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Subtle population genetic structure in the Hawaiian grouper, *Epinephelus quernus* (Serranidae) as revealed by mitochondrial DNA analyses

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The endemic Hawaiian grouper, *Epinephelus quernus*, is a commercially important species experiencing intense fishing pressure in part of its distributional range. We examined population genetic structure with 398 base pairs of the mitochondrial control region across a large portion of the range of *E. quernus*, spanning approximately 2000 km of the Hawaiian archipelago. Examination of genetic diversity shows that Gardner Island, situated midway along the island chain, harbours the most diverse haplotypes. *F*-statistics and Bayesian estimates of migration also reveal the mid-archipelago as genetically differentiated, where the first significant break among adjacent pairs of populations lies between the islands of Nihoa and Necker. Most island comparisons beyond Necker and Gardner to the northwest and among the lower five islands to the south-east show little to no genetic differences. Evidence of historical population expansion across the islands was also found by Maximum Likelihood analyses. The results suggest that management should be structured to reflect the genetic differentiation and diversity in the mid-archipelago, the patterns of which may be associated with oceanic current patterns. *Biological Journal of the Linnean Society,* 2004, **81**, 449–468.

ADDITIONAL KEYWORDS: control region – Hawaiian archipelago – migration – population growth – population structure.

INTRODUCTION

Direct estimates of migration based on tagging studies are often impossible to obtain for marine fish living in deep-water habitats. For these species, indirect inference from population genetic data can be a practical alternative to aid in the delimitation of 'stocks' for fisheries management. While direct methods have the advantage of providing a contemporary estimate of migration on an ecological timescale, traditional population genetic analyses examine historical population demography over evolutionary time, and incorporate genetic processes such as selection, recombination, drift and mutation (Slatkin, 1994). Ideally, managers should consider both when designing their fisheries management plans, but for most deep-water fishes with planktonic larvae, genetic studies are often the only feasible option for elucidating population processes.

Several classes of analytical methods now exist to assess genetic variation of populations, each of which emphasizes genetic structuring at different evolutionary timescales. For example, phylogeographic analyses based on DNA sequence data provide insights into historical aspects of geographical structure by overlaying phylogenetic patterns on to spatial distribu-

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tions of populations (Avise, 2000). These methods have greatly facilitated the application of evolutionary theory to issues of conservation, as knowledge of the genealogical relationships among populations can add a historical component to the design of conservation strategies that have long-term evolutionary goals (Waples, 1991; Moritz, 1994; Moritz, Lavery & Slade, 1995).

However, because fisheries management tends to be concerned with contemporary rather than historical aspects of population structure, analyses of allele frequency rather than allele phylogeny may be more appropriate for assessing relatively recent divergences between populations. This is because detectable changes in allele frequencies among newly formed subpopulations are likely to arise more quickly than detectable phylogeographic differences, the latter being dependent on the rate of lineage sorting, a function of effective population size (Barton & Wilson, 1996; Beerli & Felsenstein, 1999). Larger ancestral populations will take longer to reach coalescence, and in most cases, gene divergence will predate the time of population divergence (Beerli & Felsenstein, 1999).

Hence, frequency measures such as Wright's *F*-statistics and its analogues (Weir & Cockerham, 1984) should show higher sensitivity to recent changes in population structure and gene flow, and may therefore be more useful for examining the proper scale at which to delimit management boundaries. In addition, new assignment methodology has the potential to detect very small amounts of migration without assumptions of genetic equilibrium (Davies, Villablanca & Roderick,1999). Maximum Likelihood analyses allow for estimation of population growth rates (Kuhner, Yamato & Felsenstein, 1998), and Bayesian inference for simultaneous estimates of population size, ongoing migration, and recent divergence (Nielsen & Wakeley, 2001).

Because of the lack of obvious geographical barriers to migration in ocean environments, larval dispersal in the marine realm has long been assumed to be widespread, resulting in low genetic differentiation among widely distributed populations (Kay & Palumbi, 1987; Palumbi *et al*., 1997). However, recent evidence from both population studies of marine organisms and simulations of larval transport in oceanic currents highlight the potential for significant local retention within limited geographical ranges as an important component of the population dynamics of marine recruitment in island systems (Swearer *et al*., 1999; James *et al*., 2002). Both long distance dispersal and local retention of larvae can be heavily influenced by a number of key factors, including timing and duration of spawning, planktonic duration, adult life expectancy and larval behaviour (Largier, 2003).

As little information is available on characteristics of *Epinephelus quernus* larvae, recruitment patterns for this commercially important species are completely unknown. Endemic to the Hawaiian archipelago and Johnston atoll (Heemstra & Randall, 1993) and a member of the subfamily Epinephelinae (the serranid groupers), *E. quernus* adults are ambush predators which prefer to swim at depths of several hundred meters, and consume prey including smaller fishes, crustaceans and squid (Heemstra & Randall, 1993). The species is highly habitat specific, most often associated with rocky outcroppings and underwater promontories. Like most groupers, *E. quernus* is a protogynous hermaphrodite, starting life as a female and switching sex upon attainment of a relatively large size; this is thought to be stimulated by environmental conditions and surrounding sex ratios (Shapiro, 1984; Heemstra & Randall, 1993). In general, large males hold territories, and during the mating season sizeable spawning aggregations form that are both stable in space and time (i.e. over spawning seasons), making them easy targets for fishermen. Highly skewed, female-biased sex ratios are also common among groupers, with females outnumbering males by upwards of 50 : 1 in some species (Collins *et al*., 1998; Garcia-Cagide & Garcia, 1996; Zabala *et al*., 1997). Larval development is unknown for *E. quernus*, but is estimated at *c*. 40 days for a related epinepheline, the Nassau grouper (Colin, Laroche & Brothers, 1997). Eggs are buoyant and probably rise to the surface to develop, and it has been widely assumed that larvae disperse passively with ocean surface currents.

General characteristics such as slow growth rates, late age at maturity, and long life spans (Parrish, 1987; Heemstra & Randall, 1993; Williams & Lowe, 1997), in addition to having restricted depth ranges, unequal sex ratios, and a tendency to form dense, predictable spawning aggregations (Aguilar-Perera & Aguilar-Davila, 1996; Coleman, Koenig & Collins, 1996; Beets & Friedlander, 1999) have made groupers amongst the most threatened of commercially important fishes worldwide. In fact, nearly 44% of all known epinepheline species have been listed as critically endangered or threatened (Morris, Roberts & Hawkins, 2000).

In this paper we examine population differentiation of *E. quernus* across the Hawaiian archipelago using mitochondrial control region sequences. The fast rate of mutation of mtDNA has proved to be extremely useful in population level studies, where high nucleotide and haplotype diversity can provide the necessary variation to explore parameters such as subdivision and gene flow. Using traditional and simulated annealing frequency based analyses, we explore genetic subdivision among populations of *E. quernus*.

We also employ Bayesian methodology in an attempt to assess rates of migration between adjacent pairs of islands, and to estimate population sizes across the archipelago. Finally, we examine historical changes in growth rates using Markov Chain Monte Carlo (MCMC) sampling in exploited and nonexploited regions. The results of our analyses are discussed in the context of large-scale oceanic currents and their possible influence on the population structure and evolutionary history of *E. quernus*.

METHODS

Data collection

In total, 302 individual blood and/or tissue samples of the Hawaiian grouper *Epinephelus quernus* (Serranidae; Teleostei) were collected with hook and line, primarily from the southern shores of the islands, at depths of *c*.140–381 m at ten sites across the archipelago between 1999–2001 (Fig. 1, Table 1). Many samples, particularly those from the Kauai through Pearl and Hermes, were obtained with the help of commercial fishermen operating in the Northwestern Hawaiian Islands (NWHI). Samples from the Main Hawaiian Islands (MHI) were primarily caught by researchers from the Hawaii Institute of Marine Biology who were conducting habitat surveys, and additional fish were purchased at the Hilo fish auctions to supplement sample size from the island of Hawaii.

DNA was extracted using QIA DNeasy extraction kits and the mtDNA control region amplified with PCR using primers A and E (Lee *et al*., 1995), which produced a 442 base pair fragment spanning part of the t-RNA proline and the 5′ end of the control region. Amplification was carried out using the following PCR profile: 94°C held for 1 min, followed by 94°C for 30 s, 53°C for 45 s, 72°C for 30 s for 35 cycles. PCR products were visualized on a 1.5% TBE agarose gel, and positive amplifications then purified using a QIAquick PCR purification kit. Each PCR product was sequenced in both forward and reverse directions and visualized using an automatic sequencer (ABI 377 or ABI 310).

After removing primer sequences, a 398 base pair region was used for all subsequent analyses. Alignment was done manually with the aid of Sequencher ver. 3.1.1 (Genecodes Corporation) and was straightforward, as only two positions showed unambiguous, single base pair indels.

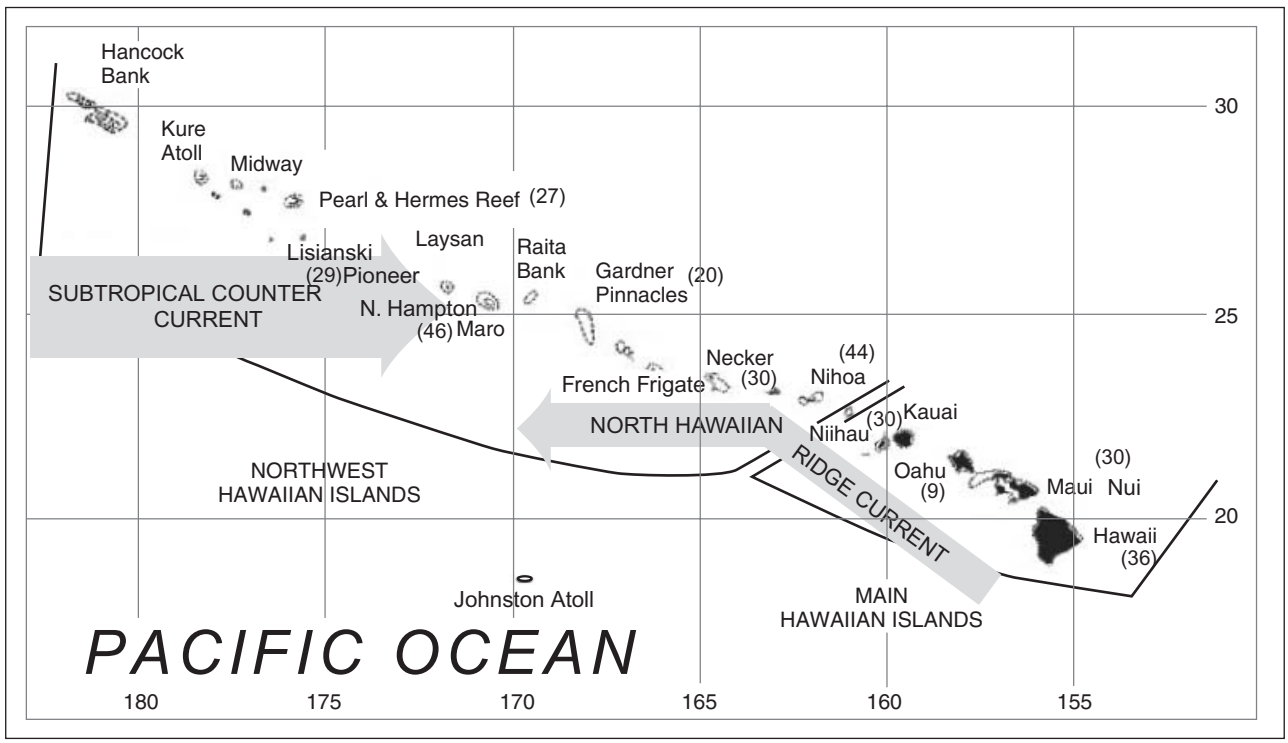

Figure 1. Map of the Hawaiian archipelago. Sample sizes from ten sites are shown in parentheses. The North Hawaiian Ridge Current in the south-east moves primarily in the north-west direction until 23 °N (Qiu *et al*., 1997) and the Subtropical Counter Current intersects the archipelago at mid-latitudes between 24 °N and 27 °N (Kobashi & Kawamura, 2002). Map by Frank Parrish, NMFS, Honolulu Lab.

Table 1. Control region diversity statistics and neutrality tests by island site and over the total archipelago. Significance of Tajima's D determined by 1000 randomizations. $*P < 0.05$, $**P < 0.01$. Tests are non-significant for individual island statistics after sequential Bonferroni correction ($\alpha = 0.05$, k = 10). Test for total sample is not corrected, and is significant at $**P < 0.01$

Island/	Depth range (m)	*N*	No. haplotypes	No. unique haplotypes	Gene diversity	Nucleotide diversity	Tajima's D
Hawaii	255–318	36	24	7	0.9619	6.084	–1.808*
Maui Nui	204–381	30	17	2	0.9195	4.749	–1.456
Oahu	240–285	9	8	0	0.9722	3.444	–1.159
Kauai/Niihau	207–390	30	19	5	0.9103	3.161	–1.691*
Nihoa	150–345	44	22	2	0.9397	5.701	–1.253
Necker	270	30	19	5	0.9632	5.515	–1.480
Gardner	240–321	30	19	8	0.9947	8.489	–1.448
Maro/N. Hampton	140–375	46	29	9	0.9507	7.156	–1.660*
Pioneer/Lisianski	210–375	29	25	15	0.9852	6.956	–1.585*
Pearl & Hermes	255–375	27	16	3	0.8661	3.407	–2.019**
TOTAL		301	92	–	1.000	5.685	–1.912**

Genetic diversity, neutrality, and parsimony reconstruction

Gene diversity (Nei, 1987) and nucleotide diversity (Tajima, 1983) were measured at each site and overall using the population genetics software package Arlequin ver. 2.000 (Schneider, Roessli & Excoffier, 2000). We also calculated both the total number of haplotypes represented, as well as the number of haplotypes that were unique to a given island. Neutrality was examined by applying Tajima's D statistic (Tajima, 1989b) for each island and overall. Significance was tested by 1000 randomizations using Arlequin and corrected using the sequential Bonferroni procedure (Rice, 1989). An estimate of rate heterogeneity (Γ distribution) was determined with likelihood ratio tests as implemented in Modeltest ver. 3.06 (Posada & Crandall, 1998).

We constructed a haplotype network using methodology described in Templeton, Crandall & Sing (1992) as implemented in TCS ver. 1.13 (Clement, Posada & Crandall, 2000), using a 95% parsimony connection limit of ten steps and considering gaps as a fifth state. Ambiguous connections (i.e. loops) were resolved according to the criterion recommended by Crandall and colleagues (Crandall & Templeton, 1993; Crandall, Templeton & Sing, 1994), namely that ambiguous haplotypes are more likely to be connected to ones that are more common and/or widespread.

Population structure, migration, divergence and population size

Using Arlequin we calculated island-by-island F_{ST} (Weir & Cockerham, 1984), assessing significance from 1000 permutations. In addition, exact tests of global differentiation and haplotype frequency differentiation among all pairs of islands (Raymond & Rousset, 1995) were performed using 20 000 and 10 000 steps of a Markov chain, respectively. After considering the results from AMOVA, we were primarily interested in the divergence between the mid-archipelago and the rest of the islands; therefore, significant outcomes among pairwise tests were corrected according to the sequential Bonferroni procedure (Rice, 1989) for comparisons between Necker and Gardner and the north-west and south-east regions combined ($\alpha = 0.05/17$).

To examine larger regional structures we adopted a simulated annealing approach to using SAMOVA ver. 1.0 (Dupanloup, Schneider & Excoffier, 2002). Unlike classical tests of population genetic structure (e.g. AMOVA), this method does not require an a priori definition of populations, but instead searches for emergent group structures based only on the genetic data. We defined the number of populations (K) and ran 100 simulated annealing processes for each possible K, ranging from K = 2 through K = 9.

Estimates of theta ($\Theta = 2N_e\mu$), ongoing migration ($M = 2N_em$), and recent divergence ($T = t/2N_e$) were obtained using a Bayesian Likelihood approach incorporating an MCMC algorithm and assuming the HKY finite sites model, as implemented in the program MDIV (Nielsen & Wakeley, 2001). We conducted a minimum of three independent runs using different random seeds to ensure consistency of results. For each run, parameters were set as follows: Mmax = 50, Tmax = 1, length of Markov chain = 2 000 000 steps, burn-in time = 500 000.

Maximum Likelihood (ML) estimates of the population growth rate (Kuhner *et al*., 1998) were estimated

for (1) the entire archipelago (2) MHI, and (3) NWHI, using the program Fluctuate ver. 1.3. These analyses use a Metropolis Hastings MCMC method to search for ML estimates of population parameters, and assume no migration, selection or recombination. In this procedure, both Θ ($2N_e\mu$) and g (growth rate) are calculated, where $N_e(t) = \Theta^{-gt}$. Present effective population sizes were also inferred under various control region mutation rates calibrated from other teleosts. Transitions and transversions were weighted equally, and calculations were performed using empirical base frequencies. Search strategies included the following: (1) 30 short and long chains, (2) 2000 steps along short chains, (3) 4000 steps along long chains, and (4) 20 short and long sampling increments. A minimum of four independent runs, each with different random seeds, were performed to check for repeatability of the estimated parameters.

RESULTS

Genetic diversity, neutrality, and parsimony reconstruction

DNA sequences (GenBank accession numbers AF540076–AF540376) were obtained for 301 out of 302 individual samples collected; only one individual from Pioneer/Lisianski (NWHI) did not amplify. Diversity statistics are summarized in Table 1. Ninety-two distinct haplotypes with 76 variable sites were represented among these 301 individuals, 56 of which were unique to single islands (variable sites of the sequence alignments are given in the Appendix). Genetic distance among haplotypes ranged from zero to approximately 5%, gene diversity among islands between 0.8661 and 0.9947 and nucleotide diversity between 3.161 and 8.489. The gamma distribution shape parameter was estimated at $\alpha = 0.3566$, suggesting that different regions of this locus evolve at varying rates.

A noteworthy result is that Gardner Island, which lies approximately at the midpoint of the Hawaiian archipelago, shows the highest gene diversity, nucleotide diversity, and the second highest proportion of unique alleles (Table 1, Fig. 2), despite being the source of a relatively smaller sample ($N = 20$). Overall, a trend of higher levels of diversity exists in NWHI, particularly from Nihoa through Pioneer/Lisianski, relative to MHI (Hawaii through Kauai/Niihau) (Fig. 2). Generally, this pattern does not appear to be confounded by sample size, except perhaps in the case of Oahu with the smallest sample ($N = 9$).

Tests for neutrality using Tajima's D, which measures the disparity between the number of segregating sites and pairwise genetic distance, show negative values for all islands and overall, although significance does not hold after Bonferroni corrections for the island tests. Negative values result from a higher number of segregating sites compared to pairwise dis-

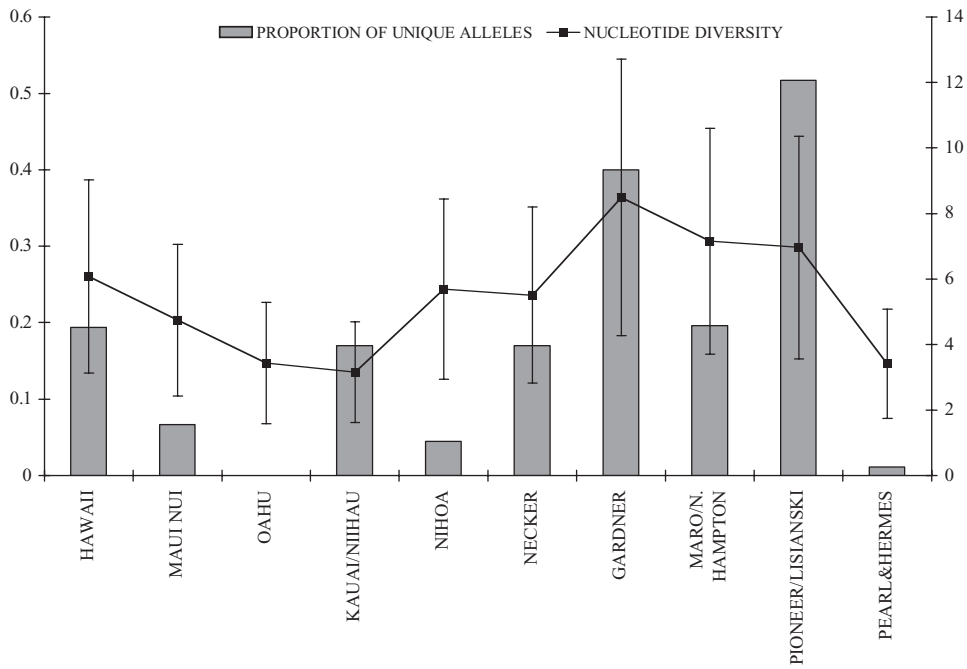

Figure 2. Proportion of unique haplotypes and nucleotide diversity across ten islands in the Hawaiian archipelago. MHI = Hawaii through Kauai/Niihau, NWHI = Nihoa through Pearl and Hermes.

tance, and may be indicative not only of selection, but of changes in population size. In particular, they are consistent with populations that are increasing (Tajima, 1989a).

A network diagram of the 92 distinct haplotypes is shown in Figure 3. Interestingly, the most common haplotype, occurring in 57 of the 301 individuals, was represented in all islands except Gardner, a site that also harbours a high genetic diversity (Table 1). In general, most haplotypes are very closely related, being connected by very few mutational steps, although there are distinct groups separated by several mutations which may be related to independent colonization events.

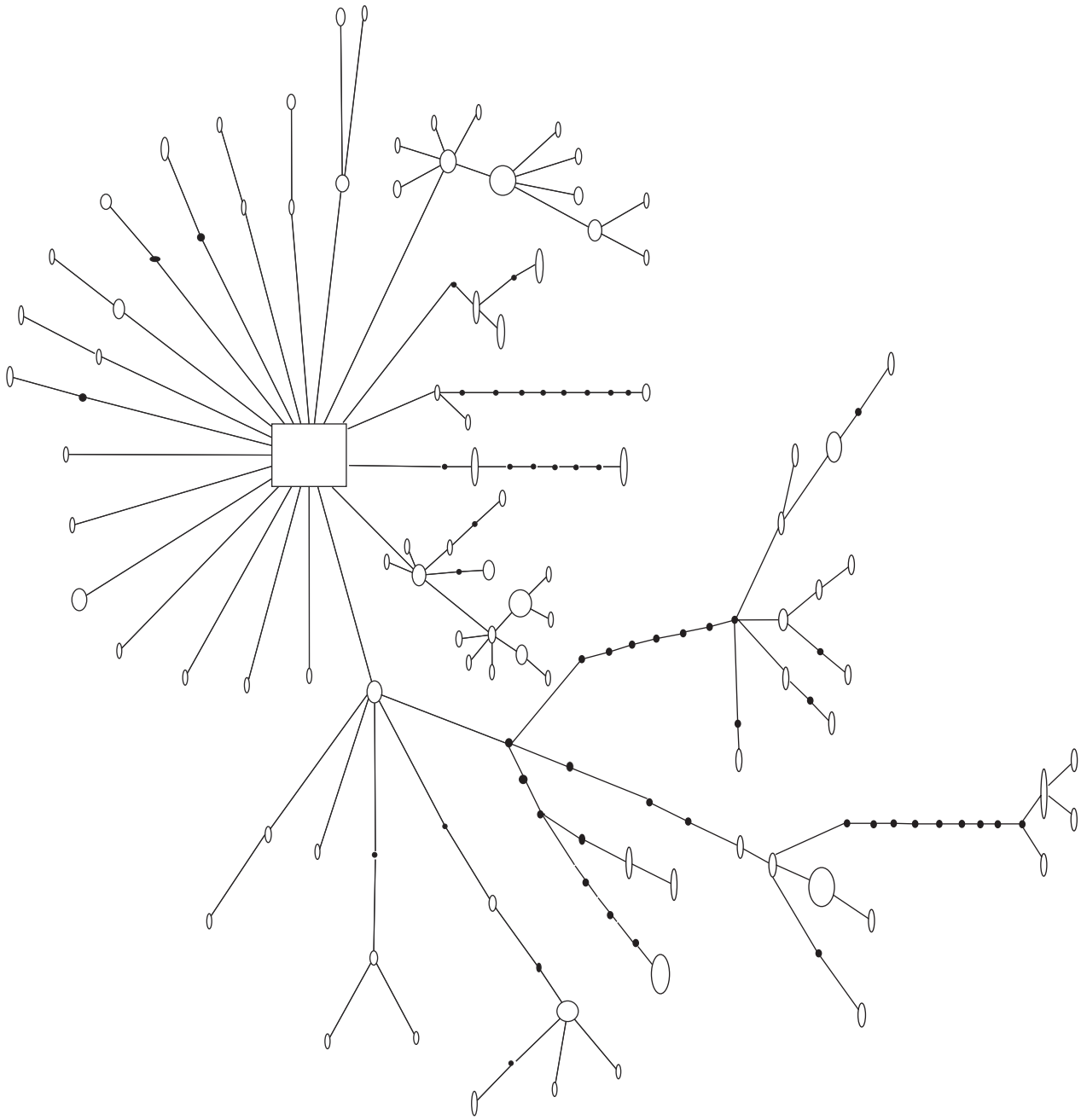

Figure 3. Network analysis of the 92 mtDNA haplotypes using TCS (Clement *et al*., 2000). Sizes of ovals are proportional to frequency of haplotypes and central rectangle represents the single most common haplotype, occurring in 57 of the 301 (18.9%) individuals. Dark circles indicate number of mutations separating haplotypes.

Population structure and SAMOVA

As global tests of among sample differentiation was highly significant ($P < 0.000001$), we further explored this variation by island pairwise comparisons. Overall, pairwise F_{ST} values were low, ranging from less than zero to a high of 0.0649 ($P < 0.01$) between Gardner and Pearl and Hermes (Table 2). Interestingly, most comparisons among the south-east islands (Hawaii through Nihoa) show very low or negative F_{ST}. That is, allele frequencies among these islands are very similar. Alternatively, many of the comparisons involving Necker and Gardner were significantly different, as shown both by permutation and/or exact tests. This trend also holds after sequential Bonferroni corrections are applied for tests between Necker and Gardner and all other islands, although with fewer statistically significant outcomes. Also noteworthy is the observation that most of NWHI sample sites (Maro/N. Hampton through Pearl and Hermes) did not show significant differences when compared to most islands furthest away in the south-east (Hawaii through Nihoa). In other words, the two extreme ends of the archipelago have allele frequencies which are more similar to each other than to those of the islands more closely situated in the mid-archipelago.

Results for analyses of regional structure using SAMOVA are shown in Table 3. With larger K values, the estimates of F_{CT} increase while those of F_{SC} decrease. This is a not unexpected outcome, given the relationship of $(1 - F_{ST}) = (1 - F_{SC})(1 - F_{CT})$, where the number of among population comparisons within groups lessens as K gets larger. A lower variance among comparisons within groups is a direct consequence of fewer populations within them (Dupanloup *et al*., 2002). However, all values of F_{CT} derived from the varying levels of K are not substantially different from each other, ranging from 0.016 (K = 2) to 0.055 (K = 9). While these estimates are all statistically significant at least at the 0.01 level, the highest levels of significance occur at K = 3 through K = 7 ($P < 0.0001$), within which the highest value of F_{CT} is 0.028 at K = 7. Simulations have shown that the highest mean F_{CT} value obtained with SAMOVA is associated with the correct (actual) number of populations (Dupanloup *et al*., 2002), which may suggest that up to seven distinct groups exist among the ten localities sampled of *E. quernus*. Necker also is the first single locality to fall out of the larger regional grouping at K = 3, consistent with pairwise F_{ST} results showing that the mid-archipelago is separated by a distinct reduction in gene flow.

Bayesian MCMC results for each pair of adjacent islands are shown in Table 4. In general, the data were insufficient to estimate migration rates or divergence times, with one exception involving the comparison between Nihoa and Necker, where M ($2N_em$) was estimated confidently at 10.84 (Fig. 4). Typical results for the rest of the comparisons show either a plateau or a continual rise in the estimate of M (see examples in Fig. 5). While a maximum migration likelihood was not achieved in these cases, in general small values of M (i.e. < 20) were excluded by extremely low likelihood scores (Table 4). Hence, for most comparisons, migration appears to be sufficiently high, that is, M between

Table 2. Pairwise conventional F_{ST} values between islands. Significance (*) was determined by 1000 permutations. Exact tests (+) of haplotype frequency differentiation based on 10 000 steps of a Markov chain were also performed (*/+ represents $P = 0.05$, **/++ $P = 0.01$). Comparisons remaining significant after sequential Bonferroni correction for multiple tests between Necker and Gardner and the rest of the islands are underlined (table wide $\alpha = 0.05$, k = 17). Global test of differentiation among samples was highly significant, with $P < 0.000001$. Islands: 1, Hawaii; 2, Maui Nui; 3, Oahu; 4, Kauai/Niihau; 5, Nihoa; 6, Necker; 7, Gardner; 8, Maro/N. Hampton; 9, Pioneer/Lisianski; 10, Pearl & Hermes

	1	2	3	4	5	6	7	8	9	10
1	–									
2	0.0077	–								
3	–0.0202	–0.0364	–							
4	0.0086	–0.0079	–0.0267	–						
5	0.0051	–0.0015	–0.0234	–0.0065	–					
6	0.0014	0.0295*++	–0.0007	0.0343**+	0.0201*++	–				
7	0.0053+	0.0367*++	0.0131	0.0431**++	0.0252*++	–0.0012	–			
8	–0.0018	0.0007	–0.0218	–0.0014	–0.0027	0.0136*+	0.0139+	–		
9	–0.0033	0.0042	–0.0223	0.0173	0.0046	0.0017	0.0041	0.0036	–	
10	0.0166	–0.0018	–0.0064	–0.0148	0.0064	0.0450**+	0.0649**++	0.0134	0.0295*	–

Table 3. Regional structure computed using SAMOVA with increasing K. Significance based on 100 simulations, where $^{*}P < 0.01$, $^{**}P < 0.00001$. Necker is the first single island to fall out of the regional grouping at K = 3

K	Group composition	F_{ST}	F_{SC}	F_{CT}
2	1. Maui Nui, Oahu, Kauai/Niihau, Pearl & Hermes 2. Hawaii, Nihoa, Necker, Gardner, Maro/N. Hampton, Pioneer/Lisianski	0.017	0.0006	0.016*
3	1. Maui Nui, Oahu, Kauai/Niihau, Pearl & Hermes 2. Hawaii, Nihoa, Gardner, Maro/N. Hampton, Pioneer/Lisianski 3. Necker	0.016	–0.004	0.019**
4	1. Oahu, Kauai/Niihau, Pearl & Hermes 2. Hawaii, Nihoa, Gardner, Maro/N. Hampton, Pioneer/Lisianski 3. Necker 4. Maui Nui	0.016	–0.007*	0.023**
5	1. Oahu, Kauai/Niihau, Pearl & Hermes 2. Hawaii, Nihoa, Gardner, Maro/N. Hampton 3. Necker 4. Maui Nui 5. Pioneer/Lisianski	0.014	–0.01	0.024**
6	1. Oahu, Kauai/Niihau, Pearl & Hermes 2. Nihoa, Gardner, Maro/N. Hampton 3. Necker 4. Maui Nui 5. Pioneer/Lisianski 6. Hawaii	0.012	–0.014*	0.025**
7	1. Oahu, Kauai/Niihau, Pearl & Hermes 2. Nihoa, Maro/N. Hampton 3. Necker 4. Maui Nui 5. Pioneer/Lisianski 6. Hawaii 7. Gardner	0.011	–0.018	0.028**
8	1. Oahu, Kauai/Niihau, Pearl & Hermes 2. Nihoa 3. Necker 4. Maui Nui 5. Pioneer/Lisianski 6. Hawaii 7. Gardner 8. Maro/N. Hampton	0.01	–0.025	0.034*
9	1. Oahu, Kauai/Niihau 2. Nihoa 3. Necker 4. Maui Nui 5. Pioneer/Lisianski 6. Hawaii 7. Gardner 8. Maro/N. Hampton 9. Pearl & Hermes	0.01	–0.049	0.055*

islands is in many cases greater than 50, the limit at which the analyses were performed.

In all tests, divergence estimates were low, but again these estimates were obtained with very low confidence. Theta ranged from 6.34 to 15.81, suggesting that N_e at each island is rather large. Because mutation rates between island pairs are expected to be the same, Θ estimates indicate the relative differences in population sizes. In general, larger population sizes characterize the islands north of Necker. However, because this method assumes constant population sizes, violations of the

Table 4. Estimates of migration ($M = 2N_em$), divergence ($T = t/2N_e$), and theta ($\Theta = 2N_e\mu$) between adjacent islands using MDIV (Nielsen & Wakeley, 2001). Posterior probabilities are shown in parentheses. Parameters were as follows: Mmax = 50, Tmax = 1, steps in Markov chain = 2 000 000, burn-in time = 500 000. Analyses were unable to estimate maximum probabilities (NE) for migration for most comparisons except between Nihoa and Necker (in bold). All estimates of Θ showed clear maximum likelihoods. A minimum of three independent runs with different random seeds was performed to confirm consistency of results

Between populations	Θ	Migration	Divergence
Hawaii–Maui Nui	10.84 (0.0015)	19.6 (NE)	0.006 (NE)
Maui Nui–Oahu	6.56 (0.0093)	>50 (NE)	0.014 (NE)
Oahu–Kauai/Niihau	6.34 (0.0084)	>50 (NE)	0.014 (NE)
Kauai/Niihau–Nihoa	8.19 (0.0129)	>50 (NE)	0.020 (NE)
Nihoa–Necker	7.59 (0.0014)	**10.84 (0.0071)**	0.320 (NE)
Necker–Gardner	12.26 (0.0100)	10.4 (NE)	0.320 (NE)
Gardner–Maro/N. Hampton	14.57 (0.0113)	>50 (NE)	0.380 (NE)
Maro/N. Hampton–Pioneer/Lisianski	15.81 (0.0109)	>50 (NE)	0.010 (NE)
Pioneer/Lisianski–Pearl & Hermes	13.38 (0.0095)	17.7 (NE)	0.022 (NE)

Table 5. Estimates of Θ, growth rate and present population size using analysis package Fluctuate (Kuhner *et al*., 1998). A minimum of four independent runs was performed using different random seeds. Analyses performed assuming Ts/Tv = 1, 30 short and long chains run. Short chains were run with 2000 steps, long chains with 4000 steps, and a sampling increment of 20. Present population size is estimated assuming a mutation rate of 1.1×10^{-7} per generation (Brown *et al*., 1993) and (2) 3.6×10^{-8} (Donaldson & Wilson, 1999)

Region	Θ	Growth rate	Likelihood	Estimated population size
Main Hawaiian Islands	0.078 ± 0.0058	108.2 ± 19.85	0.109	(1) 2.13×10^5 (2) 6.98×10^6
North-west Hawaiian Islands	0.148 ± 0.0080	186.1 ± 15.39	0.883	(1) 4.08×10^5 (2) 13.4×10^6
Hawaiian archipelago	0.202 ± 0.0084	319.7 ± 21.38	1.777	(1) 7.44×10^5 (2) 18.2×10^6

assumptions may upwardly bias estimates of both migration and Θ if populations have been increasing in size historically.

ML estimates of population growth rates (g) and population size are presented in Table 5. In all three subanalyses, positive values of g indicate expanding population sizes for *E. quernus*. Estimates of control region mutation rates have been reported in the literature for a few teleosts. Brown, Beckenbach & Smith (1993) found an approximate rate of $1.1–1.3 \times 10^{-7}$ nucleotides/site/year in white sturgeon, while Donaldson & Wilson (1999) found an approximate rate of 3.6×10^{-8} nucleotides/site/year in snook. We present in Table 5 estimates of present N_e based on both substitution rates. Estimates of Θ and g at all levels also show low standard deviations, suggesting that the compound parameter estimates are reliable. However, because implementation of this method assumes population panmixia, violations such as those due to the existence of population structure could downwardly bias estimates of Θ and g.

DISCUSSION

Genetic variation in *Epinephelus quernus*

The 5′ end of the control region has been demonstrated to have the highest observed rates of base substitutions and insertion/deletion events in vertebrates (Saccone, Pesole & Sbisa, 1987), and substitution patterns for *E. quernus* appear to be no exception. The high rate of mutation makes this region of the mitochondrial genome ideal for intraspecific studies of population structure. Gene diversity observed between 0.87 and 0.99 among islands is at the high end of the range observed in other teleosts, and is comparable to that seen in other deep-water fish such as the rosethorn rockfish, *Sebastes helvomaculatus* (Rocha-Olivares & Vetter, 1999) and the pelagic Atlantic mackerel, *Scomber scombrus* (Nesbø *et al*., 2000). Two single point indels among the 398 base pairs are also consistent with several other teleost control region observations (Lee *et al*., 1995). Network analyses show that many of the observed haplotypes are very

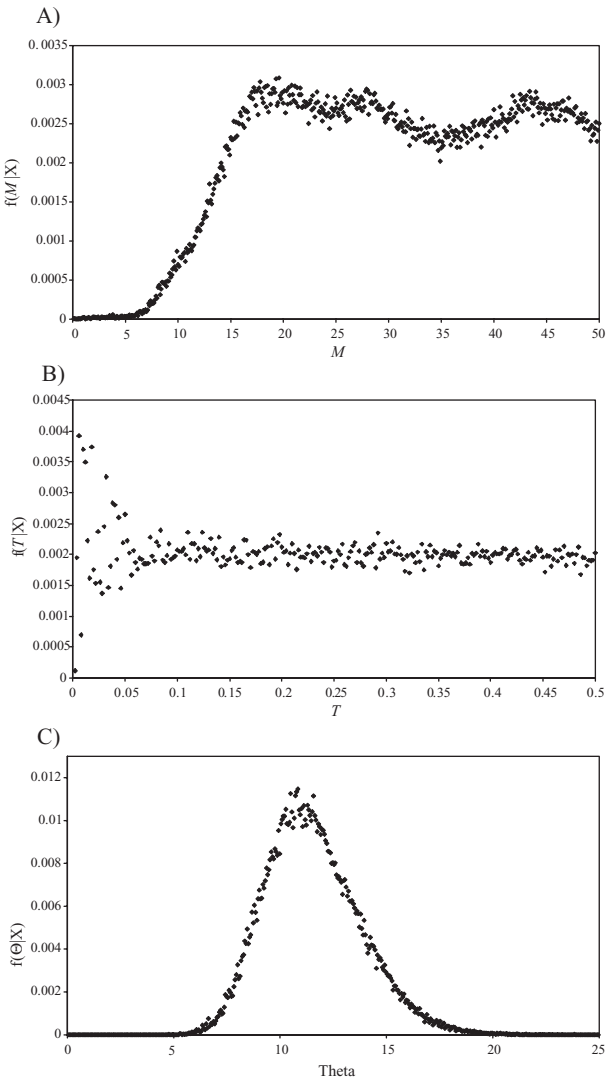

Figure 4. Typical outcomes of MDIV analyses between adjacent pairs of islands. (A) M ($2N_em$) and (B) T ($t/2N_e$) posterior probabilities did not distinguish with confidence between ongoing migration and recent divergence. (C) In all cases analyses showed clear maximum probability for estimates of Θ ($2N_e\mu$).

closely related, being separated on average by only 1–2 base pairs. Such 'star topologies' are often interpreted as a signature of recent population expansion (Slatkin & Hudson, 1991). In addition, the existence of two groups separated by several substitutions may be a result of historical independent origins from an ancestral source population.

Population structure and dispersal

Little is known of the larval ecology of *E. quernus*, making predictions of dispersal potential difficult at best. In other deep-water commercially valuable fish occurring in Hawaiian waters such as the lutjanid snappers *Etelis carbunculus* and *Etelis coruscans*, studies suggest relatively long larval development that may last upwards of 3 months (Leis & Lee, 1994). Population genetic analyses of these two species based on nuclear microsatellites and preliminary mtDNA control region data show little to no genetic difference across the Hawaiian archipelago (V. Moriwake, pers. comm.). Similarly, microsatellite analyses of the vermillion snapper *Rhomboplites aurorubens* from populations off the eastern US and Gulf of Mexico showed only very weak genetic differentiation (Bagley, Lindquist & Geller, 1999). However, groupers differ from snappers in that they tend to have a suite of identifiable life history traits such as highly specific habitat preferences, male territorial behaviour, and stable spawning aggregations (Heemstra & Randall, 1993; Morris *et al*., 2000), characteristics which are favourable to population structuring.

The genetics of marine populations, particularly for species with pelagic larval development, has often been characterized by low genetic variation among populations, a pattern driven by high dispersal capabilities and large scale oceanic mixing (Palumbi, 1994; Palumbi *et al*., 1997; Reichow & Smith, 2001). A characteristic example can be found in the three-spot reef fish *Dascyllus trimaculatus* from the islands of French Polynesia, a species that has a fairly limited pelagic larval phase (22–26 days), in which similar genetic composition is found among separate island archipelagos of the South Pacific, and marked differentiation only when compared with populations further away in the Indo-West Pacific (Bernardi, Holbrook & Schmidt, 2001).

Although the patterns found in *D. trimaculatus* probably typify many marine species, the growing attention being paid to the conservation of overexploited marine stocks and the increasing popularity of the marine protected area concept (Palumbi, 2003) has resulted in a renewed emphasis on better understanding of larval dispersal and recruitment. Recent theoretical and empirical evidence supporting the occurrence of both the significant local retention of larvae, in combination with long distance dispersal, is now being recognized as an important influencing factor in the population dynamics of marine organisms inhabiting islands. For example, simulations of regional scale hydrodynamics in the northern Great Barrier Reef complexes predict significant self-recruitment of larvae for a number of marine species (James *et al*., 2002). In addition, Swearer *et al*. (1999) used otolith trace-elements to show that the bluehead wrasse larvae from the Virgin Islands exhibited both substantial local recruitment within reefs, as well as longer distance

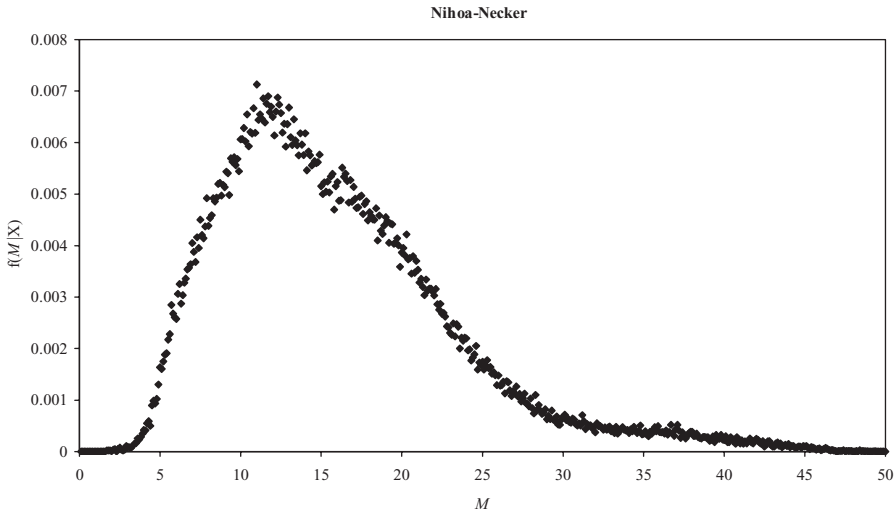

Figure 5. MDIV posterior probability distribution of *M* between Nihoa and Necker, showing a clear maximum at approximately 10.84, suggesting that ongoing migration between these two islands is limited.

dispersal between reefs on different sides of the same island.

The relative importance of larval dispersal in island complexes is also unique because, in most cases, suitable habitats are separated by considerable expanses of uninhabitable deep water, decreasing the likelihood of long distance adult migration. Genetic data can help elucidate larval dynamics in such island systems. For example, in several species of coral reef fishes, genetic patterns among South Pacific Island archipelagos support a classic island model of migration, where infrequent but chaotic movement between islands results in low but significant levels of population structuring (Fauvelot & Planes, 2002). Limited migration events such as this are sufficient to hinder genetic differentiation at small spatial scales, resulting in low observable genetic frequency differences, although any detectable geographical structure can often indicate extremely limited actual dispersal (Palumbi, 2003). Genetic differentiation observed in *E. quernus* was often low, an outcome consistent with both local retention and some amount of among-island migration, more likely by larvae rather than long-distance dispersal by adults.

Significant patterns of population structuring in the mid-archipelago based on island-by-island pairwise *F*-statistics were also observed. At the same time, the genetic composition for the majority of the south-east islands (Hawaii through Nihoa) and upper north-west islands (Maro/N. Hampton through Pearl and Hermes) was largely similar. These patterns may be explained in part by large-scale oceanic currents. A north-west flowing North Hawaiian Ridge Current (NHRC), spanning Hawaii through Nihoa, dominates the lower south-east portion of the archipelago (Qiu *et al*., 1997). At approximately 23 °N, near the latitude of Necker, the NHRC veers off due west, which is also the point at which the first significant adjacent island by island F_{ST} is observed (at Nihoa vs. Necker). Compared to the ocean in the vicinity of the MHI, studies of the physical oceanography above 23 °N are very scarce.

However, new data have revealed the existence of a west-easterly moving Subtropical Counter Current (STCC) whose southernmost border intersects the archipelago just north of Necker (Kobashi & Kawamura, 2002). The influence of the STCC may account for the significant difference in allele frequencies seen in the Necker and Gardner samples, and the NHRC for the similar allele frequencies in the lower south-east. Above this latitude, ocean current patterns are largely unknown around the islands. In the last year however, new satellite-tracked ocean current buoys have been deployed which generally show a predominantly south-west moving current (Brainard, pers. comm.; see crei.nmfs.hawaii.edu/oceanography/doc/crei_drifters_svp.html), a pattern that also supports the observed genetic structuring. However, these data are preliminary; with studies still ongoing drawing firm conclusions would be premature at this stage.

Also notable in the mid-archipelago is that Gardner shows the highest nucleotide and gene diversity. These results are consistent with recent surveys of marine species diversity in the same region. The atoll French Frigate Shoals, lying between Necker and Gardner, is host to one of the most diverse assemblages of marine sponges, algae (Maragos & Gulko, 2002), and corals (Grigg, Wells & Wallace, 1981). Several species of the table coral *Acropora*, a group absent in MHI and most of NWHI, are abundant and relatively speciose in the

mid-archipelago (Grigg *et al*., 1981; Grigg, 1981; Maragos & Gulko, 2002). They are also very common at Johnston atoll, which is the land- mass lying closest to the archipelago, situated several hundred km south-west. The shortest distance between Johnston and the Hawaiian chain is at French Frigate Shoals (Fig. 1), with Johnston also being the only other region where *E. quernus* occurs (Heemstra & Randall, 1993).

The relative proximity of these two atolls, along with the aforementioned similarity in marine species composition, has led to hypotheses that the STCC connects Johnston to the mid-archipelago at French Frigate (Grigg, 1981), which then serves as a stepping stone for colonization across the rest of the archipelago. While the high genetic diversity seen in the mid-archipelago for *E. quernus* is consistent with the stepping stone hypothesis, populations from Johnston and French Frigate should be examined to fully explore this possibility. The potential for Johnston as the ancestral source may account for the two divergent clades reflected in the network analysis (Fig. 3), where independent colonizations could contribute to the observed higher genetic diversity.

Among the north-west islands, the most interesting is Pearl and Hermes, which shows no significant frequency differences when compared to the most distant southern islands, but marginally significant differences compared to those islands closer to it in the archipelago. Inspection of the allele frequencies in these areas reveals that Pearl and Hermes and the first five southernmost islands are composed primarily of the most common haplotypes. The similar genetic composition at the two extreme ends of the archipelago might suggest that the more diverse, middle islands served as an ancestral source to the rest of the archipelago, with the most common alleles dispersing with the highest probability to colonize islands in the far south-east and north-west. Indeed, both MHI and NWHI show conformity to expectations of expanding populations (Table 5), and although Gardner lacks the most common haplotype, half of the alleles present in this sample are shared across islands throughout the archipelago (the other half are unique to Gardner, Fig. 3, Table 1).

Population dynamics

High relatedness among haplotypes likely contributed to the inability of MDIV to distinguish among ongoing migration and recent divergence in most cases, except perhaps for that involving Nihoa vs. Necker. Here, the posterior probability of $2N_em$ was estimated at 10.84 (or approximately 5.42 individuals per generation). Because *E. quernus* is a protogynous hermaphrodite with high female-skewed sex ratios, estimates of *M* may actually be biased, such that 5.42 migrants per generation may be closer to total migration, and not only female migration.

Interestingly, the confident estimate of limited migration between Nihoa and Necker using MDIV is the same adjacent comparison in which the first significant break in allele frequencies is observed based on F_{ST} and SAMOVA regional analyses, as well as the southernmost point at which the STCC intersects the archipelago. Among all other adjacent comparisons, the analyses were unable to distinguish between the relative effects of migration and divergence, although there was very little support for low levels of migration between most other sites. However, because this method also assumes constant population sizes through time, and because evidence of historical population growth was shown by the Fluctuate analyses, migration estimates may be upwardly biased. That is, historical population expansions may mask the effects of what could actually be lower migration. Nonetheless, relatively high migration and small divergences between islands probably characterize much of the archipelago due to pelagic dispersal and recent expansions. In addition, high estimates of Θ obtained in these analyses may be upwardly biased.

Genetic tests of fluctuations in N_e suggest historical population growth of *E. quernus*, with the effective population size estimated at between 0.744 and 18 million across the entire archipelago. Due to uncertainties in molecular clock calibrations and rate constancy, and because rates derived from other teleost control region studies may not be accurate for *E. quernus*, these inferences should be interpreted with caution. In addition, the derivation of N_e from the compound parameter Θ is entirely dependant on mutation rate, and as a result N_e is highly sensitive to any variation in these rates.

Given the two calibrations presented here, populations of *E. quernus* may be in the order of at least 3/4 of a million individuals. However, because of the evidence of low but significant population structure provided by *F*-statistic analysis, violations of the model may have biased our estimates of growth. This may be relevant for the analysis combining the NWHI, which includes the genetically differentiated islands of Necker and Gardner. A proposed regional structure such as this could underestimate growth rate (Kuhner *et al*., 1998). However, the results for total archipelago, MHI and NWHI all show increasing growth rates, suggesting this bias may be only marginal for our samples. In other words, if the existence of such a structure substantially affected the results, grouping of subpopulations should result in decreasing estimates of *g*, which was not observed in these analyses.

The estimates of Θ are also much smaller than those obtained from MDIV, where biases may be in the opposite direction due to population structuring. Hence,

actual Θ is more likely to fall between estimates derived by these two methods, and in both estimations is quite large. The results here are also consistent with the star-like phylogeny of alleles and the negative values obtained with Tajima's D. It is important to note, however, that tests for population growth tend to describe historical conditions, which may not necessarily reflect the current state of populations. For example, a recent study on coconut crabs fit genetic models of population expansion, yet these populations have dramatically decreased due to over-harvesting in recent decades (Lavery, Moritz & Fielder, 1996). For *E. quernus,* although declining stocks in MHI suggest a reduction in population, it has probably not substantially affected genetic characteristics.

It should be stressed that signatures of genetic differentiation among areas can take time to respond to changes in dispersal and subdivision (Slatkin, 1994). Hence, the lack of significant divergence in allele frequencies among many of the islands is not necessarily reflective of panmixia within those regions. Genetic tests of the type performed in this study, although more sensitive than phylogeographic approaches, still reflect structuring over evolutionary time. Hence, subdivision among many of these islands may exist at present but cannot be detected with genetic data (Taylor & Dizon, 1996; Taylor & Dizon, 1999). New assignment tests may have more power to detect subtle migration in marine species (Knutsen *et al*., 2003), although they are largely restricted to multilocus diploid data and not applicable to haploid mtDNA markers. Future studies of the population genetics of *E. quernus* should focus on application of these techniques.

For the mtDNA results presented here, although low differentiation between many islands was observed, it is important to keep in mind that historical population expansion will mask low migration, and may therefore bias gene flow estimates upward. In addition, relatively large estimates of effective population size could bias dispersal estimates downward. The compound parameter N_em cannot separate the relative effects of N_e and m. In other words, large effective population sizes, as evidenced in this study, will translate into lower values of dispersal (m) (Taylor *et al*., 2000).

CONCLUSIONS

The significant population structure based on *F*-statistics suggests that separate stocks of *E. quernus*, possibly influenced by oceanic currents, may exist throughout the archipelago. Because the mid-archipelago (Necker and Gardner) has genetic characteristics clearly distinct from those in MHI and upper NWHI, it is probably inadvisable to assume population panmixia across the entire island chain, and local depletion of *E. quernus* stocks should be monitored closely. The genetic patterns observed are, however, complex and delimiting boundaries based on the *F*-statistic results discussed here would be difficult. Given that the middle islands harbour the highest genetic diversity, with significant allele frequency differences distinguishing them from the rest of the chain, additional considerations may be appropriate to properly manage these areas, thereby preserving their uniqueness.

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APPENDIX

ALIGNMENT OF CONTROL REGION VARIABLE SITES FOR 301 INDIVIDUALS OF *EPINEPHELUS QUERNUS*

```
Hawaii
H5500   AAAATACTCCAGTTACCTAAACTTAATGTTAAGTGATCTATGTTGGTTTTTGGATTTTTTGATGTTATTTTGGAACTAAGGTTCAAAAGG
H5501   ...............T.............................C.....................C...................
H5502   ......T.....C...T..G......C....G.............C.......C.C-..........................
H5503   ...............T.............................CC.....................C...................
H5504   ...............T.........C...........ATA.....CC.........-CC...A.............A.........
H5505   ...............T.......G.....................................C.....................
H5506   ...............TC..........C.................CC.....................................
H5507   ...............T.............................C.............................T.............
H5508   ...............T.............................CC.....................C...................
H5509   ...............T.............................C.....................................
H5510   .............C..T....T.....A.................C...C.....................................
H5511   ...............T.......G.....................C.....................................
H5512   ..G............T.............................C.....................................
H5513   .......C.......TT.G.......C.............................-..A.C.....C...................
H5514   .............C..T....T.....A.................C...C.....................................
H5515   ...............T.............................CC.....................C...................
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H5516 ..............T.............................CC.....................C.....................
H5517 .......C........T.............................C.....................................
H5518 ..............T..................G..........CC.....................C.....................
H5519 ..............T.......G.....................................A.....................
H5520 .......C.......TT.G.......C.................................-..A.C.....C.....................
H5521 ..............T.............................CC.....................................
H5522 ........................G........A...........CC.G.......-.....................................
H5523 ..............T.............................C.....................C.....................
H5524 ..............T.......G.....................................C.....................
H5525 ........................G........A...........CC.G.......-.....................................
H5526 ..............T.........C.C.........ATA.....CC..........-C....A..............A..........
H5527 ..............T.............................C.............................G..............C.
H5528 ..............T.............................C.....................................
H5529 ..............T.............................C.....................................
H5530 ..............T.............................C.....................................
H5531 ..............T.............................A.C....A.....................................
H5532 ..............T.............................C.....................C.....................
H5533 ..............T.........C...........ATA.....CC..........-CC...A.............TTA..........
H5534 ..............T.............................CC.....................C.....................
H5535 ..............T.............................C.....................................
Maui Nui
MN5100 ..............T.............................C.....................................
MN5101 ..............T.............................C.....................................
MN5102 ..............T.............................C.....................................
MN5103 ..............T.............................CC.....................CC.....................
MN5104 ....C.T.......G.....................C......A....C.....A....-...T...CG.....................
MN5105 ..............T.......G.....................................C.....................
MN5106 ..............T.............................C.....................C.....................
MN5107 ..............T.............................C.....................C.....................
MN5108 ......T.....C...T..G......C....G.............C.......C.C-.....................................
MN5109 ..............T.......G.....................................C.....................
MN5110 ..............T.....................................................................
MN5111 ........................G........A...........CC.G.......-.....................................
MN5112 ..............T.............................C.....................................
MN5113 ..............T.......G.............T.......AC.............................G..........
MN5114 ..............T.............................AC.....................C.....................
MN5115 ..............T.......G.........A...........C..............G.....................
MN5116 ......T.....C...T..G......C....G.............C.......C.C-.....................................
MN5117 ..............T.............................C....A.....................................
MN5118 ..............T.............................C.....................................
MN5119 ..............T.............................C.....................................
MN5120 ..............T.......G.........A...........C..............G.....................
MN5900 ..............T.............................C.....................................
MN5901 ..............T.............................AC.....................C.....................
MN5902 ..............T.............................C.....................C.....................
MN5903 ..............T.............................C.....................................
MN5904 ..............T.......G.....................C.....................................
MN5905 ..............T.......G.....................................C.....................
MN5906 ..............T.......G.....................................C.....................
MN5907 ..............T.............................CC.....................................
MN5908 ..............T.......G.....................................C.....................
Oahu
O5800 ..............T.............................C.....................................
O5801 ..............T.............................C.....................C.....................
O5802 ..............T.............................C.............................T..............
O5803 ..............T.............................C.....................C.....................
O5804 ..............T.............................C.....................................
O5805 ..............T.......G.....................................C.....................
O5806 ........................G........A...........CC.G.......-.....................................
O5807 ..............T.....................C.........CC.....................................
O5808 ..............T.....................................................................
Kauai/Niihau
KN5600 ..............T.............................CC.....................................
KN5601 ..............TC.........A.C...A.............CC.........C.....................................
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KN5602  ..............T.............................CC.....................C.....................
KN5603  ..............TC.........A.C...............CC........C.............................
KN5604  ..............TC.........A.C...............CC........C.............................
KN5605  ..............T.......G.....................C.....................................
KN5606  ..............T.............................C.....................................
KN5607  ..............T.......G.....................C.....................................
KN5608  ..............T.............................C.....................................
KN5609  ..............T...........................C...C....A.............................
KN5610  ..............T.............................C.....................................
KN5611  ..............T.............................C.....................C.....................
KN5612  ..............T.............................C.....................................
KN5613  ..............T.............................C.....................................
KN5614  ...............................................................................
KN5615  ..............T.......G.........A...........C..............G.....................
KN5616  ..............T..........A...................C.....................................
KN5617  ..............T.............................C.....................................
KN5618  ..............T..............................................................
KN5619  ..............T.............................C.....................C.....................
KN5620  ..............T.............................C.....................................
KN5621  .....T.........T.............................C.....................C.....................
KN5622  ..............T.............................C.....................................
KN5623  ..G...........T.............................C.....................................
KN5624  ..............T....T..........A.............C...C..................................
KN5625  ..............T.............................C.....................................
KN5626  ........................G........A...........CC.G.......-.....................................
KN5627  ..............T.............................C.....................C.....................
KN5628  ..............T.......G.....................C.................G.....................
KN5629  .....T.........T.............................C.....................................
Nihoa
NiM01   ..............T.............................C.....................................
NiM02   ................................A...........CC.G.......-.....................................
NiM03   ..............T.............................C.....................C.....................
NiM04   ............C..T....T.....A.................C...C..................................
NiM05   ..............T.......G.....................................C.....................
NiM06   ..............T.........C...........ATA..CA.CC......-...C....A..............A.........
NiM07   ........................G........A...........CC.G.......-.....................................
NiM08   ..............T.......G.....................................C.....................
NiM09   ..............T.............................C.....................C.....................
NiM10   ....C.T.......G...................C......A....C.....A....-...T...C.....................
NiM11   ..G...........T.............................C.....................................
NiM12   ........................G........A...........CC.G.......-.....................................
NiM13   ..............T.......G.....................C.....................................
NiM14   ..............T.......G..............................................................
NiM15   ..............T.............................CC....................................
NiM16   ..............T...................G.........CC.....................C.....................
NiM17   ..............T.......G.....................................C.....................
NiM18   ..............TC.........A.C...............CC........C.............................
NiM19   ..............TC.........A.C...............CC........C.............................
NiM20   ..............T.............................C.....................................
NiM21   ..............T.............................CC....................................
NiM25   ........................G........A...........CC.G.......-.....................................
NiM61   ..............T.......G.....................C.....................................
NiM62   ..............T....T.........................C...C..................................
NiM63   ..............T.......G.....................................C.....................
NiM64   ..............T.............................C.....................................
NiM65   ..............T.............................C.....................................
NiM66   ..............TC.........A.C...............CC........C.............................
NiM67   ..............T.............................C.....................................
NiM68   ..............T.............................C.....................................
NiM69   ..............T.............................CC....................C.....................
NiM70   ..............T.........C...........ATA..C..CC.........-C....A..............A.........
NiM71   ..............T.......G.....................................C.....................
NiM72   ..............T.............................C.....................................
NiM73   ..............T.............................CC....................................
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NiM74           ................T.............................C.....................................
NiM75           ................T.............................C.....................C...............
NiM76           ................T.......G.....................C.....................................
NiM77           ................T.............................C.....................C...............
NiM78           ................T.............................C.....................................
NiM79           ................T.......G.....................................C.....................
NiMAUA          ....C.T.......G...................C......A....C.....A....-...T...C.....................
NiMAUB          ................T.........C............ATA..CA.CC.........-C....A..............A.........
NiMAUC          ................T.......G.....................................C.....................
Necker
NeM22           ................T.............................CC......................C...............
NeM23           ................T....T..........A.............C...C.....................................
NeM24           ........................G........A............CC.G.......-.....................................
NeM26           ................T.............................CC......................C...............
NeM27           ................TC...........C................CC.....................................
NeM28           ................T.........C............ATA..CA.CC.........-C....A..............A.........
NeM29           ................T.............................CC......................CC..............
NeM30           ................T.........C............ATA..CA.CC.........-C....A..............A.........
NeM31           ................T.........C............ATA..CA.CC.........-C....A..............A.........
NeM32           ................T.......G.....................................C.....................
NeM33           ................T.............................CC......................C...............
NeM34           ................T...................G..........CC......................C...............
NeM35           ................T...................G..........CC......................C...............
NeM36           ..G.............T.............................C.....................................
NeM37           ................T.......G.....................................C.....................
NeM38           ................T...G.........................................................................
NeM39           .........A......T.............................C.....................................
NeM40           ................T...................G..........CC......................C...............
NeM41           ..G.............T.............................C.....................................
NeM42           ................T...................C.........CC.....................................
NeM43           ................T.............................AC......................C...............
NeM44           ................T.....C............G..........CC......................C...............
NeM45           ................T...................G..........CC......................C...............
NeM46           ................T.............................C.....................................
NeM47           ................T.............................C.....................................
NeM48           ................T.............................C.....................................G..
NeM49           ................T.............................C......................C..................A
NeM50           ........................G........A............CC.G.......-.....................................
NeM51           ......T.........T.............................CC......................C...............
NeM52           .....................................................................................
Gardner
GH255           ................T.......G.........A...........C..............G.........................
GH256           ................T.............................CC......................C...............
GH257           ................TC.........A.C.................CC.........C.....................................
GH258           ................T.........C............ATA..CA.CC.........-C....A..............A.........
GH259           ................T.......G.....................................C.....................
GH260           ................T.......G.....................C.....................................
GH261           .......C.......TT.G.......C.............................-..A.C.....C.....................
GH301           ....C...........T.......G.....................................................................
GH302           ..........G............C..........A...........CC.G.......-.....................................
GH303           ....C.T.......G...................C......A....C.....A....-...T...C.....................
GH304           ................T.......G.............T.......AC.............................G...........
GH305           ................T.........C............ATA..CA.CC.........-C....A..............A.........
GH306           ................T.............................C.........................G.............
GH307           ......T.........T.............................CC......................C...............
GH308           ................T.............................CC......................C...............
GH312           .T......G.......TC.........A.C.................CC........C.........................NNNNNNNNN
GH313           ................T.......G.....................C.............................G...........
GH322           .TG.............T.............................C.....................................
GH323           .T..............T...................G..........CC......................C...............
GH324           .T..............T.............................CC......................C...............
Maro/N. Hampton
MNHH222         ................T.............................C.....................................
MNHH223         ................T.........C............ATA.....CC.........-CC...A..............A.........
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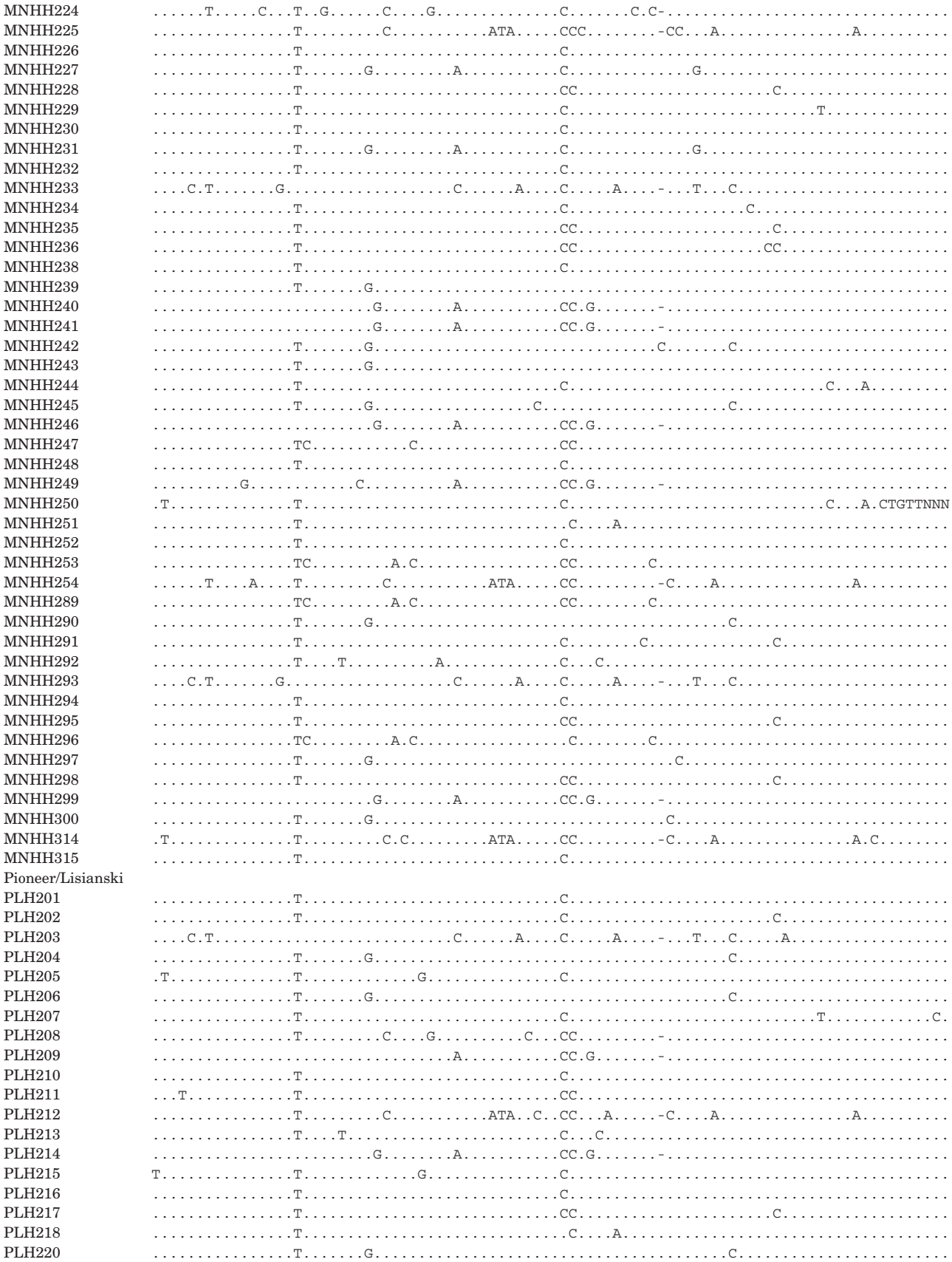
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MNHH226 ...............T.....................C...
MNHH227 ...............T.......G.........A...........C..............G.....................
MNHH228 ...............T.....................CC.....................C.....................
MNHH229 ...............T.....................C.............................T.............
MNHH230 ...............T.....................C...
MNHH231 ...............T.......G.........A...........C..............G.....................
MNHH232 ...............T.....................C...
MNHH233 ....C.T.......G...................C......A....C.....A....-...T...C.....................
MNHH234 ...............T.....................C.....................C.....................
MNHH235 ...............T.....................CC.....................C.....................
MNHH236 ...............T.....................CC.....................CC....................
MNHH238 ...............T.....................C...
MNHH239 ...............T.......G...
MNHH240 ........................G........A...........CC.G.......-.....................................
MNHH241 ........................G........A...........CC.G.......-.....................................
MNHH242 ...............T.......G.....................................C.......C.....................
MNHH243 ...............T.......G...
MNHH244 ...............T.....................C.............................C...A.........
MNHH245 ...............T.......G.................C.....................C.....................
MNHH246 ........................G........A...........CC.G.......-.....................................
MNHH247 ...............TC...........C.................CC...
MNHH248 ...............T.....................C...
MNHH249 ..........G............C..........A...........CC.G.......-.....................................
MNHH250 .T.............T.....................C.............................C...A.CTGTTNNN
MNHH251 ...............T.....................C....A...
MNHH252 ...............T.....................C...
MNHH253 ...............TC.........A.C.................CC........C.....................................
MNHH254 ......T....A....T.........C...........ATA.....CC.........-C....A..............A.........
MNHH289 ...............TC.........A.C.................CC........C.....................................
MNHH290 ...............T.......G.....................................C.....................
MNHH291 ...............T.....................C........C.............C.....................
MNHH292 ...............T....T..........A.............C...C.....................................
MNHH293 ....C.T.......G...................C......A....C.....A....-...T...C.....................
MNHH294 ...............T.....................C...
MNHH295 ...............T.....................CC.....................C.....................
MNHH296 ...............TC.........A.C.................C........C.....................................
MNHH297 ...............T.......G.....................................C.....................
MNHH298 ...............T.....................CC.....................C.....................
MNHH299 ........................G........A...........CC.G.......-.....................................
MNHH300 ...............T.......G.....................................C.....................
MNHH314 .T.............T.........C.C.........ATA.....CC.........-C....A..............A.C.........
MNHH315 ...............T.....................C...
Pioneer/Lisianski
PLH201 ...............T.....................C...
PLH202 ...............T.....................C.....................C.....................
PLH203 ....C.T.........................C......A....C.....A....-...T...C.....A.....................
PLH204 ...............T.......G.....................................C.....................
PLH205 .T.............T.............G.............C...
PLH206 ...............T.......G.....................................C.....................
PLH207 ...............T.....................C.............................T............C.
PLH208 ...............T.........C....G.........C...CC.........-.....................................
PLH209 .................................A...........CC.G.......-.....................................
PLH210 ...............T.....................C...
PLH211 ...T............T.....................CC...
PLH212 ...............T.........C...........ATA..C..CC...A.....-C....A..............A.........
PLH213 ...............T....T.....................C...C...
PLH214 ........................G........A...........CC.G.......-.....................................
PLH215 T..............T.............G.............C...
PLH216 ...............T.....................C...
PLH217 ...............T.....................CC.....................C.....................
PLH218 ...............T.....................C....A...
PLH220 ...............T.......G.....................................C.....................

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PLH221      .......C........T.............................AC.....................................
PLH309      ..............................................C.....................................
PLH310      ................T...................G.........CC.....................C...............
PLH311      ................T...............................CC.....................................
PLH316      .T..C.T.......G.....................C......A....C.....A....-...T...C.................
PLH317      .T..............T...................C.........CC.....................................
PLH318      .T..............T.........C....G..........C...CC.........-.............................
PLH319      .T........................G........A............CC.G.......-.............................
PLH320      .T..............T.........C............ATA.....CCC........-CC...A..............A.........
PLH321      .T..............T...............................C.....................................G..
Pearl & Hermes
P&HH262     ................T...............................C.....................................
P&HH263     ..............................................................................................
P&HH264     ................T...............................CC.....................CC...............
P&HH265     ................T...............................C.....................................
P&HH266     ................T.......G.......................C.....................................
P&HH267     ................T...............................C.....................C...............
P&HH268     ................T...............................C.....................................
P&HH269     ................T...................G.........CC.....................C...............
P&HH270     ................T...............................CC.....................C...............
P&HH271     ................T.........C............ATA..CA.CC.........-C....A..............A.........
P&HH272     ................T...............................C.....................C...............
P&HH273     ................T...............................C.....................................
P&HH274     ................T...............................C.....................................
P&HH275     ................T...............................C......G...............................
P&HH276     ................T...............................C.....................................
P&HH277     ................T...............................CC....................................
P&HH278     ................T...............................C.....................C.A.............
P&HH279     ............C..T....T.....A...................C...C...................................
P&HH280     ................T...............................C.....................................
P&HH281     ................T.........C............ATA.....CC.........-CC...A..............A.........
P&HH282     ................TC.........A.C.................CC........C.............................
P&HH283     ................T.................C............C.....................................
P&HH284     ................T...............................C.....................................
P&HH285     ................T...............................CC....................................
P&HH286     ................T...............................C.....................................
P&HH287     ................T...............................C.....................................
P&HH288     ..G.............T...............................C.....................................
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