpolation analysis, performed in ALLELES IN SPACE on *Pristionchus pacificus* populations from La Réunion Island. The graphic depicts a 3D surface plot, where heights represent genetic distances such that peaks/valleys exist in areas where there are large genetic differences and smooth areas represent regions with low barriers to gene flow. Population locations are approximations.

To complete the boundary analyses, WOMBSOFT was used to infer genetic boundaries among geographical locations. This method identified a large heterogeneous region in the eastern part of the island (Fig. S3a), which corresponded to an area of high candidate boundary elements significant at 0.05, again dissecting north-eastern and south-western areas (Fig. S3b).

DISCUSSION

This study investigates patterns of population structure and migration among island populations of the nematode *P. pacificus* in the Indian Ocean. Our analysis allows three major conclusions, which are outlined below.

Similarly high genetic diversity on both Mauritius and La Réunion Island, and the apparent absence of *P. pacificus* from other Indian Ocean islands

Previous population genetic and modelling approaches revealed that the presence of highly divergent STR and mt lineages in populations of *P. pacificus* on La Réunion island is due to multiple colonization events from geographically disparate source populations (Morgan *et al*., 2012; McGaughran *et al*., 2013). Here, we demonstrate that both Mauritius and La Réunion harbour a similar set of highly divergent mt and STR lineages, with mt haplotypes and STR clusters being shared between the islands. Population genetic statistics reveal a similarly high diversity of *P. pacificus* on both islands, supporting long-term establishment on Mauritius as well as La Réunion (Morgan *et al*., 2012; McGaughran *et al*., 2013).

However, although mt haplotypes and STR clusters are shared between Mauritius and La Réunion, both islands harbour a collection of unique mt haplotypes (which typically differ by between one and five mutational steps), and the Mauritius haplotypes generally cluster together within the STR network (Fig. 2). The InStruct analyses also reveal differentiation between the two islands, with the pink cluster being common on Mauritius, for example, yet represented by only two individuals on La Réunion (Fig. 3). The divergence of La Réunion and Mauritius populations is also supported by high R_{ST} values. This differentiation suggests that the Mascarene populations of *P. pacificus* follow a pattern typically seen in volcanic island chain systems, in which populations diverge from one another following colonization due to the increased strength of genetic drift in isolation (e.g. Jordan & Snell, 2008; Jordaens *et al*., 2009; Hoeck *et al*., 2010; Kuntner & Agnarsson, 2011). Collectively, this general pattern of shared lineages coupled with the presence of high genetic diversity, unique haplotypes and distinct STR clusters on both islands suggests that Mauritius and La Réunion are most likely to have been colonized on historical vs. recent time scales, allowing the accumulation of genetic diversity and inter-island differentiation.

Although negative results must be interpreted with caution, it is interesting that the two islands appear to differ with respect to the presence of lineage 'B'. However, the habitat (> 2000 m a.s.l.) and beetle species (*Amneidus godefroyi*) with which this 'high-altitude' lineage is found in association on La Réunion (Morgan *et al*., 2012) are lacking on Mauritius. Thus, it is possible that this lineage failed to disperse to Mauritius due to the reduced dispersal capacity of its associated host, or failed to persist on Mauritius due to a lack or erosion of suitable environmental conditions. Sampling of numerous scarab beetle species also failed to detect evidence of *P. pacificus* on Rodriguez, Mayotte and the Seychelles. As *P. pacificus* dispersal most likely occurs in association with host beetles, prevailing wind directions may simply not favour dispersal to these islands. Alternatively, Mauritius and La Réunion differ in a number of physical parameters from most of the other islands, being younger and/or larger than most of the other islands investigated in this study (Fig. 1) – it may be that *Pristionchus* nematodes were unsuccessful in establishing populations on smaller islands, in accordance with the classical model of island biogeography (MacArthur & Wilson, 1967). On Mayotte, many other nematodes were found, primarily consisting of Rhabditid species with a gonochoristic mode of reproduction (M. Herrmann & R. J. Sommer, unpubl. data). Interestingly, Mayotte is substantially older than La Réunion and Mauritius, further supporting our earlier observation that due to the potential for self-fertilization, hermaphroditic nematodes may have an advantage in early island colonization (Herrmann *et al*., 2010).

Bi-directional migration between Mauritius and La Réunion

The hypothesis of historical colonization of both Mauritius and La Réunion is further supported by our IM analysis. This method teases apart the signals of migration and ancestral polymorphism, which can be difficult or impossible to distinguish using more traditional methods, and estimates divergence time taking both of these factors into account (Hey & Nielsen, 2007). The estimated divergence of populations on the two islands approximately 129 421 generations ago (70 997–322 144 generations including 95% confidence intervals) falls within a similar time period to the estimated dates of spatial expansions of *P. pacificus* populations on La Réunion, approximately 133 000–191 000 years ago. These expansions are thought to have coincided with the initial colonization of the Mascarene region (McGaughran *et al*., 2013), suggesting that the two islands were most likely colonized over the same historical time scale. Although colonization of one island following establishment on the other cannot be ruled out entirely, this would have to have occurred within the distant, rather than the recent history of *P. pacificus* in the Mascarenes.

Our migration analysis supports the capacity for migration between the islands, highlighting bi-directional gene flow since population divergence. Long-distance dispersal events in *P. pacificus* have previously been supported by cases of mt haplotype sharing over large geographical distances in global populations (although this study was based on limited sampling), suggesting that such events are not limited to the Mascarene Island system (Herrmann *et al*., 2010). Homogenizing long-distance dispersal events in nematodes are generally attributed to passive dispersal, and are often associated with the movements of host individuals (e.g. Blouin *et al*., 1995; Wielgoss *et al*., 2008). In the case of *P. pacificus*, several of the host species are pests of sugarcane, and hence the transport of agricultural produce may be linked to its passive dispersal both to and from the islands, potentially over large geographical distances.

The supported long-term persistence of *P. pacificus* populations on both Mauritius and La Réunion, as well as the fact that sugarcane is exported out of the Mascarene region from both islands rather than from one island to the other, suggests the transport of agricultural produce is unlikely to be responsible for either the initial colonization events or the

bi-directional migration of *P. pacificus* between the islands. Prevailing winds between La Réunion and Mauritius probably aid in the dispersal between islands of individual host beetles, both pest and non-pest. The capacity for *P. pacificus* to disperse with these beetle hosts is probably enhanced by its ability to self-fertilize, as rare beetle movements between islands that fail to result in the establishment of beetle populations may still result in successful nematode dispersal. Thus, although the different assemblage of scarab beetle species on La Réunion Island and Mauritius suggests limited beetle movements between the islands (only one species, *Alissonotum*, was found to act as a host species on both islands), this does not rule out the beetle hosts as a potential route for *P. pacificus* dispersal. Flexibility with regard to host beetle species was reported by Morgan *et al*. (2012), and suggests that after dispersal across the ocean barrier, *P. pacificus* populations are able to opportunistically exploit the locally available suite of novel host species.

Dispersal across the landscape is limited by strong environmental barriers on La Réunion

Landscape genetic analyses performed using the more densely sampled La Réunion dataset revealed several barriers to nematode gene flow across this island. In particular, a major barrier was detected separating the eastern, windward side of the island from the west. Each of the landscape analyses supported the presence of this barrier: the AIS analysis showed genetic differentiation increasing toward the central region dissecting the east and western regions of the island; sPCA revealed higher genetic differentiation in the north-east of the island, and lower genetic differentiation encompassing sampling localities in the west and south-west; and the WOMBSOFT analysis identified a large, heterogeneous region dissecting the north-eastern and south-western regions of the island.

Rather than coinciding with an obvious geological feature, such as a mountain range, the barrier appears to coincide with the potential ecological divide discussed in Morgan *et al*. (2012) and Mila *et al*. (2010). Specifically, the north-east of the island is characterized by a humid climate with high rainfall, while the south-west is considerably more arid. Reduced gene flow between these regions may be due to either a lack of beetle movement or reduced survival of immigrant nematode individuals after their dispersal across the divide. Recent species distribution modelling analyses show a strong association between genetic structure and environmental variability in both *P. pacificus* and its beetle hosts, suggesting that local adaptation may be a particularly important driver of distribution patterns (McGaughran, Morgan & Sommer, 2014).

Genetic differentiation is generally strong between populations on La Réunion Island, indicating strong spatial structure and supporting reduced gene flow across the landscape. However, the results of the BAYESASS analysis support non-uniform, recent dispersal between several pairs of geographically distinct La Réunion populations, including pairs that are on opposite sides of the identified landscape barrier, such as GE and TB. These high levels of inferred migration may seem surprising given the strong spatial structure on La Réunion, which might be expected to become homogenized. However, the development and persistence of strong spatial structure in nematode populations despite the potential for high rates of passive dispersal has been reported in several marine species, and attributed to the occurrence of repeated founder events, followed by rapid reproduction and population growth (Derycke *et al*., 2005, 2008). Hence, *P. pacificus* population genetic patterns show little similarity to those detected in *C. elegans*, in which long-distance dispersal is thought to have been followed by repeated selective sweeps and the consequent homogenization of global spatial structure (Andersen *et al*., 2012), and rather are more similar to those detected in non-parasitic, free-living marine species, in which high spatial genetic structure is maintained despite frequent long-distance migration events.

ACKNOWLEDGEMENTS

We thank the three anonymous reviews for their comments on previous versions of this manuscript. In addition, we thank La Réunion Parc National for supplying collection permits and Jacques Rochat (La Réunion Insectarium) for his help with the planning and execution of sample collection trips. We would also like to thank members of the Sommer research group at Max Planck Institute for their discussion and advice, and in particular Christian Rödelsperger for his bioinformatic assistance. Finally, we thank the Max Planck Society and the Alexander von Humboldt Foundation (Research Fellowship for Postdoctoral Researchers to A.M.) for funding.

REFERENCES

Andersen E, Gerke J, Shapiro J, Crissman J, Ghosh R, Bloom J, Félix M-A, Kruglyak L. 2012. Chromosome-scale selective sweeps shape *Caenorhabditis elegans* genomic diversity. *Nature Genetics* **44:** 285–290.

Bandelt H-J, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16:** 37–48.

Blouin MS, Yowell CA, Courtney CH, Dame JB. 1995. Host movement and the genetic structure of populations of parasitic nematodes. *Genetics* **141:** 1007–1014.

Boucher G, Lambshead PJD. 1995. Ecological biodiversity of marine nematodes in samples from temperate, tropical, and deep-sea regions. *Conservation Biology* **9:** 1594–1604.

Brenner S. 1974. The genetics of *Caenorhabditis elegans*. *Genetics* **77:** 71–94.

Brouns G, De Wulf A, Constales D. 2003. Delaunay triangulation algorithms useful for multibeam echosounding. *Journal of Surveying Engineering* **129:** 79–84.

Courtright EM, Wall DH, Virginia RA, Frisse LM, Vida JT, Thomas WK. 2000. Nuclear and mitochondrial DNA sequence diversity in the Antarctic nematode *Scottnema lindsayae*. *Journal of Nematology* **32:** 143–153.

Crawford NG. 2010. SMOGGD: software for the measurement of genetic diversity. *Molecular Ecology Resources* **10:** 556–557.

Crida A, Manel S. 2007. WOMBSOFT: an R package that implements the Wombling method to identify genetic boundary. *Molecular Ecology Notes* **7:** 588–591.

De Ley P. 2006. A quick tour of nematode diversity and the backbone of nematode phylogeny. Available at: http:// wormbook.org. In: *Wormbook* Edited by Community TCR. Wormbook.

Derycke S, Remerie T, Backeljau T, Vierstraete A, Vincx M, Moens T. 2008. Phylogeography of the *Rhabditis* (*Pellioditis*) *marina* species complex: evidence for long-distance dispersal, and for range expansions and restricted gene flow in the northeast Atlantic. *Molecular Ecology* **17:** 3306–3322.

Derycke S, Remerie T, Vierstraete A, Backeljau T, Vanfleteren J, Vincx M, Moens T. 2005. Mitochondrial DNA variation and cryptic speciation within the free-living marine nematode *Pellioditis marina*. *Marine Ecology Progress Series* **300:** 91–103.

Excoffier LGL, Schneider S. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1:** 47–50.

Gao H, Williamson S, Bustamante CD. 2007. A Markov Chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics* **176:** 1635–1651.

Grillo V, Jackson F, Cabaret J, Gilleard JS. 2007. Population genetic analysis of the ovine parasitic nematode *Teladorsagia circumcincta* and evidence for a cryptic species. *International Journal for Parasitology* **37:** 435–447.

Herrmann M, Kienle S, Rochat J, Mayer WE, Sommer RJ. 2010. Haplotype diversity of the nematode *Pristionchus pacificus* on Réunion in the Indian Ocean suggests multiple independent invasions. *Biological Journal of the Linnean Society* **100:** 170–179.

Herrmann M, Mayer WE, 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.

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.

Hey J, Nielsen R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences of the United States of America* **104:** 2785–2790.

Hoeck PEA, Bollmer JL, Parker PG, Keller LF. 2010. Differentiation with drift: a spatio-temporal genetic analysis of Galápagos mockingbird populations (*Mimus* spp.). *Philosophical Transactions of the Royal Society B: Biological Sciences* **365:** 1127–1138.

Hong RL, Sommer RJ. 2006. *Pristionchus pacificus* – a well rounded nematode. *BioEssays* **28:** 651–659.

Huson DH, Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* **23:** 254–267.

Jombart T, Devillard S, Balloux F. 2010. Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11:** 94.

Jordaens K, Van Riel P, Frias Martins AM, Backeljau T. 2009. Speciation on the Azores islands: congruent patterns in shell morphology, genital anatomy, and molecular markers in endemic land snails (Gastropoda, Leptaxinae). *Biological Journal of the Linnean Society* **97:** 166–176.

Jordan MA, Snell HL. 2008. Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex). *Molecular Ecology* **17:** 1224–1237.

Kuntner M, Agnarsson I. 2011. Phylogeography of a successful aerial disperser: the golden orb spider *Nephila* on Indian Ocean islands. *BMC Evolutionary Biology* **11:** 119.

MacArthur R, Wilson E. 1967. *The theory of island biogeography*. Princeton: Princeton University Press.

McGaughran A, Morgan K, Sommer RJ. 2013. Unraveling the evolutionary history of the nematode *Pristionchus pacificus*: from lineage diversification to island colonization. *Ecology and Evolution* **3:** 667–675.

McGaughran A, Morgan K, Sommer RJ. 2014. Landscape genetic analyses in a hermaphroditic nematode support significant genotype-environment interactions. *PLoS One* **9:** e87317.

Mila B, Warren B, Heeb P, Thebaud C. 2010. The geographic scale of diversification on islands: genetic and morphological divergence at a very small spatial scale in the Mascarene grey white-eye (Aves: *Zosterops borbonicus*). *BMC Evolutionary Biology* **10:** 158.

Miller MP. 2005. Alleles in Space (AIS): computer software for the joint analysis of interindividual spatial and genetic information. *Journal of Heredity* **96:** 722–724.

Molnar RI, Bartelmes G, Dinkelacker I, Witte H, Sommer RJ. 2011. Mutation rates and intraspecific divergence of the mitochondrial genome of *Pristionchus pacificus*. *Molecular Biology and Evolution* **28:** 2317–2326.

Molnar RI, Witte H, Dinkelacker I, Villate L, Sommer R. 2012. Tandem-repeat patterns and mutation rates in

microsatellites in the nematode model organism *Pristionchus pacificus*. *Genes, Genomes, Genetics* **2:** 1027–1034.

Morgan K, McGaughran A, Ganeshan S, Herrmann M, Sommer RJ. 2014. Title: Data from: Landscape and oceanic barriers shape dispersal and population structure in the island nematode *Pristionchus pacificus. Dryad Digital Repository*. doi:10.5061/dryad.7194t.

Morgan K, McGaughran A, Villate L, Herrmann M, Witte H, Bartelmes G, Rochat J, Sommer RJ. 2012. Multi locus analysis of *Pristionchus pacificus* on La Réunion Island reveals an evolutionary history shaped by multiple introductions, constrained dispersal events and rare outcrossing. *Molecular Ecology* **21:** 250–266.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155:** 945–959.

Rambaut A, Drummond AJ. 2007. Tracer version 1.4. Computer program and documentation distributed by the author, website Available at: http://beast.bio.ed.ac.uk/Tracer.

Sommer RJ. 2009. The future of evo-devo: model systems and evolutionary theory. *Nature Reviews Genetics* **10:** 416–422.

Strasberg D, Rouget M, Richardson DM, Baret S, Dupont J, Cowling RM. 2005. An assessment of habitat diversity and transformation on La Réunion Island (Mascarene Islands, Indian Ocean) as a basis for identifying broad-scale conservation priorities. *Biology and Conservation* **14:** 3015–3032.

Szpiech ZA, Jakobsson M, Rosenberg NA. 2008. ADZE: a rarefaction approach for counting alleles private to combinations of populations. *Bioinformatics* **24:** 2498–2504.

Watson DF. 1992. *Contouring: a guide to the analysis and display of spatial data*. New York: Pergamon Press.

Wielgoss S, Taraschewski H, Meyer A, Wirth T. 2008. Population structure of the parasitic nematode *Anguillicola crassus*, an invader of declining North Atlantic eel stocks. *Molecular Ecology* **17:** 3478–3495.

Wilson GA, Rannala B. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163:** 1177–1191.

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

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1. The DIC values obtained here for each value of *K*.

Figure S2. Results of multivariate sPCA performed in R, identifying genetic clines among La Réunion *Pristionchus pacificus* populations, with light areas representing large genetic differences and darker colours indicating low diversity. Population locations are approximations.

Figure S3. Results of the boundary analyses performed with the WOMBSOFT package in R, inferring genetic boundaries among *Pristionchus pacificus* populations on La Réunion Island: (i) a coloured map on which each sampled point is a circle and the background is the systemic function – green corresponds to low values of the systemic function, and light pink to high values of the systemic function, i.e. zones where the population is heterogeneous; (ii) a second map, where boundaries are shown in light grey and homogeneous zones in green. The lines are the directions of the gradient.

Table S1. The variety of beetle species sampled from La Réunion, Mauritius, Rodriguez, Mayotte and the Seychelles, and the identity and dominant reproductive strategy of nematode species detected from each island.

ARCHIVED DATA

Data deposited at Dryad (Morgan *et al*., 2014).