Phylogeography of a mite, *Halozetes fulvus*, reflects the landscape history of a young volcanic island in the sub-Antarctic

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Previous studies of the microarthropods of Marion Island, Southern Ocean, documented high mitochondrial COI (cytochrome *c* oxidase subunit I) haplotype diversity and significant genetic structure, which were ascribed to landscape subdivision. In this paper we revisit these ideas in light of new geomorphological evidence indicating a major lineament orientated along N26.5°E. Using the microarthropod *Halozetes fulvus*, we test the hypothesis that the eastern and western sides of the island show different population genetic patterns, corresponding to the previously unrecognized geological separation of these regions, and perhaps also with differences in climates across the island and further landscape complexity. Mitochondrial COI data were collected for 291 *H. fulvus* individuals from 30 localities across the island. Notwithstanding our sampling effort, haplotype diversity was under-sampled as indicated by rarefaction analyses. Overall, significant genetic structure was found across the island as indicated by Φ_{ST} analyses. Nested clade phylogeographical analyses suggested that restricted gene flow (with isolation-by-distance) played a role in shaping current genetic patterns, as confirmed by Mantel tests. At the local scale, coalescent modelling revealed two different genetic patterns. The first, characterizing populations on the south-western corner of the island, was that of low effective population size and high gene flow. The converse was found on the eastern side of Marion Island. Taken together, substantial differences in spatial genetic structure characterize *H. fulvus* populations across Marion Island, in keeping with the hypothesis that the complex history of the island, including the N26.5°E geological lineament, has influenced population genetic structure.   © 2011 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2012, **105**, 131–145.

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INTRODUCTION

The complex biological and geological history of the southern hemisphere is emerging as a common biogeographical theme (see, for example, Bergstrom & Chown, 1999; Chown & Convey, 2007; Convey & Stevens, 2007). Once thought to be a consequence mostly of vicariance, geographical distributions across the hemisphere have now been shown also to include signals of dispersal and local speciation and extinction events (e.g. Sanmartín & Ronquist, 2004; de Queiroz, 2005; Allegrucci *et al.*, 2006; Grobler *et al.*, 2006, 2011a; Stevens *et al.*, 2006; Sanmartín, Wanntorp & Winkworth, 2007; McGaughran *et al.*, 2010a; Mortimer *et al.*, 2011). At smaller spatial scales, isolation-by-distance characterizes some

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species, in others a clear record of vicariance associated with glaciation is present, whilst in a few cases, recent dispersal events have been proposed (Skotnicki, Ninham & Selkirk, 2000; Fanciulli *et al.*, 2001; Frati, Spinsanti & Dallai, 2001; Skotnicki *et al.*, 2001; Coulson *et al.*, 2002; Stevens & Hogg, 2003; Myburgh *et al.*, 2007; Rogers, 2007; Hawes *et al.*, 2008).

Whether these patterns are congruent amongst taxa is not yet clear, largely owing to the paucity of investigations. However, biogeographical complexity appears to be characteristic of at least some species on the sub-Antarctic islands and, in most cases, this complexity has been ascribed to the multifaceted histories of Southern Ocean islands including repeated episodes of volcanism and glaciation. For example, Myburgh *et al.* (2007; see also McGaughran *et al.*, 2010b) documented high mitochondrial COI (cytochrome *c* oxidase subunit I) haplotype diversity and significant genetic structure in two indigenous springtail species from Marion Island (these studies included 113 individuals of *Cryptopygus antarcticus travei* from ten localities and 40 individuals of *Tullbergia bisetosa* from ten localities), and suggested that this may be a consequence of landscape subdivision by volcanic and glacial events on the island. Similar findings of significant genetic structure across Marion Island were reported for the prostigmatid mite *Eupodes minutus* (57 individuals from 11 localities; Mortimer & Jansen van Vuuren, 2007), the cushion plant *Azorella selago* (Mortimer *et al.*, 2008), and for members of the *Ectemnorhinus* group of weevils (Grobler *et al.*, 2006, 2011a, b). These studies suggest that landscape evolution has substantially affected the phylogeography of species on the island in a way similar to landscape effects on species of other islands such as Rapa and the Hawaiian and Canary archipelagos (Paulay, 1985; Wagner & Funk, 1995; Gillespie, 2004; Emerson *et al.*, 2006). In the latter cases, volcanism and the pattern and timing of lava flows have largely been responsible for genetic structure and speciation in the taxa concerned. The ways in which population genetic structure may be affected by topography, distance, and the complexity of the environment are recognized as significant for understanding spatial patterns in diversity, as is now recognized generally within landscape genetics (e.g. Storfer *et al.*, 2010).

The reasons for such highly structured patterns in haplotype diversities on Marion Island remain unknown, as do the ways in which this structure may reflect the island's geological history. Although some relationship is evident between the geological age (*c*. 450 000 years) and evolution (i.e. the timing of volcanic and glacial events) of the island (see McDougall, Verwoerd & Chevallier, 2001) and popu-

lation differentiation and demographic expansions as determined using standard molecular clock approaches (Grobler *et al.*, 2006, 2011b; Mortimer & Jansen van Vuuren, 2007; Myburgh *et al.*, 2007), no previous geological interpretations (summarized in Hall, 2002) fully explain why substantially different population processes appear to characterize the eastern and western sides of the island (as described in Mortimer & Jansen van Vuuren, 2007; Myburgh *et al.*, 2007; McGaughran *et al.*, 2010b). Nyakatya & McGeoch (2008) also report that microclimate temperatures, when considered at different altitudes as well as on the eastern and western sides of Marion Island, vary significantly. However, the extent to which these temperature differences shape genetic patterns has not been fully investigated, although a single preliminary finding has provided weak support for the idea that life-history effects via variation in metabolic rate may be involved (McGaughran *et al.*, 2010b). In addition, the original investigations were characterized by relatively small sample sizes for each locality, and for each of the studies as a whole, precluding firm inferences.

Therefore, to provide further insight into the likelihood that complex landscape evolution on Marion Island is reflected in invertebrate phylogeography, we investigate an additional species endemic to the Prince Edward Islands (comprising Marion and Prince Edward Island, the latter which lies *c*. 22 km north-east of Marion Island), namely the oribatid mite *Halozetes fulvus*, using a much larger sample size [the largest number of individuals studied to date is 113 for the springtail species *Cryptopygus antarcticus travei* (McGaughran *et al.*, 2010b) compared with the 291 *H. fulvus* individuals included in the present study]. In essence, we test the hypothesis that the eastern and western sides of the island show very different phylogeographical patterns, resulting from a previously unrecognized geological feature of these regions, namely a major linear geological feature which links the western scarp of the Sfinks/Feldmark Plateau massif and the western scarp of Long Ridge (see below). We interpret data for *H. fulvus*, and previous phylogeographical information on mites and springtails, in the context of new findings concerning the geological evolution of the Marion Island landscape. Whilst we initially established phylogeographical hypotheses based on previous interpretations of the geology of the island (such as the geographical position of lava flows and glaciations), it quickly became apparent that this approach was simplistic because of the rapid development of new geological insights that might also be informed by phylogeographical work (Boelhouwers *et al.*, 2008; Hall, Meiklejohn & Bumby, 2011). Thus, we elect to pursue a strategy of reciprocal

clarification of phylogeographical and geological history (following Emerson *et al.*, 2006).

If the geological history of the island has an impact on invertebrate populations, and particularly the considerable variability in haplotype diversity and its spatial structuring, then one or more of three major patterns might be expected. First, genetic patterns that are largely driven by adaptive processes associated with temporal and geochemical differences in eruptive events that gave rise to the eastern and western successions of grey lava. However, geochemical differences between these lava flows are only slight (Kable, Erlank & Cherry, 1971). Second, spatial segregation among populations might be expected across the N26.5°E lineament that separates the eastern and western sections of the island (see Geology of Marion Island below), largely driven by the difficulty of routinely crossing this barrier. Finally, some structure might also be related to the fact that the island was extensively glaciated during the Last Glacial Maximum (LGM) (Hall, 2002; Boelhouwers *et al.*, 2008; Hall *et al.*, 2011), with some high grey lava areas

possibly serving as refugia, culminating in a patterns of higher genetic diversity in areas where refugia might have existed with connectedness among populations as individuals re-colonize available habitat.

MATERIAL AND METHODS

GEOLOGY OF MARION ISLAND

Marion Island is a young intra-plate shield volcano, with the oldest dated lavas being approximately 450 kya (McDougall *et al.*, 2001). The island comprises a basaltic geology from eight episodes of volcanic activity, the youngest (Holocene) lavas being less than 10 kya (McDougall *et al.*, 2001). The two eruptive centres, situated some 4 km apart in the centre of the island (Verwoerd, 1971; Chevallier, 1986; McDougall *et al.*, 2001), have given rise to distinctive grey lava successions, identified as 'Eastern' (found predominantly on the northern and eastern side of Marion Island) and 'Western' (found on the western side of Marion Island) (see Fig. 1) (Verwoerd, 1971;
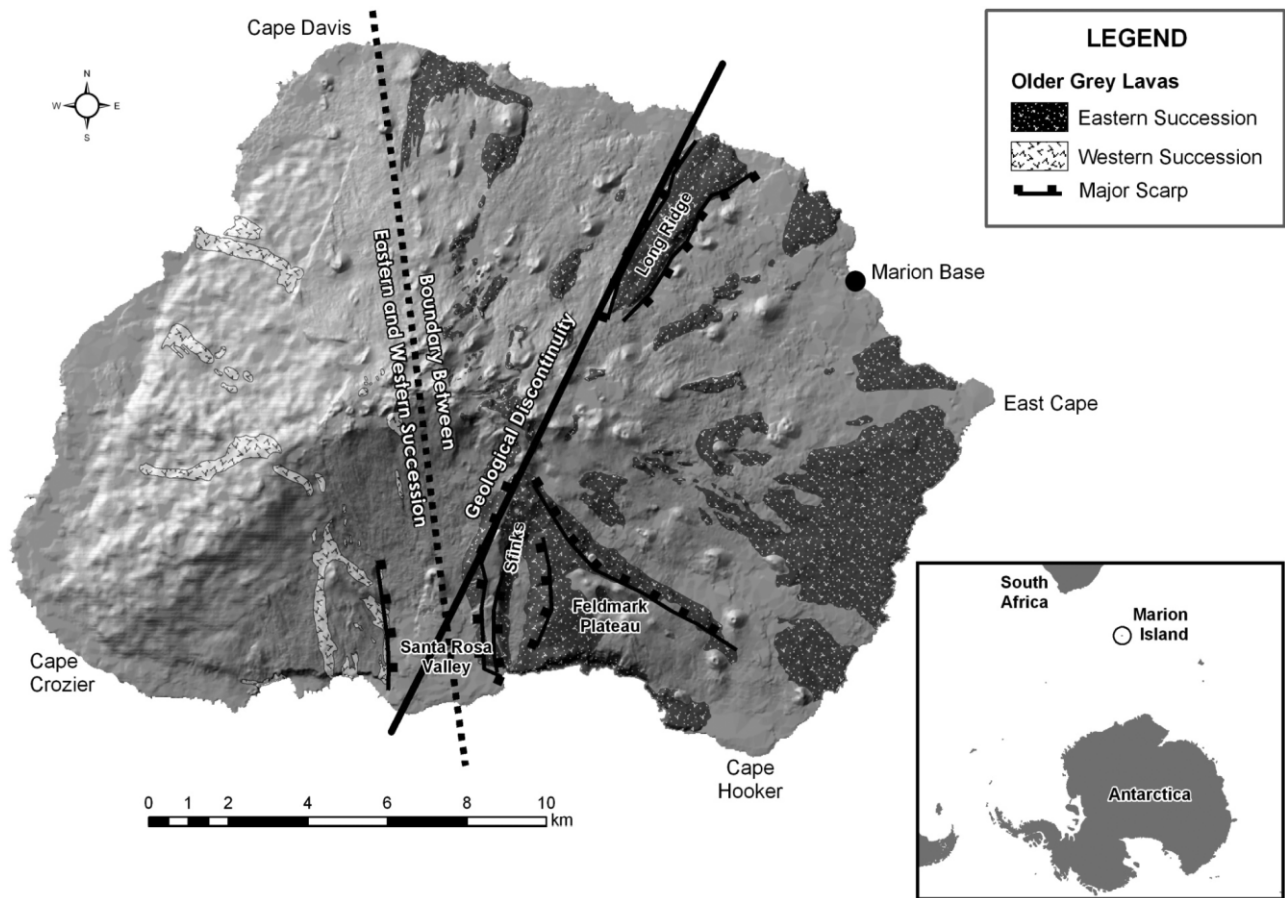

Figure 1. Schematic geological map of Marion Island overlain on a digital elevation model (from Meiklejohn & Smith, 2008), showing the N26.5°E geological discontinuity, and the boundary between eastern and western grey lava successions.

McDougall *et al.*, 2001). The surface topography of Marion Island is dominated by Holocene black lavas and scoria cones from the most recent volcanic episode.

Visually, the most prominent topographical features on Marion Island are the Santa Rosa Valley and two areas of higher relative relief, the Feldmark Plateau and Long Ridge (Fig. 1). Although the origins of these structures have been debated (see Verwoerd, 1971; Hall, 1982; Chevallier, 1986; McDougall *et al.*, 2001; Hall *et al.*, 2011), a requirement for their formation is the existence of a geological discontinuity that forms the boundary of the landform. To identify topographical lineaments (linear features that expresses an underlying geological structure, which are most often revealed by aerial photography, satellite imagery and hill-shaded digital elevation models), a 20-m-resolution shaded digital elevation model of Marion Island was built using the methods described in Meiklejohn & Smith (2008). It is clear that a major lineament links the western scarp of the Sfinks/Feldmark Plateau massif and the western scarp of Long Ridge, and which roughly corresponds to the eastern boundary of the volcanic rift zone identified by Chevallier (1986). The orientation of the lineament (N26.5°E) (Fig. 1) is close to the general structural trend of the island (N20°E, see Chevallier, 1986) and the general trend of the Prince Edward Fracture Zone, which marks the continental plate boundary.

SAMPLE COLLECTION AND LABORATORY PROTOCOLS

In total, 291 *Halozetes fulvus* individuals were collected from 30 localities across Marion Island during the 2004 and 2005 relief voyages (see Fig. 2; Table 1). They were sampled from plant material including mosses (*Ditrichum strictum*), tussock grass (*Poa cookii*), and other flowering plants (*Azorella selago, Cotula plumosa*, and *Crassula moschata*). Specimens were extracted by sieving and preserved in absolute ethanol. Because the west-central parts of the island are difficult to access and exceptionally rugged in places, and the central interior part is characterized by both surface and sub-surface ice, sampling was not undertaken here.

Total genomic DNA was extracted from individual mites using the QIAamp DNA micro kit (Qiagen, Hilden, Germany). For this study, the 5′ side of the mitochondrial COI gene was targeted using the universal primers described by Folmer *et al.* (1994). Although several papers have questioned the neutrality of mitochondrial DNA (Ballard & Kreitman, 1995; Ballard & Whitlock, 2004), the COI gene has proven to be a useful marker to describe genetic variation in microarthropods (Emerson, Oromi & Hewitt, 2000; Salomone *et al.*, 2002; Stevens & Hogg, 2003; Mor-

timer & Jansen van Vuuren, 2007; Myburgh *et al.*, 2007). All the PCR reactions were performed using 10 ng of DNA under the following cycling parameters: 94 °C for 1 min, 35 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and a final extension step at 72 °C for 5 min. PCR products were gel purified with the Wizard SV Gel and PCR clean-up system (Promega, Madison, WI, USA) and sequencing with the forward LCO1 primer (Folmer *et al.*, 1994) was performed with half-reactions (4 μL) of BigDye Terminator 3.1 mix (Applied Biosystems, Warrington, UK). Purified sequencing products were run on an ABI 3100 automated sequencer (Applied Biosystems). All sequences with ambiguous nucleotides were re-sequenced with the reverse HCO1 primer (Folmer *et al.*, 1994). In addition, 20% of all individuals were sequenced twice to verify the accuracy of the results. All the unique *H. fulvus* haplotypes generated in this study were deposited in GenBank (DQ883230–DQ883330).

SEQUENCE ANALYSIS

DNA sequences were aligned by eye and haplotypes were identified using Collapse 1.2 (Posada, 2004). In keeping with the suggestion of Lowe, Harris & Ashton (2004: 66) that rarefaction techniques can be used to estimate haplotype diversity, ecological methods were also used to estimate the extent to which the majority of haplotypes had been sampled and how many haplotypes might exist in the population. Specifically, the non-parametric Chao 2 and Jacknife 2 estimators were calculated and an individual-based rarefaction curve (see Gotelli & Colwell, 2001; Magurran, 2004) was constructed for *H. fulvus* using the EstimateS vs. 7.51 software (Colwell, 2005). These methods have traditionally been applied either for estimating the likely total species richness of a given habitat based on sampling data, or for comparing richness values among habitats or sampling periods for a given number of samples or individuals (Magurran, 2004). From the species richness perspective, the theory underlying and performance of these methods is being increasingly well explored (e.g. Brose, Martinez & Williams, 2003; Brose & Martinez, 2004; Chao *et al.*, 2005; O'Hara, 2005; Ulrich & Ollik, 2005; Walther & Moore, 2005). However, these methods are not commonly applied to haplotype data (but see Myburgh *et al.*, 2007). Individual-based rarefaction curves were used because our sampling unit was individuals (Gotelli & Colwell, 2001), and the Chao 2 and Jacknife 2 estimators were adopted because they are generally most robust (Brose *et al.*, 2003; Walther & Moore, 2005). Fifty randomizations (sampling without replacement) and 1000 randomizations (sampling with replacement) were performed to verify the results.
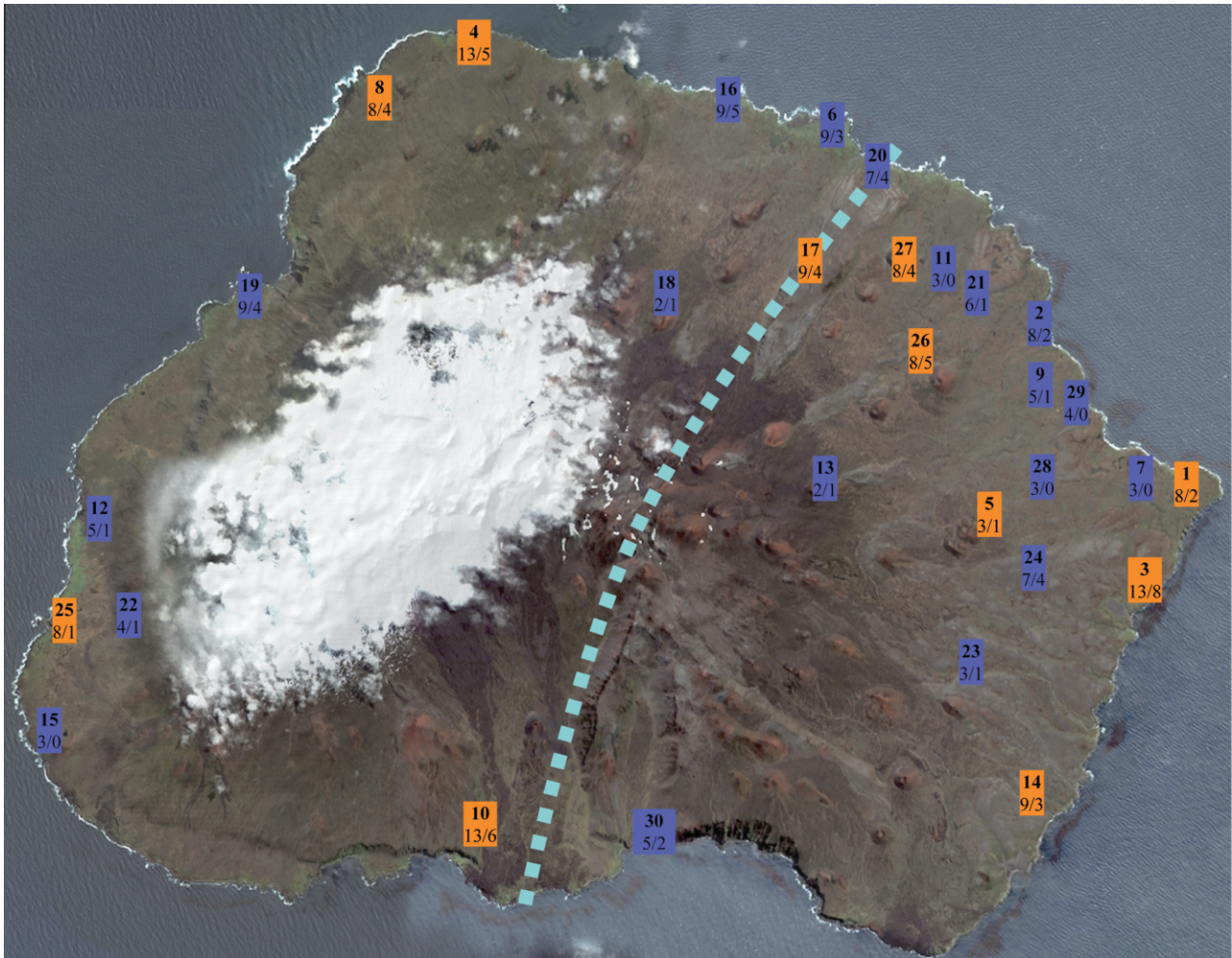
Figure 2. Map of Marion Island indicating the sampling localities (in darker grey squares). Population numbers correspond to those given in Table 1. The total number of haplotypes per locality as well as the number of singletons (total/singletons) is shown. Localities in paler grey indicate those where divergent haplotypes were found, and correspond well with suggested ice-free areas during the Last Glacial Maximum (see Hall *et al.*, 2011).

Uncorrected sequence divergences among sampling localities were calculated in DnaSP 5.1 (Librado & Rozas, 2009). Traditional phi (Φ)-statistics as well as pair-wise differences (Φ_{ST}) among populations were calculated in Arlequin 3.1 (Excoffier, Laval & Schneider, 2005) with null distributions obtained via permutational procedures (1000 randomizations). To depict the evolutionary relationship among haplotypes a statistical parsimony network was constructed using TCS 1.2 (Clement, Posada & Crandall, 2000) (for network choice see Joly, Stevens & Jansen van Vuuren, 2007). Ambiguities in the network were resolved following the criteria outlined in Crandall & Templeton (1993) and Pfenninger & Posada (2002). In short, we assumed that tip haplotypes are more likely to connect to interior haplotypes or haplotypes with higher frequency than to other tip haplotypes, and

that haplotypes were more likely to connect to haplotypes in the same geographical vicinity. The network was converted into a set of nested clades following Templeton, Boerwinkle & Sing (1987). Nested clade phylogeographical analyses (NCPAs) were performed in Geodis 2.5 (Posada, Crandall & Templeton, 2000) and results were interpreted using the most recent inference key (http://darwin.uvigo.es/ software/geodis.html). Although NCPA has been criticized (see, for example, Panchal & Beaumont, 2007; Petit, 2008; for a response see Templeton 2008) it remains one of the few methods available that statistically infer phylogeographical patterns across species distributions. The most common false positive that is returned is 'isolation-by-distance with restricted gene flow'. Thus, to assess the likelihood of a true positive result of this kind, Mantel tests were also performed

Table 1. Summary statistics (haplotype and nucleotide diversity, mean ± SD) for all sampling localities included in the present study

Number	Sampling locality	Haplotype diversity	Nucleotide diversity	Haplotypes
1	Archway Bay ($N = 10$)	0.955 ± 0.059	0.010 ± 0.006	HF18, HF21, HF37, HF41, HF42, HF55, HF66, HF80
2	Base ($N = 10$)	0.955 ± 0.059	0.008 ± 0.005	13/HF1, HF5, HF11, HF12, HF23, HF37, HF54, HF55
3	Bullard Beach ($N = 19$)	0.941 ± 0.037	0.010 ± 0.006	HF5, HF13, HF14, HF22, HF51, HF55, HF68, HF69, HF89, HF90, HF91, HF93, HF988
4	Cape Davis ($N = 19$)	0.929 ± 0.046	0.008 ± 0.004	HF5, HF9, HF18, HF33, HF37, HF39, HF46, HF55, HF82, HF91, HF92, HF95, HF99
5	Fred's Hill ($N = 4$)	0.833 ± 0.222	0.014 ± 0.010	HF55, HF83, HF95
6	Goney Plain ($N = 10$)	0.977 ± 0.054	0.005 ± 0.003	HF1, HF3, HF5, HF9, HF37, HF53, HF57, HF78, HF84
7	Albatross Lakes ($N = 3$)	1.000 ± 0.272	0.006 ± 0.005	HF37, HF55, HF65
8	Devil's Footprint ($N = 10$)	0.955 ± 0.059	0.010 ± 0.006	HF5, HF6, HF15, HF19, HF28, HF62, HF66, HF72
9	Nellie Humps ($N = 10$)	0.800 ± 0.100	0.010 ± 0.006	HF37, HF55, HF58, HF95, HF97
10	Greyheaded Albatross Ridge ($N = 19$)	0.929 ± 0.046	0.009 ± 0.005	HF5, HF16, HF18, HF37, HF49, HF50, HF55, HF63, HF65, HF68, HF70, HF74, HF76
11	Hoppie's Hell ($N = 5$)	0.700 ± 0.2184	0.002 ± 0.002	HF1, HF5, HF37
12	Kaalkoppie ($N = 11$)	0.763 ± 0.106	0.002 ± 0.001	HF5, HF18, HF25, HF37, HF80
13	Katedraalkrans ($N = 2$)	1.000 ± 0.500	0.004 ± 0.005	HF5, HF40
14	Kildalkey Bay ($N = 22$)	0.813 ± 0.059	0.008 ± 0.004	HF5, HF8, HF9, HF17, HF35, HF37, HF55, HF95, HF96
15	La Grange Kop ($N = 6$)	0.733 ± 0.155	0.003 ± 0.003	HF5, HF37, HF55
16	Log Beach ($N = 10$)	0.977 ± 0.054	0.006 ± 0.004	HF4, HF5, HF9, HF18, HF27, HF30, HF31, HF55, HF88
17	Long Ridge ($N = 16$)	0.850 ± 0.077	0.006 ± 0.004	HF5, HF26, HF37, HF38, HF43, HF64, HF85, HF87, HF99
18	Middelman ($N = 3$)	0.666 ± 0.314	0.001 ± 0.001	HF55, HF61
19	Mixed Pickle Cove ($N = 12$)	0.909 ± 0.079	0.006 ± 0.004	HF3, HF7, HF18, HF37, HF52, HF55, HF56, HF65, HF77
20	Ships Cove ($N = 10$)	0.911 ± 0.077	0.006 ± 0.004	HF18, HF20, HF34, HF37, HF65, HF71, HF79
21	Skua Ridge ($N = 10$)	0.777 ± 0.137	0.004 ± 0.003	HF18, HF35, HF37, HF55, HF59, HF85
22	Skuinskop ($N = 6$)	0.800 ± 0.172	0.006 ± 0.004	HF5, HF32, HF37, HF55
23	Soft Plume River ($N = 3$)	1.000 ± 0.272	0.004 ± 0.003	HF9, HF18, HF86
24	Stoney Ridge ($N = 8$)	0.964 ± 0.077	0.007 ± 0.005	HF2, HF5, HF7, HF36, HF44, HF55, HF67
25	Swartkops Point ($N = 10$)	0.955 ± 0.059	0.006 ± 0.004	HF5, HF9, HF18, HF37, HF45, HF55, HF80, HF101
26	Tafelberg ($N = 10$)	0.933 ± 0.077	0.007 ± 0.004	HF9, HF10, HF29, HF37, HF55, HF75, HF81, HF100
27	Third Sister ($N = 12$)	0.848 ± 0.103	0.007 ± 0.004	HF5, HF37, HF47, HF48, HF73, HF85, HF94, HF95
28	Tom, Dick & Harry ($N = 4$)	0.833 ± 0.222	0.005 ± 0.004	HF37, HF55, HF58
29	Trypot Beach ($N = 9$)	0.583 ± 0.183	0.004 ± 0.003	HF5, HF37, HF55, HF89
30	Watertunnel ($N = 8$)	0.857 ± 0.108	0.006 ± 0.004	HF5, HF24, HF45, HF55, HF60

The population number corresponds to numbers used in Table 2 and Figure 1. The number of specimens included per locality is indicated in parentheses. The haplotypes identified at each locality is shown.

in Arlequin 3.1 (Excoffier *et al.*, 2005). The western side of Marion Island is relatively inaccessible and, as such, this side of the island was less sampled than the eastern side (see Fig. 2). Mantel tests were performed on all sampling localities situated on the southern and western side of the island (Watertunnel, locality 30; Greyheaded Albatross Ridge, locality 10; La Grange Kop, locality 15; Skuinskop, locality 22; Swartkops Point, locality 25; Kaalkoppie, locality 12 and Mixed Pickle Cove, locality 19). To minimize the sampling bias towards the eastern side of the island, Mantel tests were conducted on randomly selected localities on the eastern side of the island (in each case, 12 localities were randomly selected).

A coalescent-based approach (MDIV; Nielsen & Wakeley, 2001; Nielsen, 2002) was used to calculate theta (θ; an indicator of population size), gene flow (*M*), and divergence time (*T*). As several populations (sampling localities) have not diverged (see Results), we adopted this method as opposed to others such as LAMARC (Kuhner, 2006) which assumes that all populations have fully diverged. In these calculations, a mutation rate of 1.9% per million years (following species-specific rates reported in Mortimer *et al.*, 2011) and a generation time of 1 year were assumed. These analyses were limited to the four most divergent populations on Marion Island based on population pair-wise Φ_{ST} comparisons (Kaalkoppie, population 12; Bullard Beach, population 3; Greyheaded Albatross Ridge, population 10; Long Ridge, population 17; Fig. 2). These four most divergent populations were then analysed with their neighbouring populations in a pair-wise manner with a total of 21 populations included in these comparisons, resulting in local population level as well as analyses at the regional (western, eastern, southern and northern) scale. The populations omitted from these analyses were characterized by small sample sizes (fewer than five individuals per population for populations 5, 7, 13, 18, 23, and 28) or because they were in close (< 1 km) proximity to populations included (8, 9, and 29). Overall, the populations included in the coalescent modelling provide a good coverage of the island. Upper credibility estimates for the migration parameter could not be calculated as runs did not form an asymptote and converge back to zero irrespective of the number of generations allowed for calculations. Multiple independent runs with different random seed numbers were conducted to verify the results. Simulations were run for 20 million generations with M_{max} set at 200 (this high value was specified in an attempt to reach an asymptote) while T_{max} was set at 10. In each case, M_{max} and T_{max} denote the prior distribution for the Bayesian analyses. The analyses were conducted under the HKY finite sites model (Hasegawa, Kishino & Yano, 1985) as suggested for

sequence data. The parameter values estimated with MDIV were compared with those estimated using the Isolation with Migration software (IM; Hey & Nielsen, 2004). IM was run for 100 million generations with a burn-in of 100 000.

RESULTS

We sequenced 545 bp, which translated into 181 functional amino acids. A total of 101 haplotypes were identified, of which 73 were singletons ($h = 0.92 \pm 0.01$). Haplotype and nucleotide diversities for all sampling localities are presented in Table 1 and the haplotypes recorded for each of the localities are presented in Supplementary Information Table S1. With the exceptions of Trypot Beach (locality 29) and Middelman (locality 18), haplotype diversities were high (≥ 0.7) reflecting the large number of private haplotypes found in the study. Nucleotide diversities for sampling localities were generally low (≤ 0.01) with the exceptions of Bullard Beach (locality 3) and Fred's Hill (locality 5). Taken together, this pattern might be interpreted as supporting population expansions. Rarefaction curves suggested that many haplotypes remained to be sampled (Fig. 3), with the Chao 2 estimator suggesting that a total of 290 (standard deviation of 77) haplotypes are likely to be present on the island.

The three most common haplotypes (HF37 comprising 21%, HF55 comprising 14%, and HF5 comprising 11% of the specimens) lie centrally on the haplotype network and are each connected to several haplotypes in a star-like pattern (see Fig. 4). Most haplotypes are connected by single mutational steps with only a few
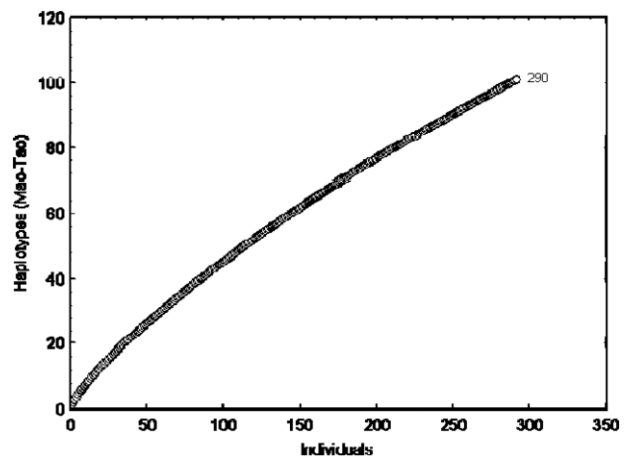

Figure 3. An individual-based rarefaction curve illustrating the increase in the number of haplotypes with increasing numbers of individuals. The number next to the curve (290) indicates the total estimated number of haplotypes using the non-parametric Chao2 estimator.
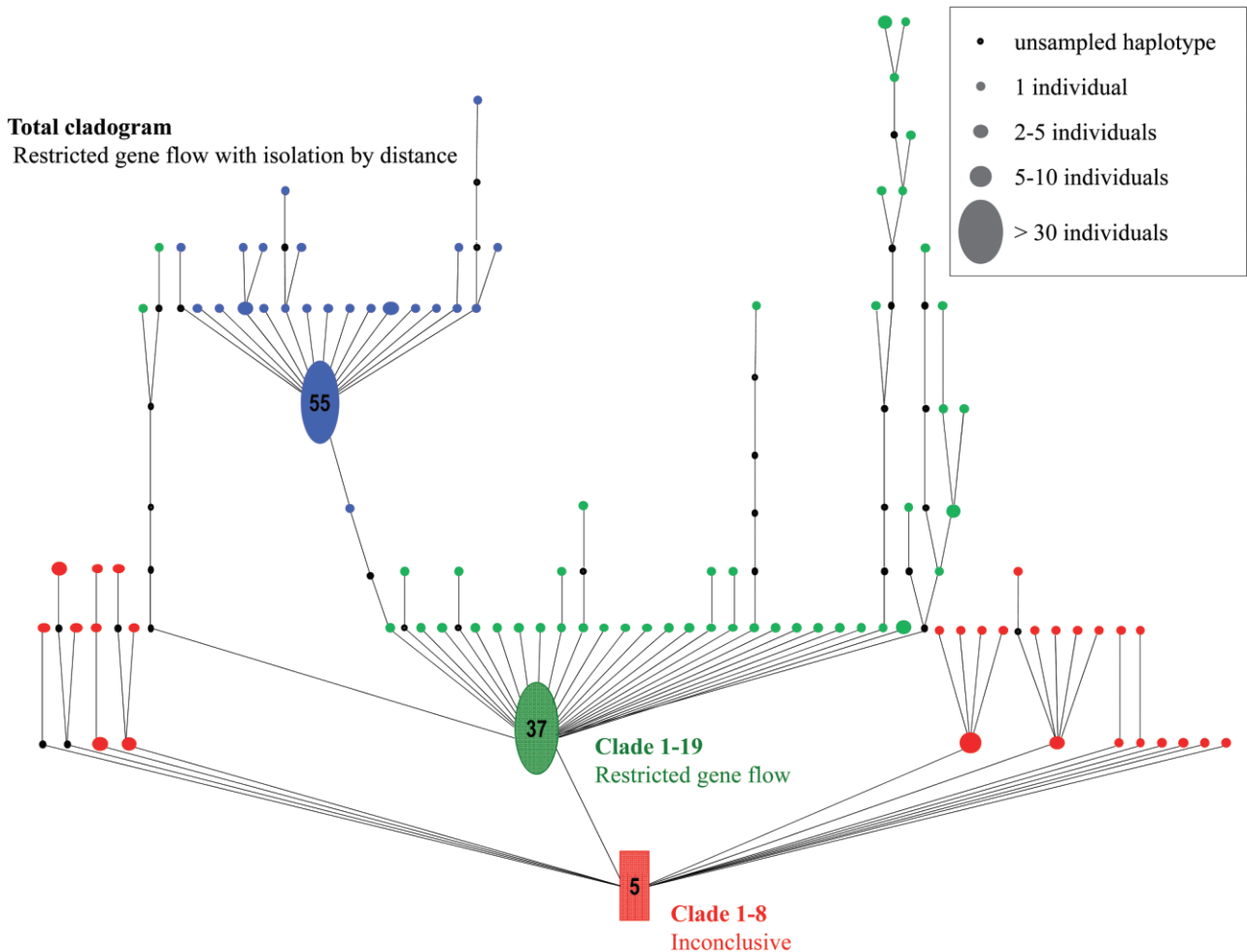
Total cladogram
Restricted gene flow with isolation by distance

Figure 4. A haplotype network constructed using TCS. The three haplogroups are indicated in blue, green and red, and correspond to the three most common haplotypes (HF37, HF55 and HF5). Three clades (1-8, 1-19 and the entire cladogram) showed a significant correlation between geography and genetic diversity (as revealed by NCPA) and the outcomes are shown.

outliers being separated by more (up to ten) mutational changes, a pattern which is typical of recent population expansion, as also suggested by haplotype and nucleotide diversity values. Thirteen loops in the network were found, all of which could be resolved by applying the criteria of Crandall & Templeton (1993) and Pfenninger & Posada (2002). Two one-step clades as well as the entire cladogram showed a significant correlation between genetic and geographical distances (see Fig. 4). Overall, the inferred population history underlying the genetic structure of *H. fulvus* suggests that restricted gene flow (with isolation-by-distance) has played a role in shaping current genetic patterns (NCPA outcome for the entire cladogram). The outcome of the NCPA for clade 1-8 was inconclusive (tip interior status could not be determined). For clade 1-19, which includes haplotypes from localities

across the island, NCPA indicated restricted gene flow.

When conducting a Mantel test on all the western and southern localities included in this study (Water-tunnel, locality 30; Greyheaded Albatross Ridge, locality 10; La Grange Kop, locality 15; Skuin-skop, locality 22; Swartkops Point, locality 25; Kaalkoppie, locality 12; Mixed Pickle Cove, locality 19), no isolation-by-distance was detected ($P = 0.21$, $r = 0.20$). To minimize the sampling bias towards the eastern side of the island, Mantel tests were conducted on randomly selected localities (in each case, 12 localities were randomly selected). In all cases, isolation-by-distance characterized localities on the eastern side of the island ($P < 0.05$).

AMOVA, including all localities, indicated significant structuring of genetic variation ($\Phi_{ST} = 0.07$,

Table 2. Coalescent results estimated for selected populations using MDIV (see text)

	θ	M	T (years BP)
(A) Kaalkoppie (pop. 12)			
Swartkops Point (pop. 25)	2.4 (1.3–5.9)	9.2 (2 – >*)	0
La Grange Kop (pop. 15)	1.2 (0.5–3.8)	17.6 (1.2 – >*)	0
Skuinskop (pop. 22)	1.4 (0.6–5.9)	1.2 (1.2 – >*)	0
Greyheaded Albatross Ridge (pop. 10)	4.1 (1.9–8.3)	1.6 (1.2 – >*)	0
Mixed Pickle Cove (pop. 19)	2.6 (1.4–6.1)	> 40 (1.6 – >*)	0
Cape Davis (pop. 4)	4.8 (2.8–9.3)	16.8 (2.4 – >*)	0
(B) Bullard Beach (pop. 3)			
Archway Bay (pop. 1)	7.6 (4.8–14.4)	2.1 (1 – >*)	~ 113 000
Kildalkey Bay (pop. 14)	5.4 (3.4–9.6)	1.9 (0.8 – >*)	~ 90 000
Stoney Ridge (pop. 24)	7.8 (4.9–14.7)	3.2 (0.6 – >*)	~ 147 000
Watertunnel (pop. 30)	5.6 (2.7–11.4)	3.1 (0.5 – >*)	~ 34 000
Base (pop. 2)	6.4 (4.0–12.4)	2.7 (0.6 – >*)	~ 79 000
(C) Greyheaded (pop. 10)			
Watertunnel (pop. 30)	4.7 (2.8–9.6)	> 40 (5.6 – >*)	0
La Grange Kop (pop. 15)	3.8 (2.3–8.3)	> 40 (5.2 – >*)	0
Skuinskop (pop. 22)	4.4 (2.5–8.9)	> 40 (7.2 – >*)	0
Kaalkoppie (pop. 12)	4.1 (1.9–8.3)	1.6 (1.2 – >*)	0
(D) Long Ridge (pop. 17)			
Long Beach (pop. 16)	5.6 (3.3–11.4)	1.6 (1.4 – >*)	~ 56 000
Goney Plain (pop. 6)	5.3 (3.1–10.7)	2.6 (1.2 – >*)	~ 56 000
Hoppie's Hell (pop. 11)	3.8 (2.0–8.0)	16.4 (4.8 – >*)	0
Third Sister (pop. 27)	5.0 (3.2–10.3)	> 40 (6.4 – >*)	0
Tafelberg (pop. 26)	4.6 (2.7–9.5)	22.2 (5.8 – >*)	0
Ships Cove (pop. 20)	4.9 (2.8–10.1)	2.4 (1.8 – >*)	0
Skua Ridge (pop. 21)	4.1 (2.3–8.3)	15.6 (5 – >*)	0
Base (pop. 2)	5.4 (3.1–10.5)	2.6 (1.4 – >*)	~ 56 000

The estimates of θ (an indicator of population size), scaled migration rates (*M*) and divergence time (*T*, given in years before present) are shown. The 95% credibility intervals are given in parentheses. *The upper credibility interval could not be estimated as the parameter did not converge back to zero.

$P < 0.001$; d.f. = 29; sum of squares = 95.14; variance component = 0.144) thereby complementing our NCPA results. Population pair-wise Φ_{ST} values indicated that five localities are significantly differentiated to more than 12 other localities (i.e. > 40% of the localities) (see Supplementary Information Table S2). These are Greyheaded Albatross Ridge (locality 10), Long Ridge (locality 17), Bullard Beach (locality 3), Middelman (locality 18), and Kaalkoppie (locality 12). Only three specimens were available for Middelman (locality 18) which does not allow meaningful statistical comparisons and this locality is therefore not included in subsequent locality-specific analyses. The remainder of the localities were not significantly differentiated from more than eight other localities (i.e. 25% of the localities).

IM and MDIV returned very similar results (for ease of representation we provide only the MDIV results). Both of these coalescent analyses indicated two different patterns across Marion Island (see Table 2). The first characterized populations on the south-western corner of the island. Pair-wise comparisons between Kaalkoppie (locality 12) with neighbouring localities indicated low effective population sizes (θ: range 1.4–4.8) and high (scaled) migration (*M*: range 1.2–45.6). As a result of the high migration, populations had not diverged (*T* = 0). The second pattern was observed for populations centred on Bullard Beach (locality 3), a locality on the eastern side of Marion Island. Here, estimates of population size were typically high (θ: range 5.4–7.9) while (scaled) migration rates were low (*M*: range 1.9–3.2). As a result, all populations had fully diverged from one another. Populations in the vicinity of Greyheaded Albatross Ridge (locality 10) and Long Ridge (locality 17), which also represented focal localities in the coalescent analyses, were largely characterized by the first pattern although some comparisons involving Long Ridge were more typical of the second pattern. Importantly, standard errors

around estimates are large, as is often typical for coalescent analyses, and divergence dates and migration estimates (given in Table 2) should not be seen as absolute, but rather as relative values where divergence times for populations on the eastern side of the island far exceed those for populations on the south-western side and where migration on the south-western side exceeds those on the eastern side of Marion Island.

Uncorrected sequence divergence values separating localities are presented in Supplementary Information Table S2. The average sequence divergence separating populations on the eastern side of Marion Island was 0.78%, that separating populations on the northern side of the island was 0.80%, while uncorrected divergence values separating populations on the south-western side of the island were notably lower and averaged 0.51% (only two populations are situated on the southern side of the island, and are therefore not included here). These results are congruent with the coalescent analyses, which suggest that populations on the south-western side of Marion Island are not as diverged as those found on the eastern and northern side of Marion Island.

DISCUSSION

Despite considerably greater sampling intensity than for any previous estimates of mitochondrial COI haplotype diversity within invertebrate species (notably springtails, mites, and weevils) on Marion Island (291 individuals vs. a maximum of 113 previously – see McGaughran *et al*., 2010b), haplotype diversity in *H. fulvus* showed no signs of asymptoting as a consequence of the considerable numbers of singletons. In consequence, it is unsurprising that the Chao2 estimator suggested a conservative value of at least another 180 haplotypes that remain to be discovered. Some of these undiscovered haplotypes (~ 20%) are accounted for in our haplotype network and their placement in the phylogeny is therefore known. Although some of these unsampled haplotypes may be extinct, and taken into account the error (standard deviation) associated with the estimate, it remains clear that haplotype diversity is notably high. Even this conservative estimate might be an underestimate as *H. fulvus* can reach densities of *c*. 700 individuals per m^2 in the cushion plant *Azorella selago* and in the epilithic biotope (Barendse & Chown, 2001). High haplotype diversity has similarly been recorded for other indigenous invertebrate species on the island, including a prostigmatid mite, two species of springtails, and three species of weevils (Grobler *et al*., 2006, 2011a, b; Mortimer & Jansen van Vuuren, 2007; Myburgh *et al*., 2007; McGaughran *et al*., 2010b). By contrast, springtail species in Antarctica show some-

what lower levels of haplotype diversity (see Frati *et al*., 2001; Stevens & Hogg, 2003; Nolan *et al*., 2006; but see McGaughran *et al*., 2010a) as do other arthropod species, such as on the Canary islands (Emerson *et al*., 2000, 2006) and Hawaiian islands (Jordan *et al*., 2005). The higher haplotype diversity found for invertebrate taxa on Marion Island compared with taxa occurring on Antarctica is further supported through rarefaction analyses which project a total of 28 haplotypes for the springtail species *Desoria klovstadi* and *Gomphiocephalus hodgsoni* (data for rarefaction analyses taken from Frati *et al*., 2001; Stevens & Hogg, 2003; Nolan *et al*., 2006) on Antarctica compared with 135 and 290 expected haplotypes for the springtail species *Cryptopygus antarcticus travei* and *H. fulvus*, respectively, on Marion Island (see Myburgh *et al*., 2007 for rarefaction analyses). Invasive invertebrate species on Marion Island are typically characterized by only a single haplotype (Lee *et al*., 2007, 2009; Myburgh *et al*., 2007).

Why such high haplotype diversity might characterize the indigenous arthropod faunas of the island is not yet clear. However, virtually all of the shapes of the haplotype networks constructed to date (star-like patterns), including this one, suggest demographic changes. This may well have been a consequence of the fact that during the LGM, Marion Island was almost entirely glaciated with the exception of a few areas mostly along the coast but also at high elevation (Hall *et al*., 2011). The presence of these proposed glacial-free areas is evidenced by glacial reconstructions based on extensive examination of glacio-depositional and erosional features across the island in combination with K–Ar dating of these (Hall, 2002; Hall *et al*., 2011, see also fig. 4.3 in Boelhouwers *et al*., 2008). For the springtails *Cryptopygus antarcticus travei* and *Tullbergia bisetosa*, it seems likely that refugia included high elevation 'nunataks' such as the grey lava ridge Katedraalkrans (locality 13), which new evidence indicates was likely to have been ice free during the LGM (see Myburgh *et al*., 2007; Boelhouwers *et al*., 2008; McGaughran *et al*., 2010b; Hall *et al*., 2011). Whether this was the case for *H. fulvus* is not clear because of our low sample size at this locality. However, it seems likely that in addition to survival in possible higher elevational refugia, this species may also have survived in coastal refugia, which may have been present especially on the western side of the island (Chown, 1990, 1994; Boelhouwers *et al*., 2008). This is supported by our data where divergent haplotypes were found in several high-altitude and coastal localities (see Fig. 2) which are situated in areas presumed to be ice-free during the LGM (see fig. 4.3 in Boelhouwers *et al*., 2008). Another reason for assuming survival in both high-elevation and coastal refugia is that the species

occurs in both habitats. Although it is not abundant in high-elevation fellfield habitats or indeed in fellfield more generally, it is common in *Azorella selago* cushions, a plant species that is thought also to have survived in inland refugia (Scott, 1985; Yeloff *et al*., 2007). *Halozetes fulvus* is also abundant in many lowland habitats including those highly influenced by coastal salt-spray (Barendse *et al*., 2002). Moreover, it is an endemic of the Prince Edward Islands (Starý & Block, 1998; Marshall & Chown, 2002), and *Halozetes belgicae*, its sister species (see Mortimer *et al*., 2011), is a typical inhabitant of the surpralittoral zone on Marion Island (and elsewhere across its distribution; Mercer, Chown & Marshall, 2000).

Although *H. fulvus* is likely to have expanded rapidly (Marion Island was extensively glaciated during the LGM and so expansion must have occurred in the last few thousand years; rapid expansion is also reflected by the haplotype network as well as haplotype and nucleotide diversity values), this expansion has not been without spatial structure. Rather, populations on the eastern and western sides of the island appear to differ in their degree of relatedness and the extent to which gene flow is currently taking place. Whether the neighbouring Prince Edward Island could act as a source for immigration is moot. However, we consider this highly unlikely for several reasons. First, in all cases where material from both islands has been examined, it is clear that very few haplotypes are shared among these islands (Grobler *et al*., 2006, 2011b; C. W. Groenewald, S. L. Chown, J. Shaw J & B. Jansen van Vuuren, unpubl. data). An inter-island comparison was also not possible in the current study owing to the infrequency of visits to Prince Edward Island for conservation reasons (see Davies, Chown & Joubert, 2007). Second, the prevailing wind direction for the last 0.5 Myr and probably much longer has been predominantly north-west with north-easterly winds being light and uncommon (see Schulze, 1971; Le Roux, 2008). Third, the species concerned here is a small oribatid that is unlikely to withstand desiccation for any length of time. Indeed, Marshall (1996) showed that 50% mortality occurs after 40 h exposure to desiccating conditions (see also Pugh, 1997). Even if this time (40 h) may be sufficient to allow movement via air currents from Prince Edward to Marion, it should be noted that laboratory experiments of this nature do not take the additional effect of wind (i.e. disruption of boundary layers) into account and survival times are therefore likely to be shorter under natural conditions. Finally, most bird species nesting in the islands are either poor dispersers (e.g. Lesser Sheathbill *Chionis minor*) or are intensely philopatric (albatrosses, petrels) (for a review see Ryan & Bester, 2008) making repeated dispersal events by that route also seem unlikely, or at least sufficiently unlikely to

explain the complex phylogeographical patterns we document.

Rather, this east–west differentiation on Marion Island seems to be closely aligned to the geological division of the island into eastern and western areas along the N26.5°E geological lineament (Fig. 1). Specifically, populations to the east of the discontinuity show considerable differentiation. By contrast, western populations show much less differentiation, and indeed cannot clearly be separated. Why this should be the case is not clear. Certainly it is probably not a consequence of current vegetation patterns because these are not structured in such a way on the island (Gremmen & Smith, 2008) and *H. fulvus* is in any case a habitat generalist (Barendse *et al*., 2002). However, geological and glacial information suggest that the discontinuity has been associated with other regularities in the history of the island which may help explain these findings. Most significantly, at least three largely grey lava refugia probably existed to the east of the discontinuity during the LGM: Feldmark Plateau, parts of Long Ridge South, and Katedraalkrans. These sites may have served as refugia for populations that differentiated earlier in the island's history, so accounting for the rather confused pattern of both substantial differentiation (with ages much older than the LGM ranging from approximately 34 000 to 134 000 years BP; see Table 2) and widespread distribution of some haplotypes. In this eastern area, the ice sheet during the LGM is likely to have extended at least to, and in some areas beyond, the current coastline (Hall, 2004; Boelhouwers *et al*., 2008; Hall *et al*., 2011), so possibly precluding coastal refugia with the possible exception of small areas around Archway Bay and Kildalkey Bay. This would have resulted in colonization of newly exposed habitats from the highland refugia, so resulting in considerable differentiation that is typical also of several other species on the island (Mortimer & Jansen van Vuuren, 2007). By contrast, the LGM ice sheet did not extend to the coast to the west of the discontinuity (Boelhouwers *et al*., 2008). Moreover, this area of the island has been much more heavily affected by more recent lava flows (including those in the 1980s) (Boelhouwers *et al*., 2008). This suggests that there has been repeated expansion of populations from coastal sites, with little opportunity for differentiation. In addition, localities where divergent haplotypes were present, in keeping with predictions for refugial populations, correspond well with areas that have been suggested as ice-free (see Fig. 2; see also fig. 4.3 in Boelhouwers *et al*., 2008).

Although our evidence for the above scenario is largely based on *post-hoc* interpretation of the molecular evidence in the context of the geological history of the island, the two sets of evidence are consistent. Moreover, this approach is in keeping with that

recommended by Emerson *et al*. (2006) in the face of complex geological history. Indeed, it seems likely that our original hypotheses were somewhat simplistic, given that a combination of glaciation, volcanic events, and the division of the island into two major areas have all probably contributed to phylogeographical structuring of *H. fulvus* across Marion Island. However, the current interpretation requires verification from other taxa given that a single species was used here. In doing so, several clearly testable hypotheses should be addressed in future studies, which emerge from consideration of likely habitat preferences among other taxa. For example, the current outcome suggests that the differentiation of populations along the east–west divide should be much less obvious for taxa that are restricted to high-elevation fellfield habitats, such as the weevil *Bothrometopus elongatus* (Chown, 1992) and the mite *Magellozetes antarcticus* (Barendse *et al*., 2002). By contrast, more eurytopic species such as the weevil *Bothrometopus huntleti* and the springtail *C. antarcticus travei* should show such differentiation after extensive sampling (to some extent, this was shown for *C. antarcticus travei*; see Myburgh *et al*., 2007; see also McGaughran *et al*., 2010b for metabolic rate variation). Similarly, purely coastal species, such as the weevil *Palirhoeus eatoni*, and the other mites in the genus *Halozetes* should show a pattern of recolonization of the eastern side of the island from the west. Further work on these species and on *H. fulvus*, using a broader range of markers, will provide a means of testing the hypotheses derived from the current study. Until then, it does seem plausible that congruence exists between the geology of the island and the evolutionary history of populations of the species occupying it. This congruence suggests a pattern of rapid evolutionary change on the island, but for the moment at the within-species level, rather than to the extent that speciation is taking place as has been the case on other islands characterized by complex land forms (Gillespie & Roderick, 2002). To what extent these hypotheses, developed for Marion Island and based on a sound understanding of the habitat preferences of the various organisms, could be extrapolated to other Southern Ocean islands remains unclear. In some cases the geology is well enough known to do so, although information on the distribution and habitat specificity of the terrestrial fauna remains less poorly developed than on Marion Island. Nonetheless, the situation is changing rapidly (e.g. Quilty, 2007; Davies *et al*., 2011) and similar landscape genetic structuring on these islands seems plausible.

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SUPPORTING INFORMATION

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

Table S1. Haplotype distribution across sampling localities. The colour of the haplotypes (red, green, blue) corresponds to the main haplogroups in Figure 4, and population numbers correspond to numbers given in Table 1.

Table S2. Pairwise F_{ST} values for all sampling localities included in the study are presented below the diagonal. # indicates significance at $P < 0.05$. Uncorrected sequence divergence values separating sampling localities are presented above the diagonal.

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