Mar Biol (2008) 153:421–429
DOI 10.1007/s00227-007-0820-z

RESEARCH ARTICLE

Completely disjunct mitochondrial DNA haplotype distribution without a phylogeographic break in a planktonic developing gastropod

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Received: 29 January 2007 / Accepted: 7 September 2007 / Published online: 2 October 2007

Abstract Planktonic developing organisms are generally assumed to be good dispersers showing little genetic structuring in neutral markers. At first glance, this also applies to the planktonic developing periwinkle *Tectarius striatus*, an endemic gastropod from Macaronesia (i.e. Azores, Madeira, Canary Islands and Cape Verde Islands), where the only sign of genetic structuring hitherto is provided by a non-significant allozyme/RAPD heterogeneity between the Cape Verde Islands and the other archipelagos. However, partial sequences of the mitochondrial cytochrome *b* and cytochrome oxidase I genes now show that the Cape Verde Islands and the three other archipelagos have no haplotypes in common, whereas the latter three do share several haplotypes. Nevertheless, this highly disjunct haplotype distribution does not entail a phylogeographic break separating the haplotypes of both areas in two reciprocally monophyletic groups. This remarkable geographic and phylogenetic structuring may be explained by assuming that *T. striatus* colonized the Macaronesian archipelagos in periods when sea levels were lower (and/or volcanic activity was higher), so that seamounts peaked above sea level and could act as stepping-stones. Yet, after the last glacial period seamounts submerged, thus preventing further stepping-stones mediated dispersal of *T. striatus* between the Cape Verde Islands and the other archipelagos, while not affecting dispersal among the latter because of their closer proximity and connectivity. Hence, these contrasting patterns of neutral genetic variation in *T. striatus* show that genetic structuring in planktonic developing species may be far more complex than is usually assumed.

Communicated by M. Wahl.

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Introduction

It still is a widely held conclusion that planktonic developing organisms are good dispersers that are characterized by extensive gene flow and very limited degrees of genetic structuring (Hedgecock 1986; Warner 1997; Avise 2004). Yet, a growing number of studies indicate that, in contrast to this general expectation, planktonic developing organisms may show surprising amounts of geographic subdivision (Reeb and Avise 1990; Palumbi 1996; Avise 2004). Particularly the application of DNA markers has been instrumental in uncovering geographic subdivision in planktonic developing species (Reeb and Avise 1990; Palumbi 1996; Avise 2000; Taylor and Hellberg 2003; Avise 2004). Such intraspecific geographic DNA surveys often show a correspondence between spatial heterogeneities in haplotype distributions and the presence of

phylogeographic breaks (i.e. concurrence of a genealogical divide and a geographic barrier) and/or cryptic taxa (Barber et al. 2002; Hellberg et al. 2002; Lee and Ó Foighil 2004; Jolly et al. 2005; Waters et al. 2005).

Tectarius striatus (King and Broderip, 1832) (Mollusca: Caenogastropoda) is an endemic periwinkle living in the rocky intertidal of Macaronesia [i.e. Azores (AZ), Madeira (MA), Canary Islands (CA) and Cape Verde Islands (CV)]. It produces pelagic larvae with planktotrophic development (Reid 1996). The species shows a considerable degree of environmentally and geographically related phenotypic variation in shell sculpture, form and size (e.g. Rosewater 1981; Reid 1996; De Wolf et al. 1997). Shells from wave exposed shores tend to be larger, are more globose, have larger apertures, and usually lack external sculpture. In contrast, shells from sheltered sites are smaller, are less globose, have smaller apertures and are more nodulose (De Wolf et al. 1997). In addition, irrespective of these microgeographic patterns of phenotypic variation, shells from CV are larger, are heavier, have larger apertures, and show a larger morphological variance, than shells from the three other archipelagos (De Wolf et al. 1998a). However, neither the microgeographic, nor the macrogeographic patterns of shell variation are accompanied by significant allozyme or RAPD heterogeneities (De Wolf et al. 1998b, c, d, 2000). Nevertheless, numbers of private alleles tend to suggest that populations from CV are genetically more diverse than elsewhere (De Wolf et al. 2000). Likewise, specimens from CV tend to express on average more electrophoretic esterase bands than elsewhere (De Wolf et al. 1998c). Finally, RAPD data suggest that CV populations tend to be more heterozygous than elsewhere (De Wolf et al. 1998b). Although none of these molecular data yielded statistically significant results, they nevertheless suggested a tentative, weak differentiation between the genetically slightly more diverse CV and the three other archipelagos (De Wolf et al. 1998b, 2000). This pattern agrees with the larger morphotypic variance in CV populations compared to elsewhere. Because (1) *T. striatus* appears genetically and morphometrically more diverse in CV, (2) Cape Verde Islands are the oldest Macaronesian formation, and (3) the oldest fossil of *T. striatus* is known from tertiary CV deposits (Reid 1996); De Wolf et al. (2000) hypothesized that CV are the centre from where *T. striatus* colonized the other Macaronesian islands. In the present contribution, we test this hypothesis and attempt to reconstruct the phylogeography of this species on a current range-wide scale by analysing mtDNA variation, since mtDNA sequences in similar studies have proven to be very suitable to this end (Avise 2000, 2004; Sá-Pinto et al. 2005; Pepper et al. 2006; Domingues et al. 2007).

Materials and methods

Sampling sites and collection

A total of 109 specimens of *T. striatus* were collected between August 1991 and January 2001 from 11 islands, representing the four Macaronesian archipelagos, and thus covering the entire geographical range of the species (between 39°41′ and 14°20′N and 31°13′ and 13°38′W) (Fig. 1). After collection, the animals were preserved in absolute ethanol or were stored at –80°C until further analysis.

DNA extraction and cyt *b* and COI mtDNA amplification

Individual genomic DNA was extracted from the radular or foot muscle following a CTAB protocol (Winnepenninckx et al. 1993), modified so that tissues were not ground under liquid nitrogen, but directly incubated in CTAB buffer. For problematic samples (i.e. poor yield with CTAB extraction) the QIAamp DNA Mini Kit (Westburg b.v., Leusden, The Netherlands) was used. Cyt *b* was amplified using "*Littorina* specific" primers 14915 (5′-TTGCAATAC ACTACACAG-3′) and 15515 (5′-ATGAGAAATTTT CAGGGTC-3′) (Reid et al. 1996). COI amplifications used general primers LCO1490 (5′-GGTCAACAAATCATAA AGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGG TGACCAAAAAATCA-3′) (Folmer et al. 1994). For the amplifications of cyt *b* and COI the same PCR cycling profile was used. It consisted of an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 1 min, annealing at 40°C for 1 min, elongation at 70°C for 2 min and a final elongation at 72°C for 5 min. PCR products were purified using the GFX^{TM} PCR DNA and Gel Band Purification Kit (Amersham Biosciences, Roosendaal, The Netherlands) and then sequenced in both directions using an automatic sequencer.

Alignment and haplotype network estimation

Sequences of both mtDNA genes were aligned using ClustalX 1.83 (Thompson et al. 1997) and checked visually. Both gene fragments were concatenated since mitochondrial gene fragments are presumed to behave as a single locus. The haplotypes for this combined dataset were identified using DnaSP version 4.00.0 (Rozas et al. 2003). Haplotype diversity was calculated using the software Arlequin 2.001 (Schneider et al. 2000), after which the Tukey–Kramer multiple comparisons test was performed using GraphPad Instat Version 3.05 for windows, 2000

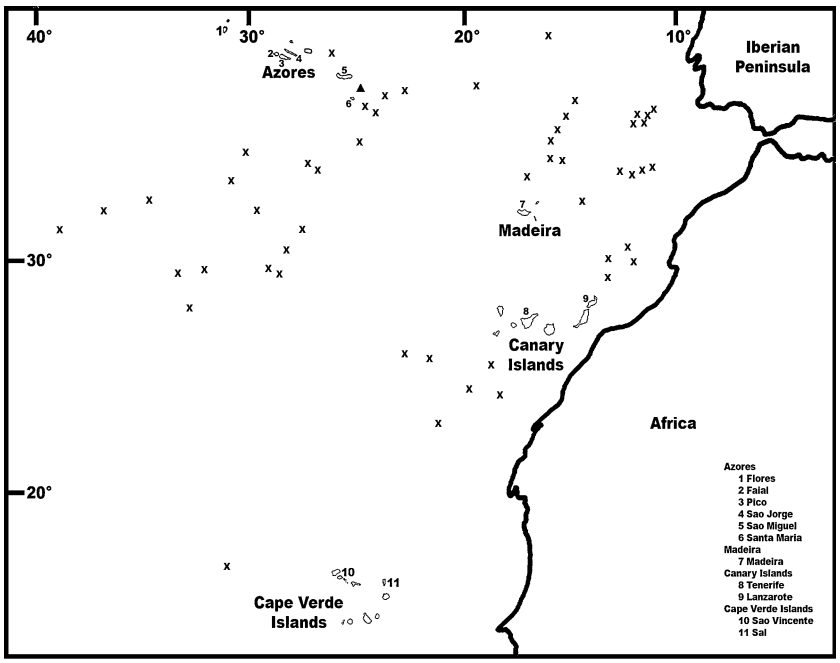

Fig. 1 Overview of Macaronesia. The locations of seamounts are indicated by *X* and the position of the Formigas Islets is shown by the *triangle*

(GraphPad Software, San Diego, California, USA) to check for significant differences between haplotype diversities. Networks of the concatenated haplotypes were constructed using the MINSPNET (http://cmpg.unibe.ch/services/software.htm) and TCS 1.18 (Clement et al. 2000) software which are respectively based on the number of pairwise nucleotide differences and the estimation of gene genealogies from DNA sequences (i.e. statistical parsimony) (Templeton et al. 1992). Loops that occurred in the constructed networks were resolved by (1) treating connections with central and frequent haplotypes to be more likely than connections with singletons and/or rare haplotypes (Posada and Crandall 2001) and (2) using the geographical criterion, favouring connections between haplotypes of the same or an adjacent population over connections with haplotypes from more distant geographical areas (Pfenninger and Posada 2002). The exclusion of loops from a haplotype network may introduce interpretative biases since (1) excluding loops by favouring connections with central or frequent haplotypes (Posada and Crandall 2001) will tend to assign affected haplotypes to interior or older clades, while (2) the geographical criterion (Pfenninger and Posada 2002) may cause a bias towards isolation by distance. Finally, Modeltest 3.5 (Posada and Crandall 1998) was used to select the sequence evolution model that best fits the data and a maximum-likelihood tree was generated using the selected evolution model. Hereafter this phylogeny was compared to the resolved network.

NCA and test for geographical association

The 95% plausible set for all haplotype linkages estimated by TCS 1.18 was converted into a nested design. Combined haplotypes separated by a single mutation were grouped together in one-step clades, carrying on from the tips to the interior of the network. Subsequently, the resulting clades were grouped again into two-step clades under the same principle, and so on, until the final level of nesting encompassed the entire network. The rules for constructing the nested cladogram are described by Templeton (1998) and references therein. Based on an exact permutational contingency test performed for every clade at each nesting level, GeoDis 2.2 (Posada et al. 2000) calculated a χ^2 statistic to test for possible associations between clades and geographical location. Furthermore, GeoDis 2.2 performed a more elaborate analysis using information on geographical distances based on the geographical coordinates of each archipelago, which can be interpreted according to the guidelines provided by Templeton (1998). In addition, hierarchical analysis of molecular variance (AMOVA) was used to partition the observed genetic variation among and within archipelagos. Pairwise F_{ST} values were used to estimate the amount of genetic differentiation among archipelagos. Estimates of genetic variance and differentiation were tested for significance using nonparametric permutational tests as implemented by Arlequin 2.001 (Schneider et al. 2000). Gene flow was estimated based on M values (= Nm for haploid populations).

Results

Characteristics of cytochrome *b* (cyt *b*) and cytochrome oxidase I (COI) mtDNA from *T. striatus*

The cyt *b* and COI fragments in this study were 482 bp and 511 bp long, respectively. The combined dataset comprised 993 bp of which 19 (1.91%) were missing data. There were 71 (7.15%) variable positions, 37 (3.73%) of which were parsimony informative. Fifty-three haplotypes were identified (Table 1) and all haplotype sequences have been deposited in the GenBank database under Accession numbers DQ021959–DQ022064. They showed 0.10–1.61% sequence divergence. The haplotype distribution over the four archipelagos is given in Table 2. Strikingly, none of the CV haplotypes was observed in the three northern archipelagos (Table 2). Yet, four haplotypes (i.e. 01, 11, 17 and 28) were shared by AZ, MA and CA, while three other haplotypes (i.e. 23, 33 and 42) were shared by two of the three northern archipelagos (Table 2). Of course, it is not excluded that these shared haplotypes still differ in the parts of the mtDNA that were not screened in this study. The most common haplotype (i.e. 01) occurred in 28.40% of individuals from the northern archipelagos. The most common haplotype of CV (i.e. 10) was observed in 21.43% of the individuals. The haplotype diversities in AZ, MA, CA and CV were 0.810 (SD 0.079), 0.929 (SD 0.030), 0.931 (SD 0.030) and 0.942 (SD 0.031), respectively. These results suggest that the highest haplotype diversity occurred in CV, and the lowest in AZ. However, only this latter value was significantly different from the others ($P < 0.001$).

Nested clade analysis (NCA)

The networks calculated by the programs MINSPNET and TCS 1.18 (95% connection limit) were identical and showed two loops. These two loops consisting of several missing haplotypes were resolved using the rules described by Posada and Crandall (2001) and Pfenninger and Posada (2002). Furthermore, Modeltest 3.5 selected the model of Hasegawa et al. (1985) as providing the best fit and therefore this model was used to generate a maximum-likelihood tree. The obtained maximum-likelihood tree showed the same structure and haplotype relationship as the resolved network. The NCA showed significant associations between geographical locations and two of the higher level clades (4-2 and 3-3), which could be interpreted using the inference key according to Templeton (1998) (Fig. 2; Table 3). No significant associations were found for clades representing recent events (i.e. one- and two-step clades), except for clade 2-9. This clade shows recent contiguous range expansion from clade 1-20 (CV) to clade 1-22 (CV) and clade 1-21 (AZ and MA). Furthermore, continuous range expansion was found for the two-step clades nested within clade 3-3 suggesting that clade 2-6, comprising of haplotypes from the four archipelagos but mainly from CV, was the origin for clades 2-7 (MA and CV) and 2-8 (MA and CA). Even though significant associations were found for ancient events (i.e. clades nested in 4-2 and the total cladogram), these associations were not so straightforward as in the lower level clades. Furthermore, the percentage of haplotypes in tip clades (Templeton 1998; Mardulyn 2001; Tiedemann et al. 2004) was calculated at the two-step level of the NCA. It was lowest in CV (3/28 = 11%) and increased through MA (12/28 = 43%) and CA (14/28 = 50%) to a maximum in AZ (14/25 = 56%). This difference among archipelagos in the frequency of haplotypes in tip clades was significant ($\chi^2 = 14.0$; $df = 3$; $P = 0.003$). A similar pattern was detected at the three-step level of the NCA (leaving clades 3-2 and 3-3 as the only interior clades). Also at this level CV had the lowest percentage of haplotypes in tip clades (10/28 = 36%), while MA and CA had intermediate values (both 19/28 = 68%), and the highest value was observed in AZ (21/25 = 84%). Again, the difference among archipelagos was significant ($\chi^2 = 14.3$; $df = 3$; $P = 0.003$).

Analysis of molecular variance

Further evidence of population genetic structure was revealed by AMOVA and F_{ST} values (Table 4). A highly significant genetic differentiation among the archipelagos was observed using AMOVA ($P < 0.001$), which was entirely due to heterogeneity between CV and the three northern island groups (Table 4). Consequently, estimates of gene flow (M) between CV and the three northern archipelagos were remarkably low (Table 4).

Discussion and conclusions

In contrast to previous allozyme/RAPD markers (De Wolf et al. 1998b, 2000), our mtDNA data suggest that present day gene flow between CV and the three other archipelagos must be very limited, if not entirely absent. Nevertheless, De Wolf et al. (2000) explained the weakly, but non-significant, higher allozyme/RAPD diversity in CV by assuming unidirectional gene flow such that CV acts as a sink accumulating genes from the northern archipelagos via prevailing southward oceanic surface currents, without exchanging genes in the opposite direction. However, the absence of shared haplotypes between CV and the northern archipelagos suggests that the barrier to gene flow must be

Table 1 *Tectarius striatus*. Variable positions in 53 haplotypes (01 to 53)

	Cytochrome b	Cytochrome oxidase subunit I
	1111111122222222333333333444444	1112222233333344444444
	1123445899901456789224567990122444567001 12	1246712501357026889223347 9
	6342703821473587951309413158863506781639257	3984217107837260131460325152
01	GATTTTGTAACTCATATCTGATCTTACTCGGTCCGATCTTGAC	ATAGCACGGTCTCTAATTCATGTTAGAC
02	.....	.G....G.....
03	.....	.G...GGA.....G.....
04	.....G.....	..G.....G.....
05	C.....G.....	.....G.....C.....
06	.....G.....	.....G.....
07	.....C.....G.....	.....C..G.....T
08	.....C..C.....G....A..	.....T...G.....
09	.....G.....G.....	.....G.....C....
10	.....G....A..	.....G.....
11	.....	.....A.....G.....
12	.....C..T.....T...G..T....AG.....	.....C.....A....T.
13	.....T.....T....T...G.....G...C..T	.....C.G.....A.....
14	.....C.....G.....	.....A.....G.....
15	.....C.....	.....G.....A...A..
16	.....G.....G.....	.....G.....
17	.....A...T.....G.....	....T.....G.....
18	.....C.....G.....	.....G.....
19	.....G..C.A..	...A.....
20	.....A.....	.....A.....G.....
21	...C.....C.....G.....	.....G.....
22	.....G.....	.....
23	.....C.....G....A..	.....T...G.....
24	..A....C..T.....T...G..T.....G.....	T.....C.....A....T.
25	.....G.....	.....G.....C.....
26	.....A.....G.....G.....	.....G..C.....
27	.....G..C....	...A.....G...
28	.....C..T.....T...G..T.....G.....	.....C.....A.....
29	.....A.....G.....T..G.....	.....G..C.....
30	.G.....	.....
31	....G....GT..G.G.....T...G.....G.....	.....C..C....A.....
32	...C.....C.....G.....	.....A.....G.....
33	.....A.....G.....G.....	.....T.G..C....C.....
34	.....T.....A.....G.....G.....	.....G..C.....
35	.....CC..T.....T...G..T.....G.....	.....C.....A.....
36	.....A.....	.....A.....G.....
37	.....G.....	.....A.....G.....
38	.....G.....	.....G.....C....
39	.....	.....A..T...G.....
40	.....G.	.....
41	.....G.....	..G.....G...T.....
42	.....C.....C.....T...G..T.G...G.....	.....C.....A.....
43	.....T.....	.....A.....G.....
44	.....C..T.....TC..G..T.....G.....	.....C.....A.....
45	...C.....C.....GC.....	.....G.....
46	.....G.....	...A.....C....G.....
47	.....G.....G.....	.....G....T...C....
48	.....C.....T.....G....A..	.....T...G.....
49	.....G.....T..G.....G.....	.....G.....
50	.....	.....C.....
51	.....T.....A.....G.T.....	...A.....G.....
52	.....C.....G....A..	.....A.....G.....
53	.....C.....G.....	.....G.....

Numbers refer to positions along the 482 bp cyt *b* and 511 bp COI fragment

very effective in both directions. If this strong barrier to gene flow has persisted for a long time, it is expected to produce a phylogeographic break involving reciprocal monophyly of the CV and the northern haplotype groups (Hellberg et al. 2002). Yet, such pattern is not apparent (Fig. 2). Therefore, we hypothesize that the barrier to gene flow between CV and the northern archipelagos must have been recently established. This scenario assumes that the planktonic stage of *T. striatus* does not last long enough to allow for long distance dispersal following the prevailing clockwise currents without stepping-stones. The fact that for similar geographic distances (about 1,200 km), there is substantial gene flow between CA and AZ, but not so between CA and CV (Table 4), indicates that the presence of stepping-stones between CA and AZ (e.g. MA) may indeed facilitate long distance dispersal, whereas at present there are no similar stepping-stones connecting CA with CV.

Despite the current barrier to gene flow between CV and the northern archipelagos, it is evident that at some stage in the past *T. striatus* must have dispersed between both areas. De Wolf et al. (2000) proposed in this context that *T. striatus* spread from CV in pre or early Miocene times via westward oceanic surface currents (i.e. the North

Table 2 *Tectarius striatus*. Sampled islands for all four archipelagos, number of individuals collected and haplotypes identified (GenBank Accession nos. DQ021959–DQ022064) are given

Island	Archipelago	Shared haplotypes									Haplotypes (no. individuals)	Sample size
		01	11	17	28	23	33	42	06	21		
Flores	AZ	1			1						05	3
Faial	AZ		1	1		1					50	4
Pico	AZ	3									08	4
São Jorge	AZ	1	1	1							22	4
São Miguel	AZ	6									24	7
Santa Maria	AZ										37, 41, 48	3
Madeira	MA	6	4	3	3	1	1	1			03, 25, 29, 30, 32, 34, 36, 39, 46	28
Tenerife	CA	1	1	2	2		2				02, 20, 27, 35, 44, 47	14
Lanzarote	CA	5	2	2				1			12, 26, 31, 40	14
São Vicente	CV								3	1	04, 07, 09, 14, 15, 18, 19, 49, 52, 53	14
Sal	CV								1	1	10 (6), 13, 16, 38, 43, 45, 51	14

The shared haplotypes are shown and the number of individuals representing these haplotypes at each island is given

Haplotypes that only occurred at a single sampled island are listed by their haplotype number and number of individuals found at this sampling site are given between brackets if it exceeds one

AZ Azores, *MA* Madeira, *CA* Canary Islands, *CV* Cape Verde Islands

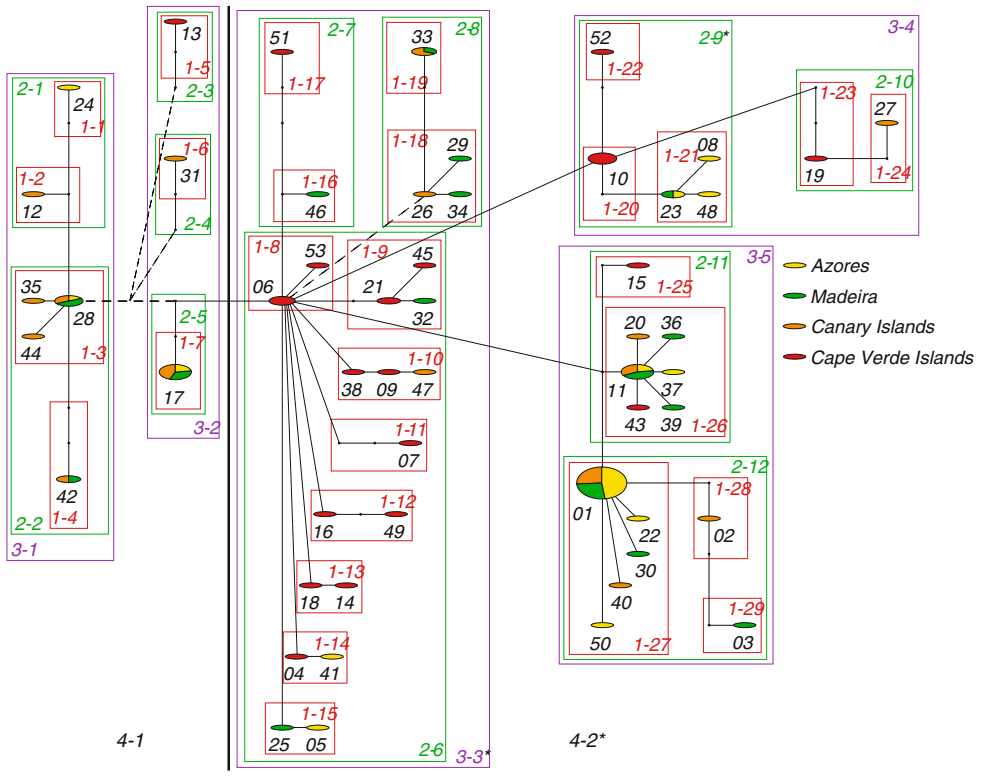

Fig. 2 Network and nested clade structure of the combined cyt *b* and COI mtDNA haplotypes of *Tectarius striatus*. Identical networks were independently calculated by the programs MINSPNET and TCS 1.18 (95% connection limit). They initially showed two loops which were resolved using the rules described by Posada and Crandall (2001) and Pfenninger and Posada (2002) and by generating a maximum likelihood tree based on the model of Hasegawa et al. (1985). The different haplotypes are represented by the *coloured circles* and their frequencies are represented by the areas in these circles. *Black dots* indicate missing haplotypes. *Full lines* connecting haplotypes represent single-mutation differences, while *striped lines* indicate that there are several mutational differences between the connected haplotypes. *$P < 0.0100$; Total Cladogram $P = 0.0130$

Table 3 Interpretation of the results of Fig. 2 using the inference key according to Templeton (1998)

Clade	Chain of inference	Inferred demographic event	Archipelagos involved/direction
2-9	1-2-11-12-NO	Contiguous range expansion	From CV to AZ, MA and CV
3-3	1-2-11-12-NO	Contiguous range expansion	From CV to all archipelagos
4-2	1-2-3-5-6-13-14-NO	Long distance colonisation and/or past fragmentation	All Archipelagos
Total	1-2-3-5-6-7-8-YES	Restricted gene flow/dispersal but with some long distance dispersal over intermediate areas not occupied by the species; or past gene flow followed by extinction of intermediate populations	All Archipelagos

AZ Azores, *MA* Madeira, *CV* Cape Verde Islands

Table 4 *Tectarius striatus*. Pairwise F_{ST} values calculated using Tamura and Nei genetic distances (Tamura and Nei 1993) are shown below the diagonal (*$P < 0.001$). Above the diagonal, *M* values calculated from genetic divergence data are given

	AZ	MA	CA	CV
AZ	–	289.91455	18.42776	3.11023
MA	0.00172	–	∞	5.11992
CA	0.02642	–0.00790	–	3.27855
CV	0.13850*	0.08897*	0.13233*	–

M equals Nm for haploid populations

AZ Azores, *MA* Madeira, *CA* Canary Islands, *CV* Cape Verde Islands

Equatorial Current), so that larvae finally reached AZ (and from there MA and CA) via the Caribbean and the Gulf Stream. This scenario is, however, untenable because: (1) there is no trace of *T. striatus* in the Caribbean (Reid 1996; personal observations), (2) in the absence of stepping-stones, the dispersal distances involved are much larger than those which at present already seem to prevent gene flow (i.e. the 1,200 km separating CV from CA), and (3) the presence of *T. striatus* in early Pliocene deposits of southern Spain (Landau et al. 2003), implies even more improbably large dispersal distances under the De Wolf et al. (2000) hypothesis.

Although the preceding colonization model seems unrealistic as a whole, we do maintain that CV is probably the ancestral area from which the other archipelagos were colonized. This assumption appears legitimate because (1) CV tends to be the genetically most diverse area (based on the congruence between allozymes, RAPD, and mtDNA data), (2) CV haplotype 06 occupies the central position in the haplotype network, suggesting that it may be ancestral to the others (Templeton 1998; Posada and Crandall 2001), (3) haplotypes at tips in a network (here mainly from AZ, MA and CA, see percentage of haplotypes in tip clades) are most likely younger than the interior haplotypes (such as haplotype 06) to which they are connected (Templeton 2004), and (4) the haplotype with the largest number of descendants (such as haplotype 06) is most likely the oldest (Posada and Crandall 2001). Nevertheless, high frequency haplotypes probably have been present in a population for a long time, having had a chance to achieve substantial copy numbers (Excoffier and Smouse 1994). Hence it cannot be ruled out that because of their higher frequencies, several northern haplotypes (particularly haplotype 01) may be older than CV haplotype 06. Nonetheless, we still regard CV as the ancestral area, since (1) CV and CA comprize the oldest Macaronesian islands (Jurassic or Cretaceous origin) (Mitchell-Thomé 1976), and (2) *T. striatus* is known from Tertiary deposits in CV (Reid 1996), i.e. long before elsewhere in Macaronesia.

Accepting CV as the ancestral area of *T. striatus* in Macaronesia and rejecting the westward dispersal routes proposed by De Wolf et al. (2000), the northern archipelagos must have been colonized via a counterclockwise, northeastward trajectory. In this way, *T. striatus* would have colonized CA from CV, as was also suggested for the seaweed *Cladophoropsis membranacea* (van der Strate et al. 2002), while CA acted as source for further northward dispersal to MA, AZ and probably even southern Spain. Seamounts may have played a crucial role in this historical long distance dispersal scenario by providing the necessary (temporary) stepping-stones (Gillet and Dauvin 2000; Gad and Schminke 2004) among the northern archipelagos and between MA and the Iberian Peninsula (Geldmacher et al. 2001), while a group of seamounts situated south of CA (Fig. 1) provided a connection with CV (Mitchell-Thomé 1976). Some seamounts were probably volcanic islands at some point in time (Geldmacher and Hoernle 2000), whereas others may have emerged above sea level during glacial periods. In either case they provided a suitable intertidal rocky shore habitat for *T. striatus*, as is currently exemplified by the Formigas islets, northeast of the island of Santa Maria (AZ) (Fig. 1) (Ávila and Azevedo 1997). However, although planktonic developers, such as *T. striatus*, may easily reach relatively distant, isolated spots, they may have difficulties to establish stable populations in

the absence of continuous surface currents (Johannesson 1988). Unfortunately, we have no data on historical oceanic current patterns in Macaronesia. Nevertheless, at present there are a number of permanent and seasonal counterclockwise sea currents connecting different archipelagos (Mourino et al. 2002; Schiebel et al. 2002; Hernandez-Guerra et al. 2003). The NCA is in line with the presumed counterclockwise, seamount mediated colonization scenario, since the four-step clades within the entire network reflect either long distance dispersal over intermediate areas not occupied by the species or past gene flow followed by extinction of intermediate populations (Table 3). The more "patchy" haplotype relationships in the lower level clades then reflect more recent long distance colonization or past fragmentation events caused by rising sea levels and the concomitant submergence of seamounts. Furthermore, the complexity of the network suggests that several historical colonization events may have taken place independently, while the F_{ST} based non-zero gene flow estimate between CV and the northern archipelagos (Table 4) is probably due to effects of past gene flow and the absence of haplotypic fixation in both areas, rather than to on-going dispersal, since CV and the northern archipelagos currently do not share any haplotype.

Finally, the contrasting patterns of neutral genetic variation in *T. striatus*, combined with the remarkably disjunct haplotype distribution between CV and the northern archipelagos without a phylogeographic break separating the haplotypes of both areas in two reciprocally monophyletic groups, confirm that planktonic developing organisms can show unexpectedly complex patterns of geographic subdivision (Reeb and Avise 1990; Palumbi 1996; Avise 2004).

Acknowledgments This work was financially supported by the following grants to T. Backeljau: EU-project EVK3-2001-00048 "EUMAR", OSTC-project MO/36/008, FWO-project G.0235.02, and UA-project RAFO/1 BACKT KP02. H. De Wolf is a Postdoctoral Fellow of the Fund for Scientific Research, Flanders (Belgium) (F.W.O.). This paper benefited from discussions during the CORONA-meeting in Plymouth (2004).

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