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Origin and post-colonization evolution of the Chatham Islands skink (*Oligosoma nigriplantare nigriplantare*)

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Abstract

Island ecosystems provide an opportunity to examine a range of evolutionary and ecological processes. The Chatham Islands are an isolated archipelago situated approximately 800 km east of New Zealand. Geological evidence indicates that the Chatham Islands re-emerged within the last 1–4 million years, following a prolonged period of marine inundation, and therefore the resident flora and fauna is the result of long-distance overwater dispersal. We examine the origin and post-colonization evolution of the Chatham Islands skink, *Oligosoma nigriplantare nigriplantare*, the sole reptile species occurring on the archipelago. We sampled *O. n. nigriplantare* from across nine islands within the Chatham Islands group, and representative samples from across the range of its closest relative, the New Zealand mainland common skink (*Oligosoma nigriplantare polychroma*). Our mitochondrial sequence data indicate that *O. n. nigriplantare* diverged from *O. n. polychroma* 5.86–7.29 million years ago. This pre-dates the emergence date for the Chatham Islands, but indicates that *O. n. nigriplantare* colonized the Chatham Islands via overwater dispersal on a single occasion. Despite the substantial morphological variability evident in *O. n. nigriplantare*, only relatively shallow genetic divergences (maximum divergence ~2%) were found across the Chatham Islands. Our analyses (haplotypic diversity, Φ_{ST}, analysis of molecular variance, and nested clade phylogeographical analysis) indicated restricted gene flow in *O. n. nigriplantare* resulting in strong differentiation between islands. However, the restrictions to gene flow might have only arisen recently as there was also a significant pattern of isolation by distance, possibly from when the Chatham Islands were a single landmass during Pleistocene glacial maxima when sea levels were lower. The level of genetic and morphological divergence between *O. n. nigriplantare* and *O. n. polychroma* might warrant their recognition as distinct species.

Keywords: island biogeography, microevolution, mitochondrial DNA, phylogeography, speciation, transoceanic dispersal

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Introduction

Island ecosystems provide ideal 'natural laboratories' to examine fundamental evolutionary processes (reviewed in Emerson 2002). Research on the biota of island archipelagos was instrumental in the formulation of evolutionary theory (Wallace 1858; Darwin 1859; Wallace 1903) and island biogeography studies continue to provide valuable insights into evolutionary and ecological processes (Whittaker 1998; Emerson 2002; Cowie & Holland 2006; Heaney 2007). Molecular genetic methods can provide a phylogenetic framework in which to examine the origin of island taxa (e.g. time of colonization, single vs. multiple colonization) and their post-colonization evolution (e.g. adaptive radiation, morphological divergence) (reviewed in Emerson 2002). For example, several studies have tested the concordance between the geological age of islands and the estimated (using molecular-clock methods) time of colonization of their resident taxa, indicating that the estimated time of

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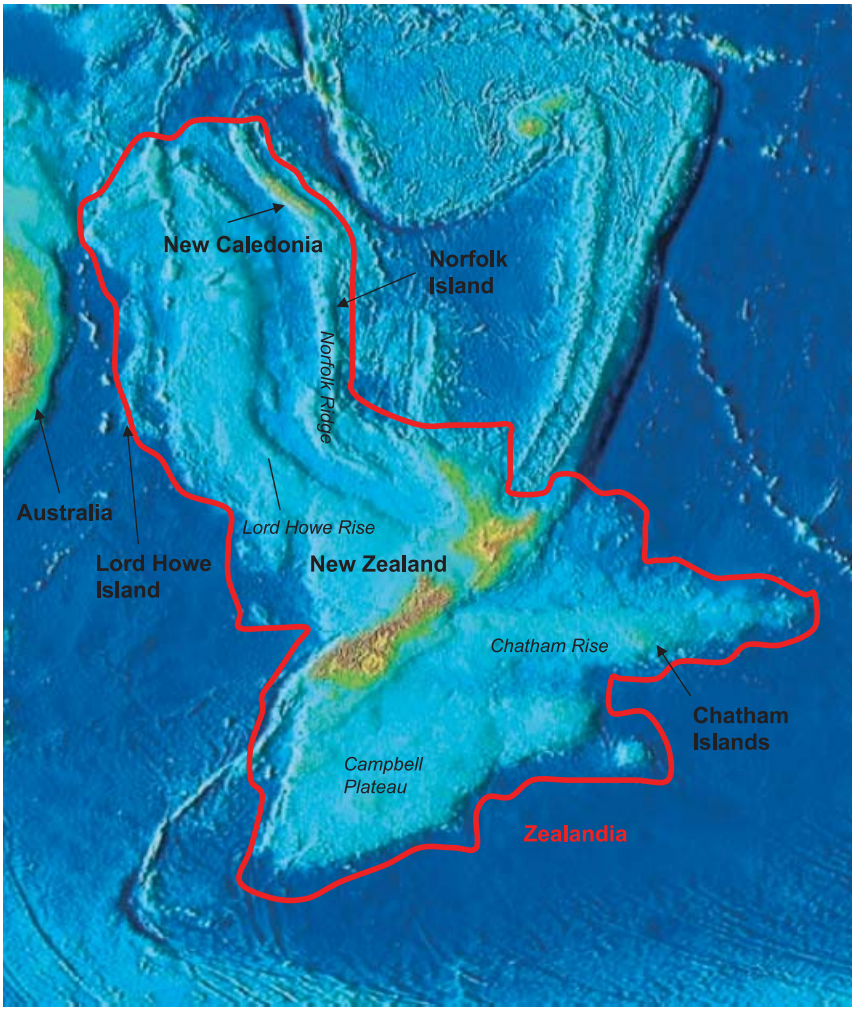

Fig. 1 Map of Zealandia. The red line indicates the approximate extent of the largely submerged continental crust of Zealandia (modified from Trewick *et al*. 2007). The location of the Chatham Islands, Chatham Rise, Campbell Plateau, New Zealand, Lord Howe Island, Lord Howe Rise, Norfolk Island, Norfolk Ridge, New Caledonia, and Australia is indicated (Base image provided by the National Oceanic and Atmospheric Administration).

colonization may (i) pre-date island emergence (e.g. DeSalle 1992; Rassman 1997; Thorpe *et al*. 2005), (ii) closely match island emergence (e.g. Thorpe *et al*. 1994; Beerli *et al*. 1996), or (iii) postdate island emergence (e.g. Censky 2006). Due to the discordance that is expected to exist between gene and species trees, especially for recent species and population divergences (e.g. Arbogast *et al*. 2002), it is possible for the estimated time of divergence of lineages to pre-date emergence. These situations might simply reflect a difference between gene divergence time and population divergence time (e.g. Edwards & Beerli 2000), and they do not necessarily arise due to errors in calibrating molecular clocks or estimating the geological age of islands.

The largely submerged continent of Zealandia (Fig. 1) provides an opportunity to examine the origin and post-colonization evolution of taxa occurring on isolated archipelagos. Zealandia separated from Gondwana ~80 million years ago (Ma), and was stretched and thinned as it rifted (Campbell *et al*. 2006; Trewick *et al*. 2007; Landis *et al*. 2008). This resulted in a relatively thin continental crust and the gradual subsidence and marine inundation of the majority of Zealandia (Campbell *et al*. 2006; Trewick *et al*. 2007; Landis *et al*. 2008). Marine inundation reached its peak in the Oligocene (~25 Ma) when the vast majority of Zealandia was believed to be submerged (Cooper & Millener 1993; Campbell *et al*. 2006; Waters & Craw 2006; Trewick *et al*. 2007; Landis *et al*. 2008). Today, only ~10% of the Zealandia landmass is emergent (Mortimer 2004; Campbell *et al*. 2006), and these regions are the result of subsequent events such as volcanic eruptions (e.g. Lord Howe Island, Chatham Islands, Auckland Islands) and tectonic activity (e.g. New Zealand, New Caledonia) (Gibbs 2006). Zealandia is approximately half the size of the Australian continent, and currently consists of New Zealand, New Caledonia, Lord Howe Island, Norfolk Island, Chatham Islands, Auckland Islands, Campbell Island and several submerged ridges (Lord Howe Rise, Norfolk Ridge, Chatham Rise, Campbell Plateau) (Fig. 1). Several isolated island archipelagos of recent geological origin are scattered throughout Zealandia (Fig. 1), and Trewick *et al*. (2007) has suggested that despite

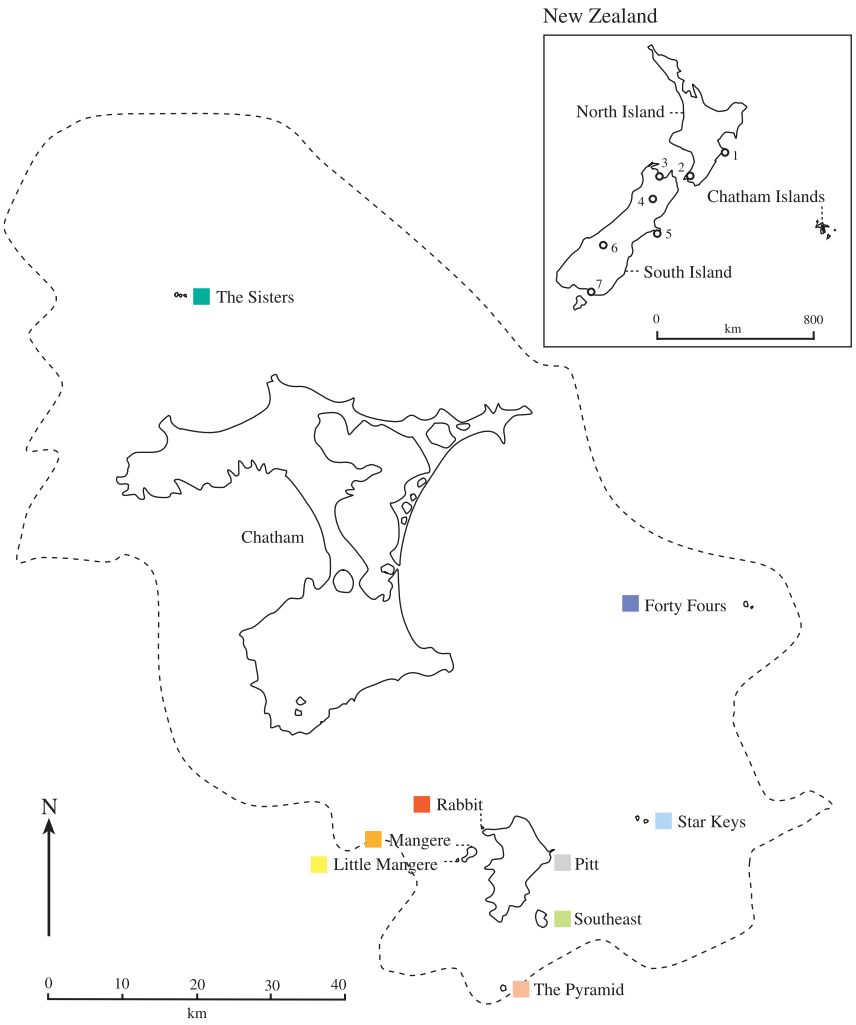

Fig. 2 The Chatham Islands, New Zealand. Colour boxes next to island names correspond to the *Oligosoma nigriplantare nigriplantare* haplotypes identified in Fig. 4. The dashed line represents the approximate coastline of the Chatham Islands during the last glacial maximum (18–22 thousand years ago). There are no records (fossil, historical, or current) of *O. n. nigriplantare* occurring on the main Chatham Island. Inset: Map of New Zealand indicating the sampling localities of *Oligosoma nigriplantare polychroma* samples listed in Table 1.

their Gondwanan heritage, these islands should be considered as oceanic islands whose biota has arisen as a result of overwater dispersal.

The Chatham Islands (44°00′S, 176°30′W) are situated at the eastern end of the Chatham Rise, approximately 800 km east of New Zealand (Figs 1 and 2), and is the most eastern edge of the Zealandia landmass. Fossil evidence indicates that the Chatham Islands supported a diverse biota (including dinosaurs) for ~20 million years (Myr) following its separation from Gondwana (Stilwell *et al*. 2006), but gradually became submerged along with the remainder of Zealandia (Campbell 1998; Campbell *et al*. 2006; Trewick *et al*. 2007). Geological evidence indicates that the Chatham Islands only recently re-emerged within the last 1–4 Myr as a result of volcanic and tectonic activity at the eastern end of the Chatham Rise (Campbell 1998; Campbell *et al*. 2006). Today, the Chatham Islands exist as an archipelago (total land area = 970 km^2) that consists of two main islands (Chatham Island, Pitt Island) and numerous smaller islets and rock formations (Fig. 2). The origin of individual islands varies, but most are the result of volcanic activity upon the continental crust (Campbell *et al*. 2006). Pleistocene glacial cycles (0.01–1.8 Ma) are believed to have resulted in the continual connection and separation of islands (Hay *et al*. 1970; Craw 1988), with only a single landmass present during the last glacial maximum (18 000–22 000 years ago; Fig. 2).

The biota of the Chatham Islands comprises a high proportion of endemics, and is believed to have recently colonized the archipelago via transoceanic dispersal, predominately from New Zealand (e.g. intertidal flora and fauna: Finlay 1928; Know 1954; invertebrates: Gaskin 1975; Emberson 1995, 1998, 2002; Trewick 2000; Arensburger *et al*. 2004; Skelley & Leschen 2007; freshwater aquatic taxa: Mitchell 1995; Stevens & Hogg 2004; McGaughran *et al*. 2006; birds: Millener & Powlesland 2001; Miller & Lambert 2006). It has been hypothesized that the transoceanic dispersal of the Chatham Islands biota has been aided by the westerly winds that have prevailed since the opening of the Drake passage (23.5 ± 2.5 Ma; Barker & Burrel 1982) and the

direction of ocean currents, which are influenced by the Chatham Rise (e.g. McGaughran *et al*. 2006). Recent molecular studies have indicated that divergences between Chatham Islands taxa and their closest mainland New Zealand relatives have occurred during the Pliocene or Pleistocene (i.e. within the last ~6 Myr; Trewick 2000; Arensburger *et al*. 2004; McGaughran *et al*. 2006; Paterson *et al*. 2006). However, although some studies have determined the level of genetic diversity within the Chatham Islands (e.g. Trewick 1998, 1999, 2000), we are not aware of any study that has examined in detail the post-colonization evolution of any species on the Chatham Islands.

Here, we examine the origin and post-colonization evolution of the Chatham Islands skink, *Oligosoma nigriplantare nigriplantare*. *Oligosoma n. nigriplantare* is part of the diverse endemic New Zealand skink fauna, which is comprised of two genera (*Oligosoma*, *Cyclodina*) and 32 described species (Gill & Whitaker 2001; Chapple & Patterson 2007; Chapple *et al*. 2008; in press a). The New Zealand common skink (*Oligosoma nigriplantare polychroma*) is widespread across New Zealand (North Island, South Island, Stewart Island), while *O. n. nigriplantare* is the sole reptile species on the Chatham Islands, where it occurs on all of the major islands except Chatham Island itself (Fig. 2). Given the geological history of the Chatham Islands, it is assumed that *O. n. nigriplantare* previously occurred on Chatham Island; however, there are no fossil records or historical reports of *O. n. nigriplantare* on Chatham Island (Hitchmough *et al*. 2005). Since *O. n. nigriplantare* is almost locally extinct on Pitt Island as a result of introduced mammals (Freeman 2000), the presence of introduced mammals on Chatham Island might have resulted in the local extinction of *O. n. nigriplantare* (Hitchmough *et al*. 2005). On vegetated islands, *O. n. nigriplantare* inhabits grassland and shrub habitat, but it also occurs on marine rock stacks with sparse vegetation (Freeman 2000).

It has been suggested that there was a single colonization of the Chatham Islands by *O. n. nigriplantare*, from New Zealand, during the Pleistocene (Fleming 1962; Towns 1974) or shortly thereafter (Hardy 1977). Such overwater dispersal appears to be widespread in squamate reptiles at both contemporary (Thomas & Whitaker 1995; Censky 2006) and evolutionary timescales (Glor *et al*. 2005; Rocha *et al*. 2006; Hare *et al*. 2008). A molecular phylogeny for the New Zealand skink radiation indicates that *O. n. nigriplantare* and *O. n. polychroma* are each other's closest relatives (D.G.C., C.H.D. and P.A.R., unpublished data). *Oligosoma n. nigriplantare* is morphologically distinct from *O. n. polychroma* (Hardy 1977; Daugherty *et al*. 1990; Patterson & Daugherty 1990), with each possibly representing distinct species.

Substantial morphological variation (body size, colour and colour pattern) is evident within *O. n. nigriplantare* (Hardy 1977; Daugherty *et al*. 1990; Patterson & Daugherty 1990; Freeman 2000), potentially indicating morphological evolution following its colonization of the Chatham Islands. Indeed, *O. n. nigriplantare* (up to 91 mm snout-vent length; SVL) has a substantially larger body size than *O. n. polychroma* (up to 77 mm SVL) (Hardy 1977; Patterson & Daugherty 1990; Gill & Whitaker 2001). The morphological diversity evident within *O. n. nigriplantare* has lead to speculation that more than one species is present within the Chatham Islands (McCann 1955). In fact, McCann (1955) recognized two species within the Chatham Islands, *Leiolopisma dendyi* and *Leiolopisma turbotti* (note that Patterson & Daugherty 1995 resurrected *Oligosoma* to accommodate all New Zealand members of *Leiolopisma*). Although both species were subsequently synonymized under *Leiolopisma n. nigriplantare* (now *O. n. nigriplantare*; Patterson & Daugherty 1990, 1995), the morphological and ecological diversity evident within *O. n. nigriplantare* might suggest the presence of substantial genetic differentiation within the species. Since all of the islands within the Chatham archipelago were repeatedly connected and separated during Pleistocene glacial cycles, it is possible that these processes have influenced the post-colonization evolutionary history of *O. n. nigriplantare*.

We use DNA sequence data from the NADH dehydrogenase subunit 2 (ND2) and NADH dehydrogenase subunit 4 (ND4) mitochondrial genes to examine the origin and post-colonization evolutionary history of *O. n. nigriplantare*. A calibrated molecular clock is used in conjunction with phylogenetic analyses to investigate the timing of colonization for *O. n. nigriplantare*. We then implement a range of phylogeographical analyses (e.g. Φ_{ST}, analysis of molecular variance, haplotype networks, nested clade phylogeographical analysis, and isolation by distance) to examine the evolution of *O. n. nigriplantare* within the Chatham Islands. We also assess the taxonomic status of *O. n. nigriplantare*.

Materials and methods

Taxonomic sampling

We obtained tissue samples from across the entire range of *Oligosoma nigriplantare nigriplantare* in the Chatham Islands (four to nine samples from nine different islands), and representative samples from across the mainland New Zealand range of *Oligosoma nigriplantare polychroma* (Fig. 2, Table 1, Table S1, Supplementary material). Samples were obtained from the Frozen Tissue Collection (FTC at Victoria University of Wellington, New Zealand) and ethanol-preserved specimens housed at the Museum of New Zealand, Te Papa Tongarewa (see Table S1). A broader phylogenetic study of the New Zealand skink fauna (D.G.C., P.A.R. and C.H.D., unpublished data) was used to select the outgroups for our analyses, the small-scaled skink (*Oligosoma microlepis*) and the robust skink (*Cyclodina alani*) (Table 1).

Table 1 Location and sample size (*n*) for taxa used in this study. The *Oligosoma nigriplantare nigriplantare* haplotypes found on each island within the Chatham Islands is indicated (see Fig. 4). Numbers in parentheses following the *Oligosoma nigriplantare polychroma* locality names correspond to the localities marked in Fig. 2

Species	*n*	Location	Latitude, Longitude	Haplotypes
O. n. nigriplantare	9	Southeast Island, Chatham Islands	44°21′S, 176°11′W	6, 10, 16–17, 22
(*n* = 54)	8	Mangere Island, Chatham Islands	44°16′S, 176°18′W	8, 13–15
	5	The Sisters, Chatham Islands	43°34′S, 176°49′W	27–28
	7	Little Mangere Island, Chatham Islands	44°17′S, 176°20′W	9, 15, 19
	6	Pyramid Island, Chatham Islands	44°26′S, 176°16′W	1–3
	6	Forty Fours, Chatham Islands	43°58′S, 175°51′W	20–21
	5	Rabbit Island, Chatham Islands	44°15′S, 176°17′W	5, 18, 24–26
	4	Star Keys, Chatham Islands	44°14′S, 176°00′W	7, 11, 23
	4	Pitt Island, Chatham Islands	44°17′S, 176°13′W	1, 4, 12
O. n. polychroma	1	Awatoto, Hawkes Bay, NI (1)	39°07′S, 177°18′E	—
(*n* = 7)	1	Pukerua Bay, Wellington, NI (2)	41°01′S, 174°53′E	—
	1	Moteuka, Nelson, SI (3)	41°06′S, 173°01′E	—
	1	Aniseed Valley, Canterbury, SI (4)	42°18′S, 172°41′E	—
	1	Banks Peninsula, Canterbury, SI (5)	43°44′S, 173°01′E	—
	1	Lake Hawea, Otago, SI (6)	44°21′S, 169°24′E	—
	1	Tiwai Point, Southland, SI (7)	46°36′S, 168°21′E	—
O. microlepis	1	Taihape, Manawatu-Wanganui, NI	39°41′S, 175°46′E	—
Cyclodina alani	1	Mercury Islands, Auckland, NI	36°39′S, 175°51′E	—

NI, North Island; SI, South Island.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from heart, muscle or liver tissue using a modified phenol–chloroform extraction protocol (Sambrook *et al*. 1989). For each sample, we targeted portions of the mitochondrial ND2 (~600 bp) and ND4 genes (~850 bp; incorporating most of the flanking-3′ tRNA cluster, including the histidine and serine tRNA genes). These regions were chosen because work at comparable taxonomic levels in New Zealand skinks, and other squamate reptile groups, have indicated useful levels of variability (e.g. Fuerst & Austin 2004; Greaves *et al*. 2007, 2008; Hare *et al*. 2008; Chapple *et al*. in press b).

The primers used to amplify and sequence these regions were L4437 (Macey *et al*. 1997) and ND2r102 (Sadlier *et al*. 2004) for ND2, and ND4I and tRNA-Leu (Forstner *et al*. 1995) for ND4. Polymerase chain reaction and sequencing were conducted as outlined in Greaves *et al*. (2007). Sequence data were edited using CONTIGEXPRESS in VECTOR NTI ADVANCE 9.1.0 (Invitrogen) and then aligned using CLUSTAL W (Thompson *et al*. 1994) executed in MEGA 3.1 (Kumar *et al*. 2004). The aligned sequences were translated into amino acid sequences using the vertebrate mitochondrial code. This was performed to determine whether these data corresponded to nuclear pseudogenes. As no premature stop codons were observed (apart from within the tRNAs flanking the ND4 sequence), we conclude that all sequences most likely represent true mitochondrial copies rather than nuclear pseudogenes. All sequences are available on GenBank under accession nos EF043106–EF043229 (see Table S1). We also used two ND2 sequences for *O. n. polychroma* (EF033052, EF033068) that were published previously in Chapple & Patterson (2007).

Phylogenetic analyses

The ND2 and ND4 data sets were concatenated, and estimates of nucleotide diversity π, and levels of uncorrected sequence divergence *d*, within and between *O. n. nigriplantare* and *O. n. polychroma* were calculated in MEGA. The base frequencies, number of segregating sites *S*, parsimony-informative sites, and the transition/transversion ratio (ti/tv) for all taxa (*O. nigriplantare* plus outgroups) were determined in PAUP*4.0b10 (Swofford 2002). We used chi-squared tests, as implemented in PAUP*, to test for equal base frequencies across sequences, using variable sites only. To test whether our sequences were evolving according to neutral expectations, we performed Tajima's *D* test (Tajima 1989, 1996) in DNASP version 3 (Rozas & Rozas 1999). To identify the model of evolution that best fit our sequence data, we performed hierarchical likelihood-ratio tests (hLRT) in MODELTEST 3.7 (Posada & Crandall 1998) from the log-likelihood scores generated in PAUP*. The Hasegawa–Kishino–Yano model with gamma-distributed rate variation (HKY85 + G, α parameter = 0.204, –lnL = 4467.646) was selected as the most appropriate.

Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian tree building methods were used. The ML

analyses were conducted in PAUP* using the parameters estimated by MODELTEST. MP and ML heuristic searches were conducted in PAUP* using random stepwise addition and the tree-bisection–reconnection branch swapping algorithm. Bayesian analyses were completed using the computer program MRBAYES version 3.1 (Ronquist & Huelsenbeck 2003). We used four Markov chains per run, started from a random tree, and ran the analysis for 1 million generations. To ensure that the analysis obtained a sampling of the full tree space rather than becoming trapped in local optima, the analysis was run twice. The chain was sampled every 100 generations to obtain 10 000 trees. The program TRACER 1.3 (Rambaut & Drummond 2004) was used to check for chain convergence. The first 2500 sampled trees were discarded as the burn-in phase, with the last 7500 trees used to estimate Bayesian posterior probabilities. To assess the statistical support for the final topology, we used both Bayesian posterior probabilities and ML bootstrap analysis (200 replicates).

Estimating divergence times

To estimate the divergence time between *O. n. nigriplantare* and *O. n. polychroma*, we calibrated the evolutionary rate of ND2 by re-analysing the data from Macey *et al*. (1998) for the agamid genus *Laudakia*. Macey *et al*. (1998) calibrated this rate through geological dating of tectonic events (mountain uplift) on the Iranian Plateau. The ND2 evolutionary rate has been demonstrated to be consistent (~1.2–1.4%) across several vertebrate groups (fish, amphibians, reptiles; reviewed in Weisrock *et al*. 2001). We re-calculated the evolutionary rate for *Laudakia* using only the 550-bp fragment of ND2 used in the present study (e.g. Greaves *et al*. 2007, 2008; Smith *et al*. 2007; Hare *et al*. 2008; Chapple *et al*., in press b). We calculated average between-group nucleotide differences across each of the calibrated nodes from Macey *et al*. (1998) (1.5, 2.5, 3.5 Ma), plotted them against time and then used the slope of the linear regression to calculate a rate of evolution for our 550-bp fragment of ND2. This resulted in an evolutionary rate of 1.4% per Myr (0.7% per lineage, per Myr) and is slightly faster than the rate of 1.3% per Myr found by Macey *et al*. (1998).

Evolution of O. n. nigriplantare *within the Chatham archipelago*

All calculations of genetic diversity (number of haplotypes: *na*; haplotype diversity: *h*; Tajima's *D*; *S*; and π) within *O. n. nigriplantare* were performed in ARLEQUIN version 3.0 (Excoffier *et al*. 2005). Genetic differentiation across the nine island populations was estimated using the Φ_{ST}, incorporating a measure of molecular distance between haplotypes, and hierarchical analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) as implemented by ARLEQUIN. The first level of the AMOVA compared individuals within each island, and the second level consisted of comparisons of individuals across all of the Chatham Islands. Significance levels of the estimated values were calculated by 10 000 permutations, and adjusted according to the Bonferroni correction procedure (Rice 1989) as described by Holm (1979). This procedure negates the potential accumulation of type I errors under during multiple comparisons.

To investigate the genealogical relationships among haplotypes of *O. n. nigriplantare*, we constructed a haplotype network using the method of Templeton *et al*. (1992) in TCS version 1.21 (Clement *et al*. 2000). We then used nested clade phylogeographical analysis (NCPA; Templeton *et al*. 1995; Templeton 1998) implemented with GEODIS version 2.4 (Posada *et al*. 2000) to investigate the association between genetic and geographical variation and infer the processes responsible. Clades within the network are nested with standard nesting rules (Templeton *et al*. 1987; Templeton & Sing 1993; Templeton 1998). Using the nested design and a pairwise distance matrix (minimum straight line distances between islands), GEODIS calculates three main statistics: the clade distance $D_{c,}$ measures the geographical range of a clade and haplotypes therein; the nested clade distance $D_{n,}$ measures how a clade is geographically distributed relative to other clades in the same higher-level nesting category; and interior-tip distances I–T_c and I–T_n, that indicate how widespread younger clades (tips) are compared to their ancestors (interiors), relative to other clades within the same nesting clade. Statistical significances were calculated by comparison with a null distribution derived from 10 000 permutations of clades against sampling locality. For clades in which geographical association was significant, inferences about population processes were made using inference keys (Templeton *et al*. 1995; Templeton 1998; November 2005 update available at http://darwin.uvigo.es/software/geodis.html). These keys provide explicit criteria for the interpretation of any significant interaction that include an assessment of sampling adequacy. The validity of NCPA for inferring population structure and historical processes has been questioned (Knowles & Maddison 2002; Panchal & Beaumont 2007; Petit 2008). However, these criticisms have been rebutted (Templeton 2004, 2008), and NCPA remains a valid phylogeographical analysis method when used with complementary analyses (e.g. Φ_{ST}, AMOVA, isolation by distance) and adequate consideration of prior expectations (e.g. predictions from geological data) (Garrick *et al*. 2008; Templeton 2008).

The association between genetic differentiation and geographical distance was examined for (genetic) isolation by (geographical) distance (Wright 1943). Genetic differentiation (pairwise Φ_{ST}) among island populations was plotted against minimum straight line geographical distance between islands using reduced major axis (RMA) regression (Sokal & Rohlf 1981) as implemented by the Isolation

Locality	*n*	π	*S*	Φ	Tajima's *D*	n_a	*h*
Southeast Island	9	0.005 ± 0.003	18	0.561	0.073	5	0.833 ± 0.098
Mangere Island	8	0.002 ± 0.001	6	0.635	1.022	4	0.821 ± 0.101
The Sisters	5	0.003 ± 0.002	10	0.619	–1.193	2	0.400 ± 0.237
Little Mangere Island	7	0.001 ± 0.001	5	0.661	–1.486	3	0.524 ± 0.209
Pyramid Island	6	0.001 ± 0.001	3	0.669	–1.233	3	0.600 ± 0.215
Forty Fours	6	0.001 ± 0.001	2	0.675	–1.132	2	0.333 ± 0.215
Rabbit Island	5	0.005 ± 0.003	15	0.572	–0.406	5	1.000 ± 0.127
Star Keys	4	0.005 ± 0.004	12	0.579	0.444	3	0.833 ± 0.222
Pitt Island	4	0.002 ± 0.002	6	0.639	–0.809	3	0.833 ± 0.222
Σ =	54	0.007 ± 0.001	74	0.623	–1.552	28	0.961 ± 0.011

Table 2 The number of *Oligosoma nigriplantare nigriplantare* individuals sequenced on each island of the Chatham archipelago and associated nucleotide diversity (π ± SD), polymorphic sites (*S*), Φ_{ST} values within, Tajima's *D*, number of observed haplotypes (n_a), and haplotype diversity (*h* ± SD).

By Distance Web Service (IBDWA; Bohonak *et al*. 2005). The significance of the association was determined by applying Mantel's permutation test (Mantel 1967) with 1000 matrix randomizations.

Results

The edited alignment comprised 1323 bp of sequence data (550 bp ND2, 773 bp ND4; no insertions or deletions were present) for 61 *Oligosoma nigriplantare* individuals and two outgroups (*Oligosoma microlepis*, *Cyclodina alani*). The data set had 334 variable sites (per site, p*S* = 0.253), of which 220 were parsimony informative. At the first position, 54 sites were polymorphic, compared with 119 and 161 polymorphisms at the second and third positions, respectively. Tajima's *D* statistic indicated that the sequences were evolving according to neutral expectations (*D* = –1.739, *P* = 0.100). Across all sites, the relative rates of each substitution type estimated under a general time-reversible model (Rodriguez *et al*. 1990) revealed a predominance of transitions (ti/tv = 4.340: A↔C = 2.603, A↔*G* = 34.716, A↔T = 2.888, C↔*G* = 1.967, C↔T = 26.670, relative to G↔T = 1.000). However, a chi-squared test indicated no significant base composition heterogeneity for variable sites among sequences (χ^2 = 83.127, d.f. = 186, *P* = 1.000, *P* > 0.05). For all variable sites, the mean base frequencies were: A = 0.31, C = 0.36, G = 0.11, T = 0.22.

Origin of O. n. nigriplantare

All three phylogenetic analysis methods [(MP (14 most parsimonious trees, 520 steps, consistency index = 0.712, RI = 0.8547), ML, Bayesian] recovered virtually identical tree topologies, indicating that *O. n. nigriplantare* and *O. n. polychroma* are reciprocally monophyletic, with relatively shallow divergences within *O. n. nigriplantare* and comparatively deeper divergences within *O. n. polychroma*. We therefore only present the ML tree (–lnL = 4523.711; HKY85 + G model of substitution) with ML bootstraps and Bayesian posterior probabilities indicating branch support (Fig. 3). All major nodes in the ML tree were extremely well supported (≥ 99 bootstrap, 1.0 posterior probability), including internal nodes within *O. n. polychroma* (≥ 64 bootstrap, ≥ 0.84 posterior probability). Despite strong support for all nodes (≥ 51 bootstrap, ≥ 0.88 posterior probability), the bifurcating tree building methods were unable to resolve all lineages within *O. n. nigriplantare*. We therefore examined the relationships among *O. n. nigriplantare* populations using haplotype networks and NCPA (see below; Fig. 4).

Substantial genetic differentiation is evident between *O. n. nigriplantare* and *O. n. polychroma*, with uncorrected sequence divergence ranging from 0.082 ± 0.007 (ONP37: Motueka and haplotype 20: Forty Fours) to 0.102 ± 0.008 (ONP8: Tiwai Point and haplotype 26: Rabbit Island), with a mean of 0.090. This level of genetic differentiation indicates that *O. n. nigriplantare* and *O. n. polychroma* diverged approximately 5.86–7.29 Ma.

Evolution of O. n. nigriplantare *within the Chatham archipelago*

Relatively shallow genetic divergences were observed in *O. n. nigriplantare*, with uncorrected sequence divergences (*d* ± SE) up to 0.020 ± 0.004 (haplotype 28: The Sisters and haplotype 23: Star Keys), with a mean divergence of 0.007. In contrast, deep genetic divergences were evident in *O. n. polychroma*, with uncorrected sequence divergences up to 0.083 ± 0.007 (ONP25: Lake Hawea and ONP1: Pukerua Bay) and a mean divergence of 0.053. Nucleotide diversity (π ± SE) was 0.007 ± 0.001 for *O. n. nigriplantare*, 0.053 ± 0.004 for *O. n. polychroma*, and 0.025 ± 0.002 for the entire *O. nigriplantare* ingroup. The limited amount of genetic variation within *O. n. nigriplantare* could account for the lack of resolution in the phylogeny for the relationships among the nine island populations.

Values for Tajima's *D* were not significant for the nine island populations of *O. n. nigriplantare* (*P* > 0.05) or *O. n. nigriplantare* overall (Table 2). Thus, the null hypothesis of neutral evolution could not be rejected. Nucleotide

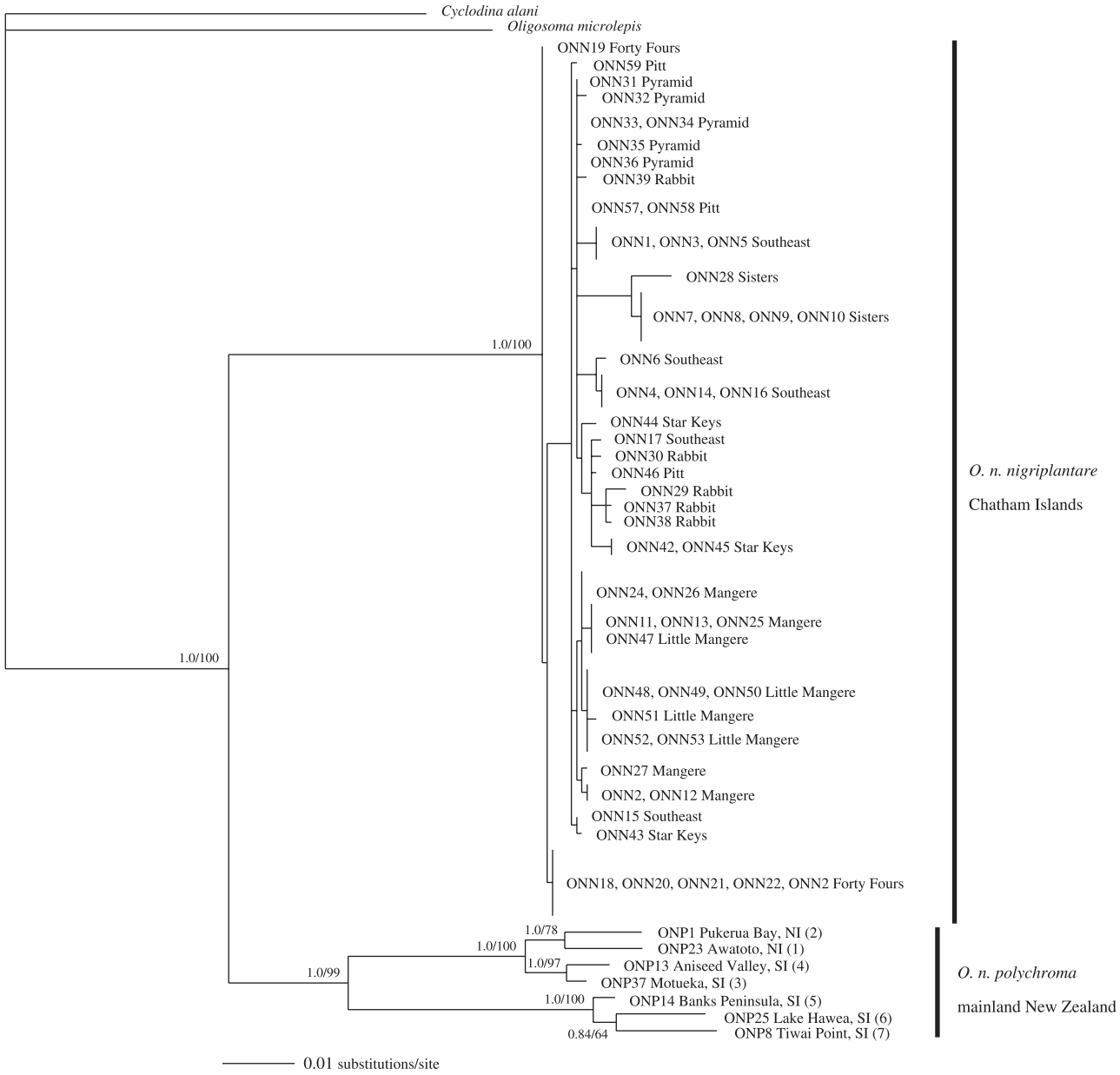

Fig. 3 Maximum likelihood (ML) phylogenetic tree (–lnL = 4523.711) based on the combined mitochondrial DNA gene regions (ND2 and ND4 = 1323 bp; HKY85 + G model of substitution), showing the relationship between the two *Oligosoma nigriplantare* subspecies (*O. n. nigriplantare* and *O. n. polychroma*). The topology of the maximum parsimony and Bayesian trees were identical to the ML tree shown. Two measures of branch support are indicated with Bayesian posterior probabilities shown on the left and ML bootstraps shown on the right (support values within *O. n. nigriplantare* are not shown as these relationships are examined in more detail in Fig. 4). The tree is rooted with two New Zealand skink species, *Oligosoma microlepis* and *Cyclodina alani*.

sequence diversity was similar for all islands (0.001: Forty Fours to 0.005: Star Keys). Twenty-eight haplotypes were identified in *O. n. nigriplantare* across the nine Chatham Islands sampled (Fig. 4, Table 1). No haplotypes were abundant, and all of the islands contained only private haplotypes (up to five), except for haplotype 15 (shared between Mangere and Little Mangere Islands) and haplotype 1 (shared between Pyramid Island and Pitt Island). Haplotype diversity ($h \pm$ SD) was high on all islands (0.333: Forty Fours to 1.000: Rabbit Island) and within *O. n. nigriplantare* (0.961 ± 0.011). AMOVA also showed high levels of genetic differentiation among *O. n. nigriplantare* on different islands (Table 3). Individuals differed from what would be expected by chance ($\Phi_{ST} = 0.623$, $P < 0.001$), and island specific Φ_{ST} values ranged from 0.561 (Southeast Island) to 0.675 (Forty Fours). Many pairwise comparisons

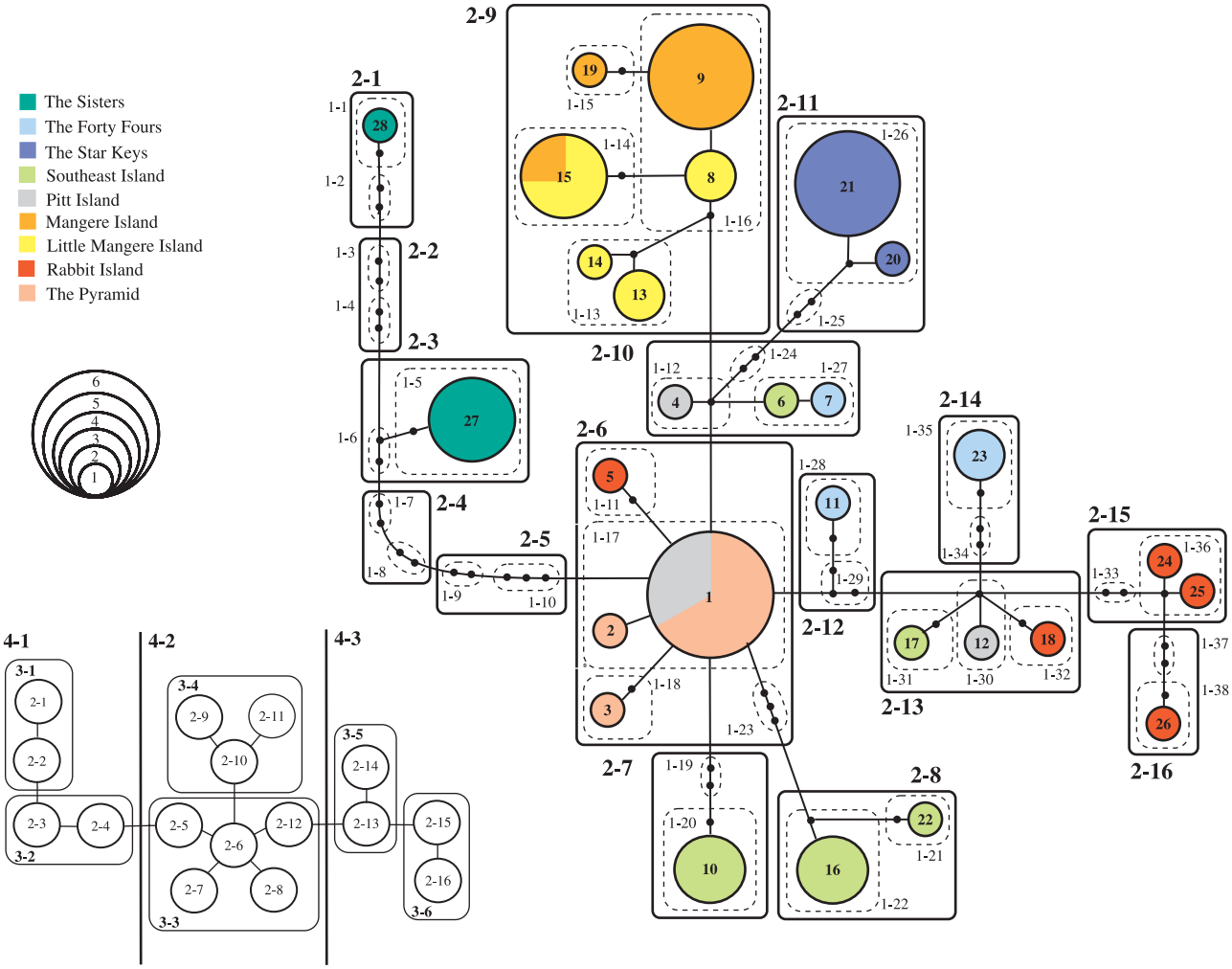

Fig. 4 Nested haplotype network representing the relationships within the Chatham Islands skink (*Oligosoma nigriplantare nigriplantare*). Each circle represents a unique haplotype (numbered; Table 1) and size refers to the frequency of that haplotype (middle key left of figure). Colour corresponds to the island/s in which the haplotype was found (top key left of figure; Fig. 2). Solid lines connect haplotypes with a single mutational difference and small solid circles represent hypothetical historical haplotypes or current haplotypes not sampled. Haplotypes are nested into 1-step and 2-step clades, designated 1-1 to 1-38, and 2-1 to 2-16, respectively. Inset bottom left: higher level nested design (3-step and 4-step clades).

Table 3 Hierarchical analysis of molecular variance (AMOVA) employing uncorrected genetic distances for *Oligosoma nigriplantare nigriplantare*. Statistical significance (*P*) was tested with 10 000 permutations

	Observed partition				
Source of variation	Percentage	Variance	*P*	d.f.	Sum of squares
Among populations	62.32	3.00	< 0.01	8	157.06
Within populations	37.68	1.81	< 0.01	45	81.57
Total		4.81	< 0.01	53	238.63

of Φ_{ST} were significant after sequential Bonferroni correction for multiple tests at $\alpha = 0.05$, although not all island populations were clearly differentiated (Table 4).

The evolutionary relationships within *O. n. nigriplantare* were well-resolved in the haplotype network constructed in TCS (Fig. 4). The haplotype network was partitioned into 38 one-step clades, 16 two-step clades, 6 three-step clades and 3 four-step clades. Because our data consisted of predominantly geographically private haplotypes, much of the data was immediately excluded from NCPA, as inference

Table 4 Pairwise Φ_{ST} estimates (below diagonal) and significance of differentiation (above diagonal) among island populations of *Oligosoma nigriplantare nigriplantare*. Asterisks denote statistical significance following sequential Bonferroni correction ($\alpha = 0.05$)

Island/island group	Southeast	Mangere	Sisters	Little Mangere	Pyramid	Forty Fours	Rabbit	Star Keys	Pitt
Southeast	—	0.0001*	0.0003*	0.0001*	0.0323	0.0003*	0.0087	0.0133	0.1795
Mangere	0.420	—	0.0010*	0.0047	0.0003*	0.0001*	0.0008*	0.0013*	0.0024
Sisters	0.697	0.818	—	0.0018*	0.0028	0.0016*	0.0092	0.0085	0.0069
Little Mangere	0.502	0.304	0.864	—	0.0008*	0.0005*	0.0011*	0.0045	0.0033
Pyramid	0.168	0.565	0.840	0.750	—	0.0018*	0.0046	0.0056	0.2202
Forty Fours	0.626	0.792	0.896	0.886	0.889	—	0.0024	0.0042	0.0050
Rabbit	0.271	0.553	0.727	0.637	0.412	0.730	—	0.0676	0.1438
Star Keys	0.255	0.518	0.729	0.605	0.398	0.736	0.193	—	0.1994
Pitt	0.092	0.437	0.772	0.629	0.051	0.803	0.187	0.145	—

can only be made for clades in which there is both genetic and geographical variation. Significantly small or large values for D_c, D_n, I–T_c or I–T_n ($P < 0.05$) indicated an association between haplotypes and geography in only clade 1-16 at the first nesting level (Table 5). Biological inference indicated allopatric divergence for the relationship between the private haplotypes 8 and 9 of Mangere and Little Mangere Islands within this clade (Table 6). At the three-step level, clades 3-3 and 3-4 both indicated a significant pattern of restricted gene flow and isolation by distance for the islands: Rabbit, Southeast, Pitt, Mangere, Little Mangere, the Pyramid and the Star Keys. Although a significant genetic–geographical relationship was found in clade 4-2, inference was ambiguous as there were too few clades at this nesting level to discriminate between range expansion, colonization and restricted dispersal/gene flow. The Sisters were brought into the analysis for the first time at the total cladogram level. According to the inference key, past fragmentation must be assumed to explain the relationship between nested clades at this level because the species is absent from intermediary geographical locations (Chatham Island). This conclusion is further supported by clades 4-1 and 4-2 being connected by a larger than average number of mutational steps.

A significant relationship was observed between Φ_{ST} and geographical distance for comparisons among all islands in both observed and log-transformed data. The highest correlation ($r = 0.714$, $Z = 854276.590$, one-sided $P = 0.007$) was among the untransformed data with an isolation by distance slope of 762×10^{-8} and intercept of 0.270 (Fig. 5).

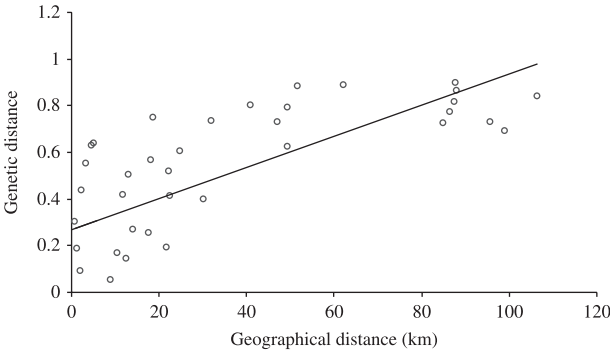

Fig. 5 The relationship between pairwise Φ_{ST} and geographical distance among island populations of *Oligosoma nigriplantare nigriplantare*. The solid line represents the regression line for all comparisons ($r^2 = 0.509$, $P = 0.007$).

Discussion

Our data indicate that *Oligosoma nigriplantare nigriplantare* colonized the Chatham Islands (via overwater dispersal) on a single occasion, diverging from the New Zealand common skink (*Oligosoma nigriplantare polychroma*) approximately 5.86–7.29 Ma. Despite the presence of only relatively shallow genetic divergences within *O. n. nigriplantare* (maximum divergence ~2%), our analyses indicated that the island populations were highly differentiated with evidence for genetic isolation by distance. This pattern might reflect the influence of Pleistocene glacial cycles where fluctuating sea levels resulted in the repeated connection and separation of islands within the Chatham archipelago. Our study represents the first detailed examination of the post-colonization evolutionary history of a species within the Chatham Islands.

Origin of the Chatham Islands skink, O. n. nigriplantare

Our phylogenetic analyses confirmed the monophyly of *O. n. nigriplantare*, indicating a divergence from *O. n. polychroma* within the last 5.86–7.29 Myr. The estimated divergence time of *O. n. nigriplantare* and *O. n. polychroma* in the Late Miocene–Early Pliocene pre-dates the re-emergence of the Chatham Islands 1–4 Ma (Campbell 1998; Campbell *et al*. 2006) and substantially pre-dates the previously hypothesized Pleistocene colonization of the Chatham

Table 5 Nested clade analysis results for *Oligosoma nigriplantare nigriplantare* mitochondrial DNA haplotypes based on 10 000 permutations. Clade distance (D_c), nested clade distance (D_n) and the average I-T distances in clades with both tip (T) and interior (I) nested clades are provided. S indicates that the distance is significantly small at the level and L indicates that the distance is significantly large ($\alpha = 0.05$)

0 step			1 step			2 step			3 step			4 step		
Haplotype	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n	Clade	D_c	D_n
1	6195.2	5527.6	1-17	6195.2	7228.6	2-6	8733.9	10034.0	3-3	9902.6S	18057.9	4-2	21433.7S	27752.2S
2	0.0	2968.5												
I-T	6195.2	2559.1												
5			1-11	0.0	16455.9									
3			1-18	0.0	5757.4									
			I-T	6195.2	–3878.0									
16			1-22			2-8	0.0S	6810.2						
22			1-21											
11			1-28			2-12	0.0	21969.4						
10			1-20			2-7	0.0	6583.2						
						I-T	7860.5L	4514.6L						
4			1-12	0.0	7208.9	2-10	10698.7	20098.5	3-4	24337.7	23617.1L			
6	0.0	17678.2	1-27	17678.2	12443.6									
7	0.0	17678.2												
I-T	0.0	0.0	I-T	–17678.2	–5234.7									
8	0.0	724.5L	1-16	514.2	494.7	2-9	554.4S	17622.1S						
9	0.0S	398.5S												
13			1-13	0.0	429.1									
14														
15			1-14	531.3	436.1									
19			1-15	0.0	455.4									
			I-T	248.5	58.8									
20			1-26			2-11	0.0S	42876.9L						
21														
						I-T	10302.7	–4739.3	I-T	–14435.1S	–5559.2			
12			1-30	0.0	1656.2	2-13	5827.0	11547.6	3-5	12746.3	12314.8	4-3	12147.5	22980.7
17			1-31	0.0	8055.8									
18			1-32	0.0	7769.0									
			I-T	0.0	–6256.2									
23			1-35			2-14	0.0	14801.3						
						I-T	5827.0	–3253.7						
24			1-36			2-15			3-6	0.0	9806.8			
25														
26			1-38			2-16								
									I-T	12746.3	2508.0			
27			1-5			2-3			3-2			4-1	0.0S	88768.0L
28			1-1			2-1			3-1					
												I-T	13958.3	–20531.3S

Islands (Fleming 1962; Towns 1974; Hardy 1977). Our result is concordant with other molecular studies that have indicated that the divergence between Chatham Islands taxa and their closest mainland relative pre-dates the hypothesized re-emergence of the islands (e.g. Coleoptera, *Geodorcus*: 4.9–5.6 Ma, Trewick 2000; up to 6 Ma for several plant and animal taxa, Paterson *et al*. 2006). This might result from the predicted discordance between gene trees and species trees, which appear to be especially common for recent species and population divergences as would be likely for species that have colonized the Chatham Islands (e.g. Edwards & Beerli 2000; Arbogast *et al*. 2002). However, for several other taxa the estimated colonization time was 1–4 Ma (Coleoptera, *Mecodema*: 1.2–1.4 Ma, Trewick 2000; Orthoptera, *Talitroposis*: 1.3–1.5 Ma, Trewick 2000; Blattoidea, *Celatoblatta*: 2.1–2.45 Ma, Trewick 2000; freshwater isopods, *Austridotea lacustris*: 2.6–4.5 Ma, *Austridotea annectens*: 1.9–2.2 Ma, McGaughran *et al*. 2006; cicada, *Kikihia longula*, 1.5 Ma, Arensburger *et al*. 2004), which corresponds to the hypothesized re-emergence of the islands.

Table 6 Chi-squared test of geographical association and biological inference for clades and haplotypes therein from the nested clade analysis of *Oligosoma nigriplantare nigriplantare*. *P* is the probability of obtaining a chi-squared statistic larger than or equal to the observed statistic by randomly permuting the nested contingency results 10 000 times. *Too few clades to determine concordance and discriminate between range expansion, colonization and restricted dispersal/gene flow

Clade	χ^2	*P*	Chain of inference	Inference
1-16	7	0.049	1-19 No	Allopatric fragmentation
3-3	34	0.001	1-2-3-4 No	Restricted gene flow/isolation by
3-4	48	< 0.001	1-2-3-4 No	Restricted gene flow/isolation by
4-2	32.588	< 0.001	1-2-3-5-6	Insufficient genetic resolution*
Total Cladogram	80.258	< 0.001	1-2-3-5-6-13-14- No	Past fragmentation

Given the geological history of the Chatham Islands, and the estimated colonization times of Chatham Islands taxa, it appears that the resident biota reached the Chatham Islands via overwater dispersal, predominately from New Zealand (e.g. Trewick *et al*. 2007; Landis *et al*. 2008). Long-distance overwater dispersal is increasingly being recognized as an important process in the biogeography of many animal taxa (de Queiroz 2005; Cowie & Holland 2006; Heaney 2007). Indeed, such overwater dispersal has been demonstrated in squamate reptiles over both contemporary (Thomas & Whitaker 1995; Censky 2006) and evolutionary timescales (Glor *et al*. 2005; Rocha *et al*. 2006; Hare *et al*. 2008). Several New Zealand skink species exhibit behavioural, ecological and physiological traits that might enable them to survive being transported on driftwood during long-distance overwater travel. For example, some New Zealand skink species (*Oligosoma acrinasum*, *O. smithi*, *O. suteri*) live in coastal or intertidal habitats, readily enter the water, can tolerate saltwater, and can hold their breath for up to 20 min (Thomas 1985; Thomas & Whitaker 1995; Miller 2007). *Oligosoma n. polychroma* commonly occurs in coastal regions (Gill & Whitaker 2001), and *O. n. nigriplantare* occurs in both coastal regions and small isolated islets, placing both subspecies in areas where there is the potential for them to be transported between islands via overwater dispersal.

Evolution of O. n. nigriplantare *within the Chatham archipelago*

Although several studies have examined the level of molecular divergence in species within the Chatham Islands (e.g. Trewick 1998, 1999, 2000; Miller & Lambert 2006), there has been no previous examination of the post-colonization evolution of a Chatham Islands species. The maximum genetic divergence within *O. n. nigriplantare* was only ~2%, indicating that it is unlikely that rapid speciation or radiation occurred following colonization, as observed in several other taxa inhabiting other isolated archipelagos (reviewed in Emerson 2002). However, substantial morphological divergence appears to have occurred in *O. n. nigriplantare* following its colonization of the Chatham Islands (Hardy 1977; Daugherty *et al*. 1990; Patterson & Daugherty 1990). Rapid shifts in body size (e.g. dwarfism, gigantism) are common in squamate reptiles inhabiting islands (Keogh *et al*. 2005). Indeed, *O. n. nigriplantare* (up to 91 mm SVL) is substantially larger in body size compared to *O. n. polychroma* (up to 77 mm SVL) (Hardy 1977; Patterson & Daugherty 1990; Gill & Whitaker 2001). However, *O. n. nigriplantare* also exhibits substantial variation in body size and colour pattern both within and between islands (Hardy 1977; Freeman 2000). Hardy (1977) suggested that the relaxation of selection pressures following colonization might have resulted in the rapid morphological diversification of *O. n. nigriplantare*. Indeed, rapid morphological diversification in response to variation in the available niches and resources in island habitats is common across a wide range of animal taxa (e.g. Lomolino *et al*. 2006). The results of our study suggest that morphological divergence and genetic divergence are decoupled in *O. n. nigriplantare*, as is common in other squamate reptile species (e.g. Bruna *et al*. 1996; Malhotra & Thorpe 2000).

We found only shallow genetic differentiation (maximum genetic divergence ~2%) across the nine islands within the Chatham archipelago inhabited by *O. n. nigriplantare* (compared to ~8% maximum divergence within *O. n. polychroma*). Given the substantial genetic divergence between *O. n. nigriplantare* and *O. n. polychroma* (~9%), the limited level of genetic differentiation within *O. n. nigriplantare* (~2%) suggests an intriguing post-colonization evolutionary history. Since a drop in sea level of ~130 m (as occurred during the last glacial maxima) would be sufficient to transform the Chatham Islands from an island archipelago to a single landmass (Fig. 2), Pleistocene glacial cycles might have had a significant influence on the evolution of *O. n. nigriplantare* within the Chatham Islands. Given that *O. n. nigriplantare* can persist even on small rock stacks, where they feed on invertebrates and seabird regurgitation (McCann 1955), sea level changes might have resulted in repeated fragmentation. Severe fragmentation on small rock stacks might have resulted in small population size and genetic bottlenecks, leading to the erosion of genetic

variation. Dispersal between islands might have also resulted in founder effects and the loss of genetic variation. This might act to explain the limited genetic differentiation currently present among *O. n. nigriplantare* on different islands. Alternatively, the repeated re-connection of the islands might have acted to enhance gene flow across the archipelago and limit genetic divergence within *O. n. nigriplantare*. Indeed, our genetic analyses reveal an interesting combination of restricted gene flow and isolation by distance within *O. n. nigriplantare*.

Several of our analyses indicated restricted gene flow for *O. n. nigriplantare* between islands within the Chatham archipelago. Haplotypic diversity was high in *O. n. nigriplantare*, with no widespread haplotypes and a predominance of private haplotypes. Where haplotypes were shared between islands, it was limited to adjacent islands (Mangere and Little Mangere) or islands within close proximity to each other (Pyramid and Pitt) (Fig. 2). Our analyses (NCPA, Φ_{ST}, AMOVA) indicated extremely high levels of population differentiation between islands. The pairwise Φ_{ST} values confirm that strong differentiation even exists among populations separated by less than ~1 km (Mangere and Little Mangere Islands), a result interpreted as allopatric divergence by our NCPA. Restricted gene flow and/or allopatric divergence were observed across all islands, suggesting that water between islands represents a significant barrier to dispersal for *O. n. nigriplantare* within the Chatham archipelago. This indicates that overwater dispersal might not be common over short evolutionary timescales for *O. n. nigriplantare*.

Given the shallow genetic divergences and lack of well-resolved clades within *O. n. nigriplantare,* it is likely that there has been some degree of gene flow between islands within the Chathams archipelago. The Mantel tests of genetic and geographical distance, along with the inferences from NCPA, indicate a significant pattern of isolation by distance within *O. n. nigriplantare*. This suggests that restricted gene flow has only recently influenced the evolutionary history of *O. n. nigriplantare*, possibly since sea level rises after the last glacial maximum (18 000–22 000 years ago), or even previous glacial maxima, isolated islands within the Chatham archipelago (e.g. Hay *et al*. 1970; Craw 1988).

The Sisters were both the most genetically divergent and geographically isolated *O. n. nigriplantare* population (Figs 2 and 4). Our NCPA indicated that this was a result of past fragmentation. Although it appears that The Sisters would have been part of a single Chathams landmass during Pleistocene glacial maxima (Fig. 2), it is possible that it was not always connected to this single landmass during periods of lowered sea level. Alternatively, the genetic divergence of The Sisters population might be explained by the presumed local extinction (due to introduced mammals) of the Chatham Island *O. n. nigriplantare* population (Freeman 2000; Hitchmough *et al*. 2005). If this is in fact the case, it might suggest that introduced mammals are a significant conservation concern for the persistence of *O. n. nigriplantare* on the Chatham Islands.

Although this study inferred the post-colonization evolutionary history of *O. n. nigriplantare* solely on the basis of mitochondrial DNA, future studies could employ other molecular markers such as microsatellite DNA to examine gene flow (i.e. migration rates between islands), historical population sizes, and differentiation between islands. However, the present study has highlighted that the isolated archipelagos within the largely submerged continent of Zealandia provide an ideal opportunity to examine the origin and post-colonization evolutionary history of island species.

Taxonomic implications

Our genetic analyses of *O. n. nigriplantare* do not support the previous morphologically based species boundaries within the Chatham Islands suggested by McCann (1955). McCann (1955) listed *Leiolopisma dendyi* as occurring on Pitt Island, The Sisters, Southeast Island, Mangere Island, Rabbit Island, and The Star Keys, while *Leiolopisma turbotti* was believed to be restricted to Little Mangere Island, The Forty Fours and the Pyramid. Our analyses do not support such a division, and instead indicate that there is only a single variable species on the Chatham Islands, *O. n. nigriplantare*. However, since *O. n. nigriplantare* and *O. n. polychroma* can be distinguished on the basis of both morphological (Hardy 1977; Daugherty *et al*. 1990; Patterson & Daugherty 1990) and genetic divergence (this study), the two subspecies might represent distinct species.

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This study formed part of the MSc research of Libby Liggins. Her research is interested in biogeography and the recent evolutionary history of New Zealand's terrestrial and marine life. David Chapple's research is focused on the phylogenetics, phylogeography and evolutionary history of squamate reptiles. Charles Daugherty has research interests on the evolutionary and population biology of vertebrates, conservation genetics and ecological restoration. Peter Ritchie's research focuses on the evolutionary genetics of species in New Zealand, the Southern Ocean and Antarctica.

Supplementary material

The following supplementary material is available for this article:

Table S1 GenBank accession numbers, tissue codes, sources and location for Oligosoma and Cyclodina taxa used in this study. Museum acronyms are: CD/FT codes, National Frozen Tissue Collection (NFTC), Victoria University of Wellington; RE/S codes, specimen collection from Museum of New Zealand, Te Papa Tongarewa.

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