



Parasites and the island syndrome: the colonization of the western Mediterranean islands by *Heligmosomoides polygyrus* (Dujardin, 1845)

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ABSTRACT

Aim Populations of free-living vertebrates on islands frequently differ from their mainland counterparts by a series of changes in morphometric, life-history, behavioural, physiological and genetic traits, collectively referred to as the 'island syndrome'. It is not known, however, whether the 'island syndrome' also affects parasitic organisms. The present study establishes the colonization pattern of the Mediterranean islands by the nematode *Heligmosomoides polygyrus*, a direct and specific parasite of rodent hosts of the *Apodemus* genus, and evaluates the effects of island colonization by this species on two components of the island syndrome: the loss of genetic diversity and the enlargement of the ecological niche.

Location *Heligmosomoides polygyrus* was sampled on seven western Mediterranean islands – Corsica, Crete, Elba, Majorca, Minorca, Sardinia and Sicily – as well as in 20 continental locations covering the Mediterranean basin.

Methods The mitochondrial cytochrome *b* gene (690 base pairs) was sequenced in 166 adult *H. polygyrus* individuals sampled in the 27 continental and island locations. Phylogenetic reconstructions in distance, parsimony, maximum likelihood and Bayesian posterior probabilities were carried out on the whole cytochrome *b* gene data set. The levels of nucleotide, haplotype and genetic divergence (Kimura two-parameter distance estimator) diversities were estimated in each island population and in the various continental lineages.

Results Phylogenetic reconstructions show that the mainland origins of *H. polygyrus* were continental Spain for the Balearic Islands (Majorca, Minorca), northern Italy for the Tyrrhenian Islands (Corsica, Sardinia, Elba), southern Italy for Sicily, and the Balkan region for Crete. A comparison of island *H. polygyrus* populations with their mainland source populations revealed two characteristic components of the island syndrome in this parasite. First, island *H. polygyrus* populations display a significant loss of genetic diversity, which is related ($r^2 = 0.73$) to the distance separating the island from the mainland source region. Second, *H. polygyrus* exhibits a niche enlargement following insularization. Indeed, *H. polygyrus* in Corsica is present in both *A. sylvaticus* and *Mus musculus domesticus*, while mainland *H. polygyrus* populations are present exclusively in *Apodemus* hosts.

Main conclusions Our results show that *H. polygyrus* has undergone a loss of genetic diversity and a niche (host) enlargement following colonization of the western Mediterranean islands. To our knowledge, this study provides the first evidence for components of the 'island syndrome' in a parasitic nematode species.

Keywords

Corsica, Heligmosomoidea, *Heligmosomoides polygyrus corsicum*, island biogeography, island syndrome, mitochondrial cytochrome *b*, nematode, niche enlargement.

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INTRODUCTION

Parasites and the island syndrome

In comparison with mainland environments, the isolated nature of islands allows the identification and quantification of the relative importance of factors involved in the rate of evolutionary differentiation of populations. From this comparison, key factors determining the differentiation of island populations appear to be (1) the area of the island, and (2) the distance of the island from the mainland source population. These two factors, combined with the loss of genetic diversity at the foundation of the population, determine the degree of isolation, that is, the decrease in genetic exchange, between island and mainland source populations, and therefore the rate of island population differentiation (Whittaker, 1998; Blondel, 2000).

The study of island populations has mainly focused on lizards, mammals and birds. In these taxa, and in some groups of insects as well, islands show lower species diversity than areas of similar size on the nearby mainland (Whittaker, 1998; Blondel, 2000). At the intraspecific level, island populations are often characterized by a series of changes such as loss of genetic variability and changes in morphometric (size and shape), life-history (niche expansion, demography, etc.), behavioural (decrease in aggressiveness, etc.) and physiological traits. Together, these differences define the phenomenon known as the 'island syndrome' (Blondel, 2000). In this context, Frankham (1996) showed that a significant majority of island populations presented less genetic variability than their mainland counterparts, with an average reduction of 29%. These changes are the result of non-selective (mutation, genetic drift) and selective (adaptation to a new environment) microevolutionary processes (Adler & Levins, 1994; Blondel, 2000; Pergams & Ashley, 2001; Michaux *et al.*, 2002), and may occur rapidly (Frankham, 1997). For example, Roth (1990) reported that elephants on Sicily and Malta decreased in body mass by one order of magnitude within a few thousand years.

Island populations of parasites, on the other hand, have been little studied. Among the few existing studies, Mas-Coma *et al.* (1998, 2000) and Goüy de Bellocq *et al.* (2002) reported a loss of helminth species richness in micromammals of the Mediterranean islands. This decrease was correlated with the area of the island and with its distance from the mainland (Miquel *et al.*, 1996; Goüy de Bellocq *et al.*, 2002; Milazzo *et al.*, 2003). At the intraspecific level, Mas-Coma & Feliu (1984) and Goüy de Bellocq *et al.* (2002) showed that the host specificity of helminth parasites in the rodents *Mus musculus* and *Rattus rattus* decreased on Mediterranean islands compared with that on the nearby mainland. Similar examples of parasite niche expansion in island populations of *R. rattus* were reported for *Fasciola hepatica* in Corsica (Mas-Coma *et al.*, 1988) and *Schistosoma mansoni* in Guadeloupe (Theron & Pointier, 1995).

Consequently, it might be expected that the effects of insularity should exist in parasite populations because founder

hosts may reach islands with only a partial subset of their parasitic fauna. This should lead to a decrease in parasite species richness on islands and to a subsequent niche expansion, with parasitic species increasing their number of host species. At the intraspecific level, the possible existence of an 'island syndrome' affecting island parasite populations remains unexplored.

State of knowledge of the phylogeographies of *Heligmosomoides polygyrus* and *Apodemus sylvaticus*

Heligmosomoides polygyrus (Dujardin, 1845) is a specific and direct (i.e. without intermediate host) parasite of rodent hosts of the *Apodemus* genus in continental Europe. *Heligmosomoides polygyrus* reproduces exclusively sexually, and its first larval stages are free-living, requiring 4–6 days to become infective. Host infection occurs following ingestion of parasitized faeces. Adult parasites live in the intestine and produce eggs in the host faeces from about nine days after ingestion for up to nine months (Ehrenford, 1954).

A comparison of the phylogeographies of *H. polygyrus* and *A. sylvaticus* revealed significant temporal and spatial co-differentiation of the lineages of the two species in south-western Europe (Michaux *et al.*, 2003; Nieberding *et al.*, 2004, 2005). Indeed, both *H. polygyrus* and its host display similar genetic and geographical lineages in Spain, France, Belgium, Italy and North Africa, and both species survived the Quaternary ice ages in the Iberian and Italian refuges. Moreover, the island of Sicily, colonized from southern continental Italy, revealed a supplementary ancient refuge during the Quaternary cold stages for both species. At the end of the last ice age, both species recolonized Western Europe and North Africa from the Iberian refuge, while the Italian (and Balkan) populations of both species remained confined to their zone of origin, as a consequence of the Alpine and Carpathian mountain barriers, with low population densities.

Apodemus sylvaticus is the only *Apodemus* species currently and historically present on most of the Mediterranean islands (Majorca, Minorca, Corsica, Elba, Sardinia and Sicily), with the exception of Crete, where *A. mystacinus* is also present (Vigne, 1987, 1999; Flowerdew, 1991). It is, therefore, most likely that *H. polygyrus* colonized these islands by following only *A. sylvaticus*. On the basis of palaeontological and archaeozoological data, the colonization of the western Mediterranean islands by *A. sylvaticus* is known to date back to between 6000 and 4000 yr BP (Vigne, 1987) and to be of anthropogenic origin (Vigne, 1999). Indeed, the successive ancient human civilizations that practised commercial trade and presided over political affairs in the Mediterranean basin during the Holocene period were instrumental in the extinction of the endemic mammalian island faunas and in their replacement by species of continental origin, including *A. sylvaticus*. Moreover, genetic surveys of these island populations of *A. sylvaticus*, on the basis of cytochrome *b* (cyt *b*) gene sequences and restriction fragment length polymorphism

(RFLP) data, show that the Balearic populations (Majorca, Minorca) formed part of the Western European clade, while the Tyrrhenian islands (Corsica, Elba, Sardinia) and Crete formed part of the Italo-Balkan clade limited to the Italian, Balkan and Turkish regions (J. Michaux, unpubl. data; Michaux *et al.*, 1996a,b, 1998; Libois *et al.*, 2001).

Within this framework, the aim of this study was to evaluate whether a parasite species such as the nematode *H. polygyrus* could also present components of the 'island syndrome' in terms of loss of genetic diversity and (host) niche expansion on islands. For this purpose, the island colonization history of *H. polygyrus* was first established on the basis of mitochondrial *cyt b* gene sequences in seven western Mediterranean islands: Corsica, Crete, Elba, Majorca, Minorca, Sardinia and Sicily. Second, the levels of genetic diversity and host specificity were compared between island and mainland source populations of *H. polygyrus*.

MATERIALS AND METHODS

Collection of samples

A total of 100 *H. polygyrus* adult individuals (from 20 localities) were sampled on *A. sylvaticus* throughout the continental Mediterranean basin. Seventy of these had been collected in a previous study (Nieberding *et al.*, 2005), and this study adds samples from Slovenia (4), Morocco (9) and Turkey (17). In addition, 56 individuals were sampled from seven western Mediterranean islands: Corsica (6), Crete (10), Elba (10), Majorca (10), Minorca (10), Sardinia (1) and Sicily (9) (Table 1, Fig. 1). A further 10 individuals of the subspecies *H. p. corsicum*, originally described by Durette-Desset (1968), were sampled on *Mus musculus domesticus* in Corsica. All animals were identified at the species level by morphological determination. The *H. polygyrus* collection is conserved in the

Table 1 Geographical distribution, tissue collection codes, and EMBL accession codes of *H. polygyrus* (Dujardin, 1845) individuals sequenced for 690 bp of *cyt b* gene

Country	Region	Symbol	Sampling	Tissue collection code	EMBL accession code
France	Hérault	F1	5	CN 980-1 to 987-1	AJ608829-AJ608833
	Pyrénées Orientales (Banyuls/mer)	F2	6	CN 968-1 to 969-5	AJ608834-AJ608840
	Pyrénées Orientales (Py)	F3	6	CN 1139-1 to 1144-1	AJ608841-AJ608846
	Pyrénées Atlantiques	F4	2	CN 1295-1, 1295-6	AJ630643-AJ630644
Greece	Peloponnisos	Gr1	2	CN 1246-1 to 1246-3	AJ608916-AJ608917
	Thessalia	Gr2	1	CN 1221-1	AJ608915
Italy	Liguria	It1	13	CN 988-1 to 996-1	AJ608890-AJ608902
	Calabria	It2	5	CN 839-1 to 867-1	AJ608884-AJ608889
	Tuscany	It3	4	CN 1268-1 to 1268-4	AJ608880-AJ608883
Morocco	Haut Atlas (Setti Fatma)	Mr	9	CN 2539-1 to 2541-5	AJ971147-AJ971155
Slovaquia	Kosický kraj	Sa	6	CN 1103-1 to 1121-1	AJ608903-AJ608908
Slovenia	Vipava	Sl1	2	CN 1516-1 and 1516-2	AJ971193- AJ971194
	Pohorje, Goteniška gora	Sl2	2	CN 1524-1 and 1528-2	AJ971195- AJ971196
Spain	Avila	S1	4	CN 1211-1 to 1212-3	AJ608815-AJ608818
	Almería	S2	5	CN 1213-1 to 1214-2	AJ608819-AJ608823
	Cataluña	S3	6	CN 780-1 to 821-1	AJ608805-AJ608814
	Navarra	S4	5	CN 1215-1 to 1216-3	AJ608824-AJ608828
Turkey	Ayder	Tu1	9	CN 2237 to 2306	AJ971207-AJ971212, AJ971219-AJ971221
	Bozdag	Tu2	2	CN 2361 and 2367	AJ971213-AJ971214
	Ortan and Yolkiy	Tu3	6	CN 2396 to 2412	AJ971215-AJ971218, AJ971222-AJ971223
Islands	Crete	Cr	10	CN 1197-2 to 1218-1	AJ971197- AJ971206
	Elba	El	10	CN 1306-1 to 1306-10	AJ971182- AJ971191
	Corsica	CoA	6	CN 1299-1 to 1300-2	AJ971176- AJ971181
	Corsica on <i>M. m. domesticus</i>	CoM	10	CN 970-1 to 1336-1	AJ971224- AJ971233
	Majorca	Ma	10	CN 737-1 to 741-3	AJ971156- AJ971165
	Minorca	Mi	10	CN 760-1 to 770-2	AJ971166- AJ971175
	Sardinia	Sa	1	CN 1355-4	AJ971192
	Sicily	Si	9	CN 842-1 to 891-1	AJ608871-AJ608879
Outgroups	<i>H. kurilensis kobayashii</i>	Japan	1	CN 1451	AJ971146
	<i>Heligmosomum costellatum</i>	Spain	1	CN 1340	AJ971145

Newly obtained sequences are represented in bold italic. For symbols see Fig. 1. All *H. polygyrus* samples were obtained on *A. sylvaticus* hosts, with the exception of the Corsican population present on *M. m. domesticus* (CoM). CoA refers to *H. polygyrus* samples present on Corsican *A. sylvaticus* hosts.

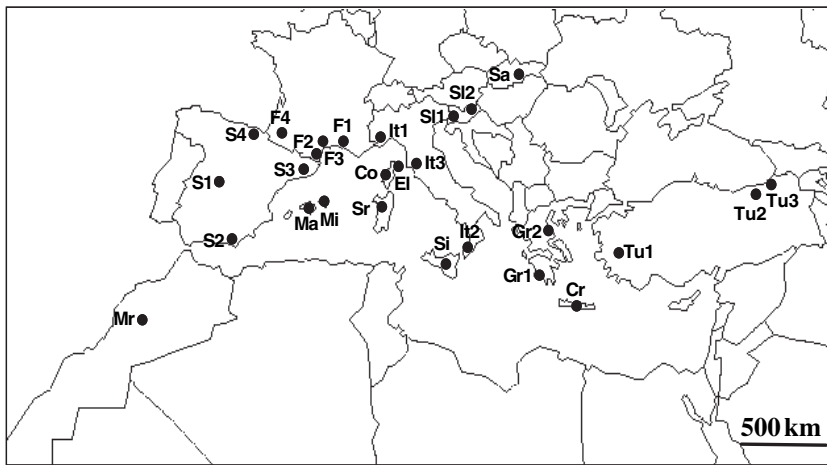


Figure 1 Geographical distribution of *H. polygyrus* (Dujardin, 1845) samples. The codes are as in Table 1.

collection of C. Nieberding housed at the Zoogeographical Research Unit (University of Liège, Belgium).

Morphological analyses

The subspecies *H. p. corsicum* was originally described by Durette-Desset in 1968 on *Mus musculus domesticus* in Corsica on the basis of its greater total body length and its higher number of longitudinal cuticular ridges (estimated at the middle of the nematode body) in comparison with *H. p. polygyrus sensu stricto*. In this study, these same morphological features were estimated on the Corsican *H. polygyrus* individuals in order to distinguish *H. p. polygyrus* from the *H. p. corsicum* subspecies on this island.

Molecular data analyses

Sequences data

Heligmosomoides polygyrus DNA extraction, amplification and sequencing of 690 base pairs (bp) of the *cyt b* gene were carried out as described in Nieberding *et al.* (2005). The polymerase chain reaction specific primers 1Fc (5'-GRAATTTTGGTAG-TATRTTRG-3') and 1R (5'-AGMACGYAAAATWGYAW-AAGC-3') were used for the island populations of Majorca, Minorca and Crete, the primers 4F (5'-TTCAGATTGTYACYGGYAC-3') and 4R (5'-ACGGTAAAATTGTATAAGC-3') for the island populations of Elba, Corsica, Sardinia and Sicily, and the primers 3F (5'-TTCAGATTGTGACAGGTA-3') and 3R (5'-TCACTCAGGSACAATATGAAC-3') for the subspecies *H. p. corsicum*. The primer 1F (5'-KCWGTGTRTTATYACTAG-3') was used with the primer 1R to amplify the last 400 bp of the 690 bp of the *cyt b* gene of the Moroccan and Turkish populations.

Phylogenetic and network analyses

Cyt b gene sequences were aligned using the MUST package (Philippe, 1993). The *H. polygyrus* data matrix comprised 166

sequences; *Heligmosomoides kurilensis kobayashii* Asakawa and Ohbayashi 1986, and *Heligmosomum costellatum* (Dujardin, 1845) were used as outgroups. *Heligmosomoides polygyrus* data were analysed by distance (neighbour joining, NJ) and maximum parsimony (MP) using the PAUP 4.0b8 package, by maximum likelihood using the PhyML package (Guindon & Gascuel, 2003) and by Bayesian-based inference as implemented in MrBayes 3.0 (Huelsenbeck & Ronquist, 2001). AIC in Model test version 3.06 (Posada & Crandall, 1998) was used to determine the best-fitting substitution model for the parasite data in the NJ and ML reconstructions, which was GTR + I + G. The Bayesian analysis was performed using the Metropolis-coupled Markov-chain Monte Carlo algorithm. The tree-space was explored four times by using one chain run during 1,000,000 generations. We used a general-time-reversible (GTR) model of sequence evolution allowing four among-site rate-variation categories. The robustness of inferences was assessed by bootstrap resampling (BT) (Felsenstein, 1985) using 10,000 NJ, MP and ML random repetitions. Bayesian posterior probabilities were obtained from the 50% majority rule consensus of trees sampled every 1000 generations, after removing trees obtained before chains reached apparent stationarity ('burn in' determined by empirical checking of likelihood values ranging between 120,000 and 400,000 generations).

Diversity analyses

From the complete *H. polygyrus* data matrix, nucleotide (π) and haplotype (h) diversities were estimated using the DnaSP vers. 4.0 program (Rozas & Rozas, 1997), while genetic divergence (GD) estimations were obtained using a distance analysis (Kimura's two-parameter distance estimator, K_2P) using the MUST package (Philippe, 1993). π and h diversities and mean GD were estimated for each (sub)clade defined by the phylogenetic and networks analyses, and for each island population, to estimate the possible loss of genetic diversity linked to the island colonization by *H. polygyrus*.

RESULTS

Heligmosomoides p. corsicum morphological identification

The total body length and the number of longitudinal cuticular ridges were estimated for *H. polygyrus* from Corsica in order to assign them to either the *H. p. polygyrus* or the *H. p. corsicum* subspecies (Fig. 2) (Durette-Desset, 1968). Individuals sampled on Corsican *M. m. domesticus* hosts displayed statistically greater body lengths and higher numbers of cuticular ridges than those sampled on Corsican or continental *A. sylvaticus* hosts (ANOVA test, $P = 0.04$) and were, therefore, assigned to the *H. p. corsicum* subspecies (CoM code in this study), while Corsican *H. polygyrus* individuals on *A. sylvaticus* hosts (CoA code) were considered to belong to the *H. p. polygyrus* subspecies, according to Durette-Desset (1968).

Phylogenetic relationships of *cyt b* gene haplotypes

A total of 142 haplotypes were identified among the 166 *H. polygyrus* *cyt b* sequences (EMBL accession numbers AJ608805 to AJ608917, AJ630628 to AJ630644, and AJ971145 to AJ971233). The matrix provided 690 bp, of which 270 sites were variable and 168 were parsimony-informative. The NJ, MP, ML and Bayesian reconstruction analyses were performed on the complete *H. polygyrus* data matrix with the outgroups *H. k. kobayashii* and *H. costellatum* (Fig. 3). As shown previously (Nieberding *et al.*, 2005), the continental populations of *H. polygyrus* form four genetic and geographical groups well supported by BT values (Fig. 3). The first group comprises the Italian populations (clade 1; BT of 99%, 90%, 94% and 1.00 in NJ, MP, ML and Bayesian analyses respectively). The endemic Iberian populations form the second clade (clade 2; BT of 100%, 91%, 94% and 0.99 respectively). The third clade covers south-western Europe and north Africa (Spain, France and

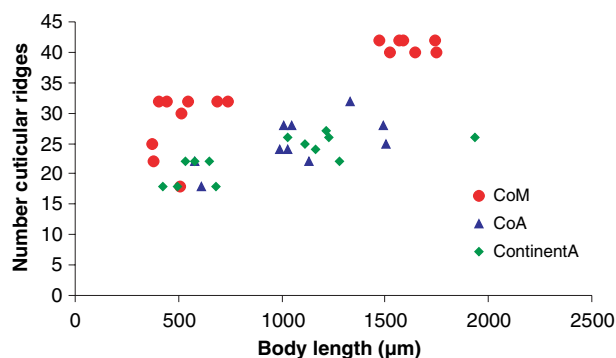


Figure 2 Graph comparing the body length (*x*-axis) and number of longitudinal cuticular ridges (*y*-axis) of adult *H. polygyrus*. Three groups of samples were considered: *H. polygyrus* on Corsican *M. m. domesticus* (CoM population); *H. polygyrus* on Corsican *A. sylvaticus* (CoA population); and *H. polygyrus* on continental *A. sylvaticus* (ContinentA population).

Morocco) (clade 3; BT of 100%, 96%, 93% and 1.00 respectively), and the fourth clade includes all Balkan populations (clade 4; BT of 100%, 100%, 98% and 1.00 respectively). In this study, a fifth clade appears, comprising the Turkish populations on *A. sylvaticus* and the Corsican populations on *M. m. domesticus* (clade 5; BT of 100%, 82%, 86% in NJ, MP, ML analyses respectively). A signal was found to link the Western European (clades 2 and 3) and Balkan clades (clade 4) (BT of 82%, 79%, 81% and 1.00 respectively); the relationships between clades 1, 5 and the complex (2, 3, 4) remain unclear. The first clade is further divided into two allopatric subclades: a first one corresponding to the northern part of Italy (subclade 1a; BT of 91%, 93%, 89% and 0.76 respectively), and a second one including the populations from southern Italy (subclade 1b; BT of 61%, 71%, 73% and 0.99 respectively). In addition, the Moroccan and southern Spanish (population S2) populations form a particular subclade within clade 3 (subclade 3a; BT of 95%, 98%, 95% and 1.00 respectively). The Moroccan population is monophyletic within this particular subclade (subclade 3a1; BT of 94%, 68%, 93% and 1.00 respectively).

The island parasite populations join the continental clades as follows (Fig. 3): the Majorcan (Ma) and Minorcan (Mi) populations fit into the Spanish endemic clade (clade 2) but do not form a specific subclade within it. The Sardinian, Elban and the Sicilian populations are part of the southern Italian subclade (subclade 1b), while the Corsican population present on *A. sylvaticus* is included in the northern Italian subclade (subclade 1a). More specifically, the Sardinian haplotype joins the Tuscan haplotypes (It3) (BT of 71%, 62%, 65% and 0.89 respectively); and the populations of Sicily (subclade 1b1; BT of 86%, 88%, 92% and 1.00 respectively) and Elba (subclade 1b2; BT of 89%, 88%, 85% and 1.00 respectively) form two distinct subclades that are well supported by bootstrap. In contrast, the Corsican *H. polygyrus* sequences from *A. sylvaticus* (CoA) are dispersed within the northern Italian clade. The population of Crete (Cr) is included within the Balkan clade (clade 4). Interestingly, the population of *H. p. corsicum* (CoM) present specifically on *M. m. domesticus* in Corsica forms a distinct, but weakly supported, subclade (subclade 5a; BT of < 50%, < 50%, 64% and 0.98 respectively) within the Turkish clade of *H. polygyrus* populations hosted by *A. sylvaticus*.

Diversity analyses

Comparison of the genetic diversity values on islands with the mainland source populations

Estimations of mean GD, π and *h* diversities for each continental (sub)clade, as defined by the phylogenetic analyses, and for the island populations were performed to assess whether the colonization of islands induced a loss of genetic diversity in *H. polygyrus* (Table 2). The continental populations of Italy (clade 1) and western Europe (clades 2 and 3) show high levels of mean GD, π and *h* typical of stable

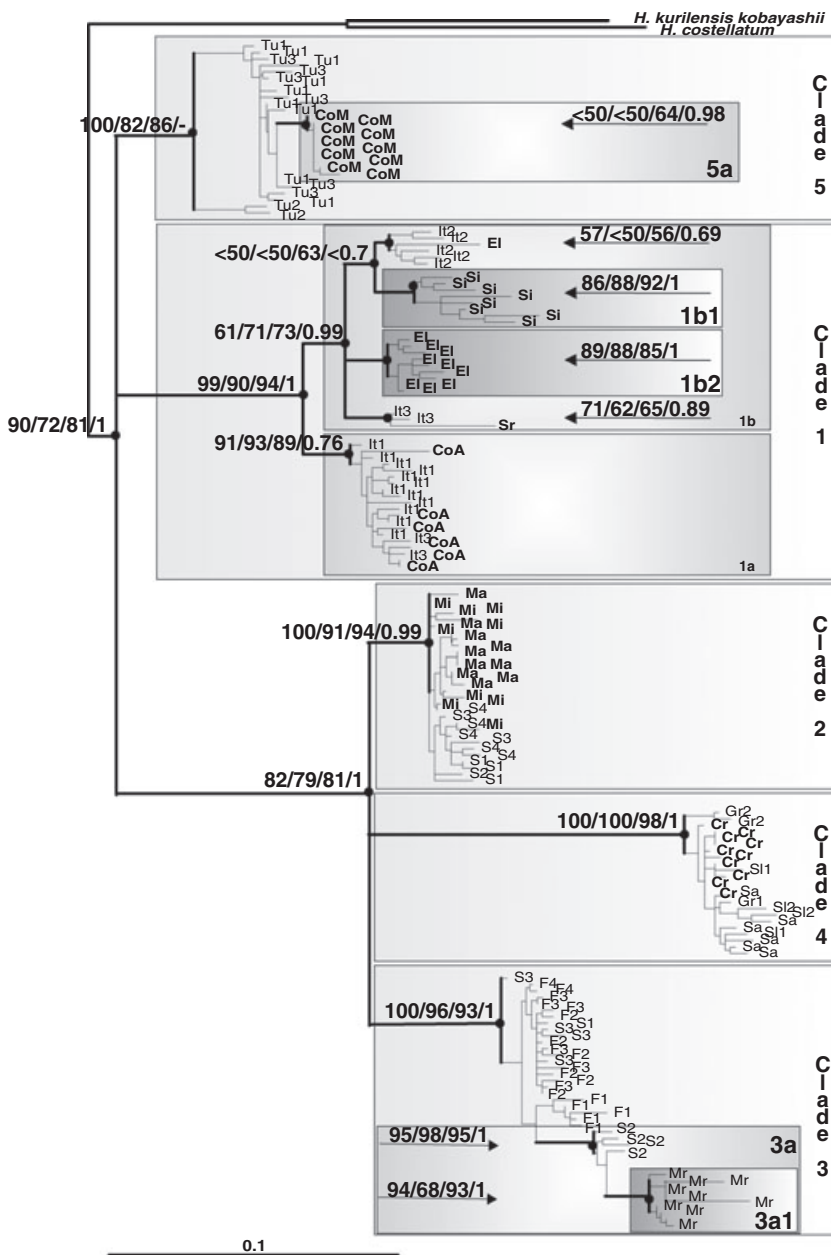


Figure 3 Most likely tree of the PhyML reconstruction for the 166 mtDNA sequences and the outgroups *H. kurilensis kobayashii* Asakawa and Ohbayashi 1986 and *H. costellatum* (Dujardin, 1845), represented by their geographical origin (see Table 1 and Fig. 1). Numbers on branches indicate, from left to right, bootstrap support obtained in the (a) NJ analysis (GTR + I + G); (b) MP analysis; (c) PhyML analysis; and (d) posterior probabilities in the MrBayes analysis. Note that bootstrap values under 50% and posterior probabilities under 0.7 were not considered.

populations with large long-term population sizes (Avice, 2000). In contrast, the Balkan (clade 4), Turkish (clade 5) and Moroccan (subclade 3a1) populations show lower levels of GD and π , which may reflect past genetic bottlenecks or recent population expansion from a small number of founder individuals. Within the islands, only Sicily displays levels of GD, π and h that are comparable to the Italian and western European values (Student's *t*-test, Table 2). All other Mediterranean islands display lower values of GD and π than mainland source populations ($P < 0.01$, Student's *t*-test). The average reduction of the genetic variability of *H. polygyrus* is 55.5% of GD, 54.8% of nucleotide (π) diversity, and 21.82% of haplotype (h) diversity. The Corsican (CoA) and Elban parasite populations show intermediate GD, π and h values, ranging between 1.47 and 1.61, 0.13 and 0.19, and 0.933 and

1.000, respectively; their h diversity values are not significantly lower than mainland source ones (Student's *t*-test). In contrast, the Corsican population on *M. musculus* (CoM), and the Cretan and Majorcan populations show significantly lower GD, π and h diversities values than mainland source populations ($P < 0.01$, Student's *t*-test). GD, π and h values range between 0.20 and 0.67, 0.002 and 0.005, and 0.533 and 0.844, respectively. The case of Minorca is noteworthy: GD and π values are intermediate, but a low h ($P < 0.01$, Student's *t*-test) diversity is observed; this could result from a transient bottleneck in a large ancestral population, because a sudden reduction in population size can eliminate many haplotypes without necessarily impacting π severely (Avice, 2000). It is noteworthy that no island presents genetic diversity values higher than those of mainland source fauna, which confirms

Table 2 Genetic variability observed within the continental lineages and the various islands of *H. polygyrus* (Dujardin, 1845). Student's *t*-tests were performed to test for the significance of the differences in GD, π and *h* diversity values observed between island and mainland source populations

Mainland	Sampling	Nb		GD (%)	π (SD)	<i>h</i> (SD)	Island	Sampling	Nb		GD (%)	Student's <i>t</i> -test	π (SD)	<i>h</i> (SD)	Student's <i>t</i> -test
		Sampling	Ht						Sampling	Ht					
Clade 1	22	21	21	2.59 (0.01)	0.024 (0.003)	0.996 (0.015)	-	-	-	-	-	-	-	-	-
Clade 1a	15	14	14	2.67 (0.01)	0.025 (0.001)	0.996 (0.028)	Corsica (CoA)	7	6	1.47 (0.01)	0.019 (0.003)	<i>P</i> < 0.01	0.933 (0.122)	n.s.	
Clade 1b	7	7	7	2.38 (0.01)	0.023 (0.003)	1.000 (0.076)	Elba	10	10	1.61 (0.01)	0.013 (0.005)	<i>P</i> < 0.01	1.000 (0.040)	n.s.	
							Sardinia	1	1	-	-	-	-	-	-
							Sicily	10	10	2.57 (0.01)	0.021 (0.002)	n.s.	1.000 (0.040)	n.s.	
Clade 2	11	11	11	1.99 (0.01)	0.018 (0.001)	1.000 (0.040)	Majorca	10	5	0.67 (0.01)	0.005 (0.001)	<i>P</i> < 0.01	0.844 (0.080)	<i>P</i> < 0.01	
							Minorca	10	5	1.43 (0.02)	0.013 (0.001)	<i>P</i> < 0.01	0.667 (0.163)	<i>P</i> < 0.01	
Clade3	35	26	26	2.84 (0.02)	0.027 (0.002)	0.965 (0.017)	-	-	-	-	-	-	-	-	
Clade 3a1	9	5	5	1.12 (0.01)	0.009 (0.003)	0.833 (0.098)	-	-	-	-	-	-	-	-	
Clade 4	13	13	13	1.79 (0.01)	0.017 (0.001)	1.000 (0.003)	Crete	10	7	0.47 (0.01)	0.004 (0.001)	<i>P</i> < 0.01	0.911 (0.077)	<i>P</i> < 0.01	
Clade 5	17	15	15	1.57 (0.01)	0.010 (0.001)	0.889 (0.046)	Corsica (CoM)	10	4	0.20 (0.01)	0.002 (0.001)	<i>P</i> < 0.01	0.533 (0.180)	<i>P</i> < 0.01	

Nb, number; Ht, haplotype; GD, genetic divergence; π , nucleotide diversity; *h*, haplotype diversity; SD, standard deviation. CoM, *H. polygyrus* population present on Corsican *M. m. domesticus* hosts; CoA, *H. polygyrus* population present on Corsican *A. sylvaticus* hosts.

that the colonization of these islands by *H. polygyrus* took place from a unique mainland source region.

Relationships between genetic diversity and the size and degree of isolation of islands

Correlation degrees were estimated between the genetic diversity values (GD, π and *h*) of island *H. polygyrus* populations and islands' area or distance from their mainland source populations (Tables 2 and 3). No significant correlation was observed either between *H. polygyrus* GD, π or *h* island diversity values and island area, or between *H. polygyrus* GD or π diversity values and distance from source mainland (the determination coefficient, *r*², ranged between 0.08 and 0.43; data not shown). In contrast, a significant negative relationship was found between island haplotype diversity values (*h*) and the logarithm of the islands' distance from source mainland (Fig. 4; the determination coefficient, *r*², was 0.73, *P* < 0.02). This result was confirmed by a permutation test of island haplotype diversity values against island isolation (*r*² = 0.69; *P* = 0.003). This result shows that the decrease in the genetic variability of island populations of *H. polygyrus* is correlated with the geographical isolation of the corresponding islands.

DISCUSSION

Colonization pattern of the western Mediterranean islands by *Heligmosomoides polygyrus*

As shown by the phylogenetic analyses (Fig. 3), the continental origins of *H. polygyrus* settlements in the Balearic and Tyrrhenian islands, in Sicily and in Crete, are the same as those of its host *A. sylvaticus* (see Introduction): south-western Europe is the continental source for the Balearic populations, and the Italo-Balkan region is the continental source for the Tyrrhenian, Sicilian and Cretan populations of both species. These results extend the continental spatial congruence in the seven western Mediterranean islands for both species (Nieberding *et al.*, 2004).

Table 3 Area (column 2) and distance from mainland source regions (column 3) of the seven Mediterranean island *H. polygyrus* populations sampled

Island	Area (km ²)	Distance (km)	Source-mainland
Corsica (CoA)	8722	85	Clade 1a
Crete	8335	98	Clade 4
Elba	223	25	Clade 1b
Majorca	3640	173	Clade 2
Minorca	702	200	Clade 2
Sicily	25,460	3	Clade 1b
Corsica (CoM)	8722	1600	Clade 5

CoM, *H. polygyrus* population present on Corsican *M. m. domesticus* hosts; CoA, *H. polygyrus* population present on Corsican *A. sylvaticus* hosts.

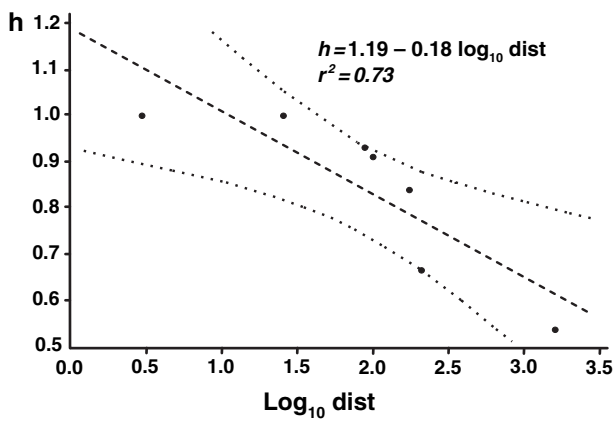


Figure 4 Relationship between *H. polygyrus* (Dujardin, 1845) haplotype diversity values (h) and the decimal logarithm of the distance of the island from the mainland source (dist) (see Tables 2 and 3). The coefficient of determination (r^2) and the equation of the correlation line are given. The variation of the slope of the correlation line within the 95% confidence interval is represented (dashed lines).

Heligmosomoides polygyrus presents a more diversified and more complex differentiation pattern than its host, which allows the specification of the continental geographical origin of the island populations of this parasite. Indeed, *H. polygyrus* data highlighted that the Balearic populations clustered within the endemic Iberian clade (clade 2), rather than within the western European one (clade 3) that is also present in France. In addition, neither of the two island populations of the parasite formed distinct subclades within clade 2, or were differentiated from it, and both displayed low intrapopulation genetic diversity values. These results indicate a recent parasite – and host – colonization event of these islands from the Iberian Peninsula. In the same way, genetic differentiation between populations of *H. polygyrus* from northern Italy (from Genoa to Sienna) and southern Italy (from Sienna to Calabria) allow us to specify the affinity of the Corsican *H. p. polygyrus* population on *A. sylvaticus* (CoA) with the northern part of Italy. Sardinia joined Tuscan sequences, and Elba formed a distinct subclade (subclade 1b2) within the southern Italian subclade. The Sicilian phylogeographical history of both species is distinctive: the differentiation of the Sicilian populations of both species was shown to be ancient, occurring from 0.55 ± 0.24 to 0.90 ± 0.15 Ma in both species. An old lineage in both species probably survived in southern Italy and entered Sicily during the last glaciation, 70,000 yr BP. The level of genetic diversity is very high in both species in Sicily, suggesting that this island may act as a hot spot of intraspecific biodiversity (for more details, see Michaux *et al.*, 2003; Nieberding *et al.*, 2004, 2005). Finally, the Cretan population of *H. polygyrus* belongs to the Balkan clade, which spreads from continental Greece to Slovenia. As the Cretan populations of both species present a low level of genetic diversity and are not genetically differentiated from the continental Balkan populations, they were probably recently introduced from mainland Greece, following anthropogenic

colonization of the island. It is noteworthy that one other *Apodemus* species is present on Crete, namely *A. mystacinus* (Flowerdew, 1991). Cretan *A. mystacinus*, however, originates from the Middle East, and not from the Balkan region (Michaux *et al.*, 2005). Consequently, *A. mystacinus* is unlikely to have been involved in the colonization of Crete by *H. polygyrus*.

Parasites and the ‘island syndrome’

At the intraspecific level, the colonization of islands has been shown to induce important changes in several species-specific traits, such as the level of population genetic diversity, or the enlargement of the ecological niche, in numerous free-living species. These evolutionary changes are known as the ‘island syndrome’ (e.g. Whittaker, 1998; Blondel, 2000). In this study, the determination of the precise continental origin of each *H. polygyrus* island population has permitted evaluation of the consequences of colonization of the Mediterranean islands on two species-specific traits: the level of genetic diversity and the extension of the ecological niche.

Loss of genetic diversity in island populations of *Heligmosomoides polygyrus*

In comparison with mainland source populations, all Mediterranean island populations of this parasitic nematode species present lower levels of genetic diversity (except Sicily, see Table 2) and no genetic differentiation (except Sicily, Elba and Sardinia, see Table 2), as could be expected for recently isolated populations. The average reduction of *H. polygyrus* genetic variability is 55.5% for GD, 54.8% for π diversity, and 21.82% for h diversity. In comparison, the average reduction of genetic diversity on islands of free-living vertebrate species was 29% according to Frankham (1996). To our knowledge, this is the first empirical evidence for the existence of this component of the ‘island syndrome’ in a parasite nematode species. Loss of genetic diversity in island populations is usually explained by a founder effect: as island colonization is carried out by only a small number of individuals, the founding event involves a loss of part of the continental genetic pool (Frankham, 1996).

In contrast to this general scenario, Sicily presumably acted as a refuge during Pleistocene glaciations (see discussion point 1). This theory is supported by Goüy de Bellocq *et al.* (2003), who showed that the parasite species richness of *A. sylvaticus* was higher in Sicily (14 species) than in Calabria (11 species) and other Mediterranean islands. These results confirm the role of Sicily as a ‘hotspot’ of genetic diversity for the woodmouse and its associated endofauna. The differentiation of Elban parasites may reflect the fact that the island was recently colonized by a number of mainland haplotypes of the parasite. No conclusion can be proposed for Sardinia, from where a unique sample was analysed in the present study.

The loss of genetic haplotype diversity was related to the geographical distance between island and mainland source populations ($r^2 = 0.73$) but not to island area ($r^2 = 0.08$ –

0.43) (Fig. 4). In agreement with the model of island biogeography proposed by MacArthur & Wilson (1967), this result indicates a decrease in the immigration flow of *H. polygyrus* proportional to the distance of the island from the mainland source area. This result is rather surprising because the colonization of the western Mediterranean islands by *H. polygyrus* and its host was not natural but took advantage of various anthropogenic influences.

Enlargement of the ecological niche of Heligmosomoides polygyrus in Corsica

The ecological niche of a parasite is defined by the range of hosts that it is able to infest. From extensive parasitological data, *H. polygyrus* is known to be specific for the *Apodemus* genus throughout the continental western Palaearctic distribution range (Durette-Desset, 1968; Göüy de Bellocq *et al.*, 2002; C. Nieberding, unpubl. data); notably, the prevalence of (i.e. the percentage of hosts infected by) *H. polygyrus* and its abundance (i.e. the average number of parasites per host) on *A. sylvaticus* are respectively 50% and 11 (Göüy de Bellocq *et al.*, 2002, 2003). Infestation of other species by *H. polygyrus* is extremely rare on the mainland (Durette-Desset, 1968, pers. comm.; C. Nieberding, unpubl. data). However, a significant exception to this host specificity is observed in Corsica, where *H. polygyrus* is abundant on both *A. sylvaticus* and *M. m. domesticus* (Durette-Desset, 1968; C. Nieberding, unpubl. data). The general infestation of these two distinct host species suggests that *H. polygyrus* underwent an enlargement of its ecological niche during colonization of Corsica. Analysis of the populations of *H. polygyrus* present in the two hosts revealed that they are genetically different (Fig. 3; *c.* 10% of GD in K_2P distance estimation), as a result of their different continental origins. Indeed, *H. polygyrus* populations present on *A. sylvaticus* originated in continental Italy, while Corsican parasite populations on *M. m. domesticus* came from Turkey. This study shows that the *H. polygyrus* populations on Corsican *M. m. domesticus* and Turkish *A. sylvaticus* represent the *H. p. corsicum* subspecies described by Durette-Desset (1968) (Fig. 4).

Which scenario might explain the passage of *H. polygyrus* on *M. m. domesticus* in Corsica? On the basis of palaeontological and archaeozoological data, it is known that *A. sylvaticus* and *M. musculus* colonized the island of Corsica, respectively, *c.* 6000 and *c.* 3000 yr BP, when the local ancestral and archaic endemic fauna was replaced by modern species of anthropogenic origin (Vigne, 1987, 1999). Therefore, both lineages of *H. polygyrus* must have reached Corsica within the last few thousand years. The *H. p. corsicum* lineage may have reached Corsica from Turkey with *A. sylvaticus*, the native local host, or, less likely, with an atypically contaminated *M. m. domesticus* host. Several pieces of evidence support the first hypothesis.

(1) As mentioned earlier, *M. musculus* is not infected by *H. polygyrus* on the mainland; Middle East *M. musculus* populations are instead parasitized by *Heligmonoides josephi*

(Wertheim & Durette-Desset, 1976), which out-competes *Heligmosomoides* in *M. musculus* as a consequence of niche competition (Wertheim & Durette-Desset, 1976).

(2) *Mus musculus domesticus* probably colonized Corsica through continental Italy rather than directly from the Middle East (Navajas Y Navarro & Britton-Davidian, 1989; Vigne, 1999). Therefore, invading *M. m. domesticus* individuals would probably have lost the Turkish *H. polygyrus* population before reaching Corsica.

(3) Turkish *A. sylvaticus* and their *H. p. corsicum* parasites might have reached Corsica with the Phoenicians, who had coastal trading settlements in Corsica and Turkey *c.* 3000 yr BP, as continental Italian *A. sylvaticus* and *H. polygyrus* were already established in Corsica at that time.

Whatever the scenario, the presence of abundant *H. polygyrus* populations on both *A. sylvaticus* and *M. m. musculus* host species in Corsica is an interesting case of niche enlargement of a nematode parasite, and the second line of evidence of an island syndrome in *H. polygyrus*.

CONCLUSIONS

In conclusion, the phylogeographical study of the colonization by *H. polygyrus* of seven western Mediterranean islands extends the congruence previously observed between the phylogeographies of the parasite and its host, *A. sylvaticus*, in parts of continental Europe. The determination of the precise continental origin of the island populations of this parasite also sheds light on how a nematode parasite may evolve components of the island syndrome at the intra-specific level, namely (1) a general loss of genetic diversity in six of the seven studied islands, where loss is related to island distance from the mainland source area, and (2) a niche enlargement on the island of Corsica, where both Corsican *M. m. domesticus* and *A. sylvaticus* populations are parasitized by *H. polygyrus*. To our knowledge, this study is the first to highlight strong empirical genetic evidence for the existence of an island syndrome in a parasitic nematode species.

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BIOSKETCHES

This work represents part of **Caroline Nieberding's** PhD work, which deals with a comparison of the European phylogeographical patterns of the nematode *Heligmosomoides polygyrus* and the rodent *Apodemus sylvaticus*. C. Nieberding is currently a postdoctoral fellow in Professor Paul Brakefield's laboratory (Leiden University, the Netherlands), working on the genetic basis of the variability in pheromone production of the African butterfly *Bicyclus anynana*.

Serge Morand is interested in host–parasite co-evolution and diversification and has contributed throughout this PhD program.

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