

Population Structure and Comparative Phylogeography of Jack Species (*Caranx ignobilis* and *C. melampygyus*) in the High Hawaiian Islands

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Abstract

Members of the family Carangidae are top-level predators and highly prized food and sport fishes. Although ecologically and economically important, little is known about the biology of numerous species in the family. This is particularly true of the jacks *Caranx ignobilis* and *C. melampygyus*, which have experienced recent population reductions around the high Hawaiian Islands due to overfishing. Previous studies have documented territorial tendencies as well as cases of long-distance excursions in both species, suggesting populations may exhibit a range of structure at the genetic level. To explore this possibility, mitochondrial DNA ATPase6 and ATPase8 gene sequence variation was assessed from 91 individuals (33 *C. ignobilis* and 58 *C. melampygyus*) spanning the islands of Kaua'i, O'ahu, Moloka'i, Maui, and Hawai'i. Although a total of 20 distinct haplotypes (8 for *C. ignobilis*; 12 for *C. melampygyus*) were recovered, no evidence of population structure was found for either species across the examined geographic range. However, distinct demographic patterns were identified, implying differing evolutionary histories and/or population dynamics. Additionally, ~6% of the examined *C. ignobilis* were *C. ignobilis* × *C. melampygyus* hybrids because they harbored mitochondrial haplotypes typical of *C. melampygyus*. These hybrids contribute to measurable gene flow between the species and may play a significant role in the evolution of the genus.

Key words: Carangidae, Caranx, Hawai'i, jacks, phylogeography, population genetics

Humans have historically exploited fish species for consumption, economics, and recreation (e.g., Pringle 1997). Unfortunately, intensive commercial fishing as well as varying indirect anthropogenic stresses (i.e., eutrophication, habitat change, the proliferation of invasive species, etc.) have driven freshwater and marine fisheries on a global scale into sharp declines over recent decades (reviewed by Hilborn et al. 2003; Pauly 2008). Impacting the spectrum of species from small pelagic foragers (e.g., anchovies, herring, mackerel, sardines, etc.; Tacon and Metian 2009) to large top-level predators (e.g., tuna, billfishes, swordfish, etc.; Myers and Worm 2003, 2004), this precarious trend has ignited concern around the world due to issues ranging from the loss of intrinsic and/or cultural value (Lewin et al. 2006) to collapses in ecosystem function and health (Mullon et al. 2005). For future management practices to be successful will require the integration of sociopolitical, physical (i.e., oceanographic,

habitat, etc.), and organismal (i.e., natural history, behavior, etc.) information into a holistic framework (reviewed by Botsford et al. 1997; Thrush and Dayton 2010). However, knowledge concerning the basic biology of many fisheries-related species, such as their genetic structure, is lacking (Reiss et al. 2009), thus hindering such efforts.

The perciform family Carangidae consists of ~200 species of predominately marine fishes found in temperate and tropical waters around the world. Known as jacks, trevallies, pompanos, jack mackerels, and scads, many larger species are important top-level predators and are highly prized food and sport fishes (Shomura 1987; Honebrink 2000; Tagawa and Tam 2006). Twenty-seven species of carangids in 13 genera are listed as occurring in the Hawaiian Archipelago (Froese and Pauly 2010); of these, the jacks *Caranx ignobilis* Forsskål, 1775 and *C. melampygyus* Cuvier, 1833 (called ulua aukea and omilu, respectively, by

the native Hawaiian people) are the most frequently encountered. The 2 species have easily identifiable phenotypes (i.e., coloration and size), with *C. ignobilis* being a silvery color with occasional dark spots and reaching up to 170 cm in fork length (FL) (90 kg), whereas *C. melampygyus* is an iridescent color of brassy or gray hues speckled with brilliant blue spots and is considerably smaller (maximum of 91 cm FL; 14 kg). Although *C. ignobilis* and *C. melampygyus* occur sympatrically and have been observed in mixed schools (Honebrink 2000; Murakami et al. 2007), catch data imply that *C. melampygyus* is more common than any other jack species on Hawaiian coral reefs (e.g., Meyer et al. 2001; Tagawa and Tam 2006) and interspecific dietary differences suggest resource partitioning between them (Meyer et al. 2001).

Due to the ecological and economical importance of *C. ignobilis* and *C. melampygyus*, concerns have been raised over population reductions around the Hawaiian Islands due to overfishing (Shomura 1987; Friedlander and Dalzell 2004). This has spurred efforts toward developing a better biological understanding of these species. A particular area that has received focus is their short- and long-distance movement patterns because such information is fundamental for establishing effective marine protected areas (reviewed by Halpern and Warner 2003; Palumbi 2004). Using acoustic telemetry in the Northwestern Hawaiian Islands Marine National Monument, Meyer et al. (2007) found that *C. ignobilis* typically occupies core areas of activity with occasional travel of up to 29 km on the same atoll. Likewise, sonic tracking and tag-and-release studies of *C. melampygyus* in Kaneohe Bay, Hawai'i, suggest that most individuals (~75%) range >0.5 km from their release points (Holland et al. 1996) and other tag-and-release studies of *C. ignobilis* and *C. melampygyus* around the high (i.e., Kaua'i, O'ahu, Moloka'i, Maui, and Hawai'i) Hawaiian Islands have also documented similar patterns (Tagawa and Tam 2006). Thus, given this territorial tendency, it could be proposed that *C. ignobilis* and *C. melampygyus* exhibit some level of population genetic structuring in the high Hawaiian Islands. On the other hand, some of these same studies have also identified cases of long-distance excursions for both species, with a few individuals of *C. ignobilis* and *C. melampygyus* traveling 60–338 km between sites (Tagawa and Tam 2006; Tagawa AW, unpublished data). Such movements, even on limited occasions, could reduce the population structuring of *C. ignobilis* and *C. melampygyus* in the high Hawaiian Islands. Surprisingly, no population genetic studies for either species, in Hawai'i or elsewhere across their range (i.e., Indian and Pacific Oceans, Honebrink 2000; Froese and Pauly 2010), have been previously conducted to distinguish between such alternative hypotheses. Studies like these would not only arm resource managers with additional information useful for developing management strategies for the fishery but also will further our limited knowledge on the ecology and evolution of *Caranx* species in general.

The goal of the present study was to determine the degree of genetic structure among populations of *C. ignobilis* and *C. melampygyus* in the high Hawaiian Islands. Previously, Murakami et al. (2007) found that these species were 98.5%

and 90.9% similar in their mitochondrial DNA (mtDNA) large subunit ribosomal (16S ribosomal DNA) and cytochrome *c* oxidase subunit I (COI) gene sequences, respectively, during an examination of 2 *Caranx* hybrid individuals caught off O'ahu, Hawai'i. Thus, mitochondrial gene sequences have sufficient discriminating power to distinguish mtDNA haplotypes within, as well as between, the species. For this reason, we targeted a ~900 bp fragment of the mtDNA genome from multiple individuals of *C. ignobilis* and *C. melampygyus* collected across the high Hawaiian Islands. Using this approach, this report represents the first population genetic study of any *Caranx* species conducted to date. Along with this, comparing the demographic patterns from these species provides novel insight into the evolutionary histories of these ecologically and economically important predators in the Hawaiian Islands.

Materials and Methods

Biological Materials

All specimens of *C. ignobilis* and *C. melampygyus* were sampled as part of the State of Hawai'i Division of Aquatic Resources' Uluia Tagging Project, a statewide, angler-based program where volunteers were asked to assist in the capture and tagging of various jack species found in Hawaiian waters. Twenty anglers among the 2000 volunteers who fish on a regular basis were tasked with collecting secondary (soft) dorsal fin clip samples for this study. These volunteers were supplied with a fin clip kit consisting of a scissors, forceps, vials with 95% ethanol for sample preservation, data card, and a postage paid self-addressed stamped envelope for shipping of samples. All fish were landed using a rod and reel, and along with tagging and tissue sampling, measurements of FL and phenotypic appearance were collected. From this effort, samples from 33 *C. ignobilis* and 58 *C. melampygyus* were obtained from 25 localities spanning 5 Hawaiian Islands (Figure 1 and Supplementary Material S1) between September 2006 and June 2007.

DNA Extraction, Polymerase Chain Reaction, and Sequencing

Total genomic DNA was extracted from each fin clip sample using 2× cetyltrimethyl ammonium bromide/chloroform and utilized as template (~10 to 30 ng DNA per reaction) to amplify a ~900-bp fragment of the mtDNA genome encompassing the ATPase6 and ATPase8 genes. This fragment, spanning 2 genes, was selected for analysis to avoid inadvertent recovery of mtDNA pseudogenes translocated to the nuclear genome in a singular fashion (e.g., Lopez et al. 1994). Polymerase chain reactions (PCRs) were done in 25 µl volumes containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 1.5 mM MgCl₂, 200 µM dNTPs, 0.4 µM each of primers L8331 (5'-AAA GCR TYR GCC TTT TAA GC-3') and H9236 (5'-GTT AGT GGT CAK GGG CTT GGR TC-3') (Quenouille et al. 2004), and 1 U *Taq* DNA polymerase. Reactions were conducted in a PTC-100 thermocycler (MJ Research) under the following

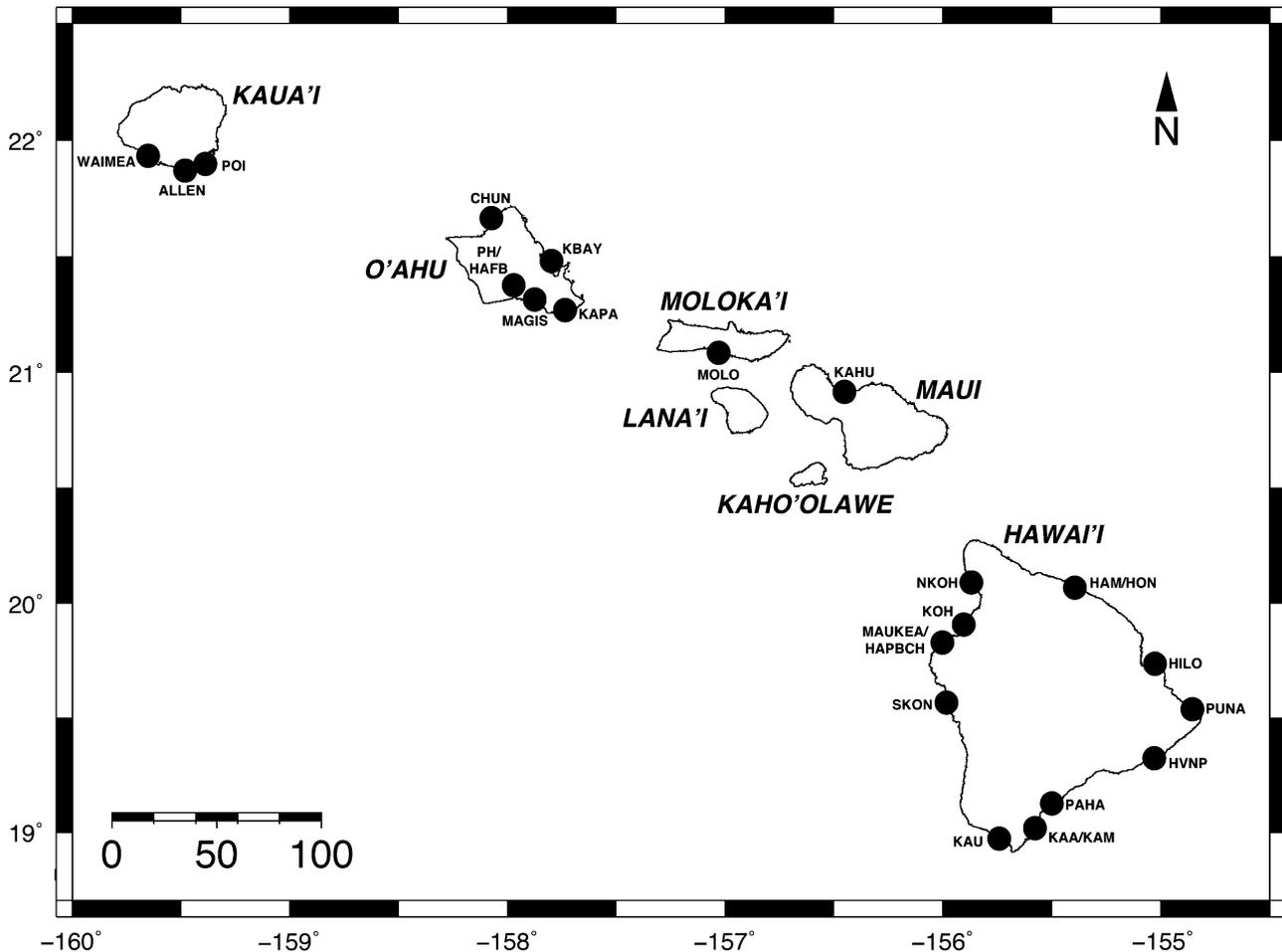


Figure 1. Map of the high Hawaiian Islands depicting sampling locations for *Caranx ignobilis* and *C. melampyngus* examined in this study. Key to sampling location codes is given in Supplementary Material S1.

“touchdown” profile: initial denaturing step of 94 °C for 4:30 min; 11 cycles of 94 °C for 45 s, 60 °C for 45 s (−1 °C per cycle), 72 °C for 60 s; 26 cycles of 94 °C for 45 s, 50 °C for 45 s, 72 °C for 60 s; and a final extension of 72 °C for 5 min. Amplifications were confirmed by electrophoresis in a 1% agarose gel, followed by staining and viewing with ethidium bromide and shortwave (265 nm) UV, respectively.

Amplicons were purified with Montage PCR Filter Units (Millipore) according to the supplier’s recommendations, cycle-sequenced in both directions using Big-Dye Terminators v3.1, and read on a PRISM 3100 Genetic Analyzer (Applied Biosystems). Corrections of ambiguities in the chromatograms were accomplished by comparison with the complement DNA strand in SEQUENCHER v4.7 (Gene Codes Corporation). Finished sequences were aligned manually using SE-AL v2.0a11 (available at <http://tree.bio.ed.ac.uk/software/seal/>).

Haplotype Diversity, Population Structure, and Demographic Analyses

Nucleotide (π) and haplotype (h) diversity estimates for *C. ignobilis* and *C. melampyngus* were calculated according to the

methods of Nei (1987). To test for genetic differentiation between islands in each species, pairwise Φ_{ST} statistics (based on haplotype frequency and molecular divergence) were conducted by pooling all individuals of a species and from a given island into a single “population.” Kimura’s (1980) 2-parameter model of evolution (K2P; as selected by the Akaike information criterion [AIC] in MODELTEST v3.6 [Posada and Crandall 1998]) was utilized for the pairwise Φ_{ST} statistics. Tajima’s D (Tajima 1989) and Fu’s F_s (Fu 1997) tests were conducted to determine whether patterns of mitochondrial sequence variation were consistent with predictions of the neutral model. Along with detecting the influence of selection on a gene, these tests can also be potentially informative about the demographic forces that have affected a population or species (Tajima 1989; Fu 1997). Lastly, Harpending’s raggedness index (Hri, Harpending 1994), based on mismatch distributions (i.e., the frequency distribution of pairwise differences among all haplotypes in a sample), was estimated to test whether the sequence data from each population deviated from what is expected under a sudden expansion model. A significant Hri value ($P < 0.05$) is taken as evidence for rejecting the sudden population

expansion model (Schneider and Excoffier 1999). All population structure and demographic analyses were conducted with the program ARLEQUIN v3.11 (Excoffier et al. 2005) and significance of the above tests assessed by 10 000 permutations.

Haplotype Network and Nested Clade Analysis

Relationships among mitochondrial haplotypes of *C. ignobilis* and *C. melampygyus* were visualized as networks constructed with the program TCS v1.21 (Clement et al. 2000). The analysis was conducted using the 95% parsimoniously plausible branch connections between haplotypes. Haplotypes within each network were then nested according to the approach of Crandall (1996) 3 independent times (to ensure a consistent nesting design) prior to conducting nested clade analyses (NCA; Templeton et al. 1995). The NCA, which allows separation of population history from population structure (reviewed in Templeton 1998), was done with GEODIS v2.6 (Posada et al. 2000) and 5000 permutations were employed in order to detect any significant associations between particular clades and their geographic (i.e., island) locations. Inferences regarding the historical processes giving rise to the current genetic patterns were made using the December 2008 NCA inference key (Templeton 2004; available at <http://darwin.uvigo.es/>).

Results

Genetic Diversity of *C. ignobilis* and *C. melampygyus* in the High Hawaiian Islands

A total of 656 bp from an overlapping (i.e., forward and reverse sequence reads) region of the ATPase6 and ATPase8 genes was analyzed from each of the 91 individuals (33 *C. ignobilis* and 58 *C. melampygyus*) examined here. From these, 20 distinct haplotypes (8 for *C. ignobilis*; 12 for *C. melampygyus*) were identified, with sequences deposited into GenBank under accession numbers EU131643–EU131662. For each species, 3 haplotypes were recovered more than once while the remaining ones occurred as singletons (Supplementary Material S1). Uncorrected (p) pairwise genetic distances between ATPase6/ATPase8 haplotypes of *C. ignobilis* and *C. melampygyus* were 10.4–11.8%, consistent with divergence levels of mtDNA COI haplotypes previously reported from these species (Murakami et al. 2007). Although haplotype (h) diversity values were similar for each species, nucleotide diversity (π) values were 3 \times higher for *C. ignobilis* than *C. melampygyus* (Table 1). Additionally, there was an \sim 3 \times difference in the mean number of pairwise differences between haplotypes in each species (Table 1). Numbers of transitions, transversions, and private substitution sites were similar for both species (Table 1). Notably, 2 of the 33 individuals identified phenotypically as being *C. ignobilis* were found to possess identical mitochondrial haplotypes to the 2 most commonly recovered from *C. melampygyus* (haplotypes Cm1 and Cm2, Supplementary Material S1). To confirm this, a second independent DNA

Table 1 Summary of mitochondrial genetic data for *Caranx ignobilis* and *C. melampygyus* from the high Hawaiian Islands

	<i>C. ignobilis</i>	<i>C. melampygyus</i>
Sample size	31	58
No. of unique haplotypes	8	12
Haplotype diversity	0.697 (\pm 0.075)	0.692 (\pm 0.041)
Nucleotide diversity (K2P)	0.006 (\pm 0.003)	0.002 (\pm 0.002)
Mean number of pairwise differences between haplotypes (K2P)	3.8 (\pm 2.0)	1.5 (\pm 1.0)
No. of transitions	12	11
No. of transversions	1	2
No. of private substitution sites	11	10
Tajima's D test	0.557	-1.34
Fu's F_s test	0.984	-4.93*
Harpending's raggedness index (Hri)	0.098*	0.031

K2P, Kimura 2-parameter model of evolution (Kimura 1980).

* $P < 0.05$.

extraction was conducted from the fin clip samples of these 2 individuals, followed by PCR and sequencing. In both cases, the mitochondrial haplotypes were identical in sequence to those initially recovered, suggesting the original results were not due to contamination. Instead, this finding is consistent with a previous report that *C. ignobilis* and *C. melampygyus* are capable of hybridizing, with *C. melampygyus* being the maternal (i.e., mitochondrial-contributing) lineage in such a cross (Murakami et al. 2007). These *C. ignobilis* \times *C. melampygyus* hybrids represented \sim 6% of the *C. ignobilis* individuals examined here.

Population Structure and Demographic Analyses of *C. ignobilis* and *C. melampygyus* in the High Hawaiian Islands

Pairwise Φ_{ST} statistics between the species *C. ignobilis* and *C. melampygyus* that excluded or included the 2 hybrid individuals were 0.971 and 0.925, respectively. Although significant genetic structure remains between the 2 species ($P < 0.001$), this difference of \sim 0.05 does imply low, but measurable, gene flow is occurring between them. Overall, pairwise Φ_{ST} statistics provided no evidence of genetic structure between *C. ignobilis* or *C. melampygyus* populations sampled from the different islands ($P > 0.05$ for all comparisons, Table 2). For this reason, the Tajima's D , Fu's F_s , and Hri tests were conducted on a per species basis. Both the Tajima's D and Fu's F_s tests of neutrality were positive, but not significant ($P > 0.1$), for *C. ignobilis*, whereas for *C. melampygyus*, the tests were both negative, with Fu's F_s being significant ($P = 0.007$; Table 1). A significant negative value in neutrality tests such as Fu's F_s reflects an excess of rare polymorphisms and is often obtained from populations that have experienced a recent expansion (Fu 1997). Further support for demographic differences between the species comes from the Hri of the mismatch distributions, with the absence of a significant Hri value (0.031, $P > 0.05$) for *C. melampygyus* but a significant Hri value (0.098, $P < 0.05$)

Table 2 Φ_{ST} statistics for *Caranx ignobilis* (above diagonal) and *C. melampygyus* (below diagonal) by Hawaiian island

	Hawaiian Island			
	Kaua'i	O'ahu	Maui/Moloka'i	Hawai'i
Kauai	—	<0.0001 (0.783 ± 0.011)	n/d	<0.0001 (0.999 ± 0.001)
Oahu	<0.0001 (0.866 ± 0.013)	—	n/d	0.013 (0.368 ± 0.016)
Maui/Molokai	<0.0001 (0.999 ± 0.001)	<0.0001 (0.999 ± 0.001)	—	n/d
Hawai'i	<0.0001 (0.844 ± 0.011)	<0.0001 (0.803 ± 0.011)	<0.0001 (0.999 ± 0.001)	—

P values presented in parentheses, with $P \gg 0.05$ for all comparisons. n/d, not determined.

for *C. ignobilis* (Table 1). This implies that the sudden population expansion model cannot be rejected for *C. melampygyus*. Taken together, it appears that *C. melampygyus* has experienced recent population expansion in the high Hawaiian Islands, whereas *C. ignobilis* has not.

Haplotype Network and NCA of *C. ignobilis* and *C. melampygyus* in the High Hawaiian Islands

The TCS analysis produced discrete networks for *C. ignobilis* and *C. melampygyus* (Figure 2). The nesting procedure revealed two 2-step clades in each species (Figure 2), with distinct frequencies of individuals within these clades. For example, $\sim 2\times$ more individuals occurred in clade 2-1 ($n = 20$) compared with clade 2-2 ($n = 11$) of *C. ignobilis* (Figure 2). On the other hand, the frequencies of individuals were nearly identical ($n = 28$ vs. 30) for clades 2-1 and 2-2 of *C. melampygyus* (Figure 2). The NCA found no significant associations between clades and geography in *C. ignobilis*. For *C. melampygyus*, a significant association was recovered from clade 1-1 in the form of large and small nested clade (D_n) values. In this case, however, the inference key utilized in the interpretation of the NCA output came to an “inconclusive

outcome” due to an absence of satisfied conditions at the end of the chain (i.e., 1, 2, 11, 17, No). Thus, no evolutionary process could be inferred for this association.

Discussion

Population Structure in *C. ignobilis* and *C. melampygyus* of the High Hawaiian Islands

Based on mitochondrial sequence data, we found no evidence of genetic structure in *C. ignobilis* and *C. melampygyus* of the high Hawaiian Islands. Thus, in spite of exhibiting territorial behaviors that might favor structuring, populations appear to be homogenized, at least over the examined geographic range. Although only a few studies have examined the genetic population structure of species in the family Carangidae to date, similar results from mitochondrial and nuclear sequence data have been reported from the carangid genus *Decapterus* in Southeast Asia and the Indo-Malay archipelago (Arnaud et al. 1999; Borsa 2003).

Although the development of, and screening of allelic variation at, potentially higher resolution nuclear loci (i.e., microsatellites) may reveal structure among populations of

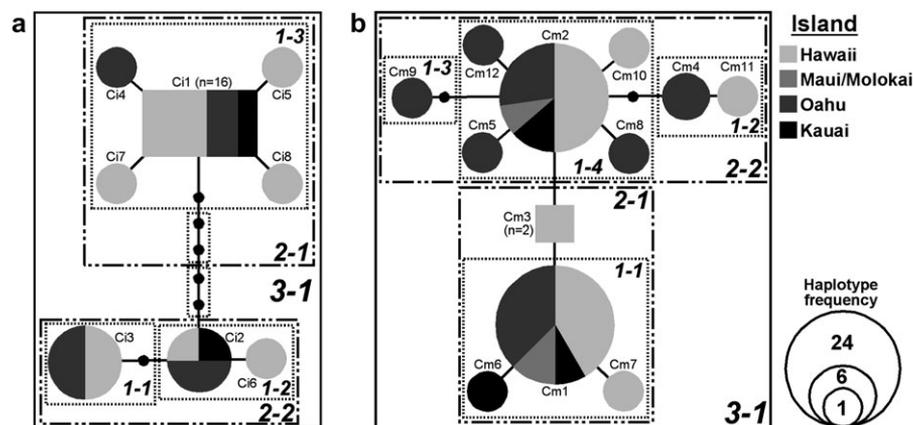


Figure 2. mtDNA haplotype networks of (a) *Caranx ignobilis* and (b) *C. melampygyus* from the high Hawaiian Islands depicting the nesting levels used to infer historical processes in each species. Sampled haplotypes are indicated by a name (i.e., CiX or CmX), whereas black dots represent unsampled (i.e., missing) haplotypes. The rectangle represents the haplotype with the highest outgroup probability according to the TCS (Clement et al. 2000) analysis. The size of ovals and the rectangle are proportional to the frequency at which a haplotype was recovered (for exact haplotype frequencies, see Supplementary Material S1). Color codes for each island population are presented in the legend. Note that in spite of variable lengths, each branch implies a single mutational difference between haplotypes.

C. ignobilis and/or *C. melampygyus* in the islands, we hypothesize that data from such genetic markers would likely result in similar conclusions as the mitochondrial sequence data presented here. One reason for this line of thought is that long-distance movements among sites have been recorded for both *C. ignobilis* and *C. melampygyus* (see Introduction), which would facilitate mixing among populations around and between the islands. Along with migration, the transport of eggs and/or larvae via oceanic currents can foster population homogenization at the genetic level in marine organisms (reviewed by Caley et al. 1996). Unfortunately, little is known regarding the reproductive biology of these species (e.g., Leis et al. 2004). For the carangid *Gnathanodon speciosus*, hatching has been observed 18 h after spawning (Watson and Leis 1974). Assuming a comparable duration for *C. ignobilis* and *C. melampygyus*, this would allow some amount of passive dispersal prior to hatching. Along with this, carangid fish do not settle (Leis 1991) and early juveniles (i.e., <21 mm standard length) often associate with floating or drifting objects (Honebrink 2000). Such behavior would also facilitate population mixing in these species because juveniles have an opportunity to disperse for longer durations and/or distances. Taken together, we conclude that the absence of genetic structure reported here for *C. ignobilis* and *C. melampygyus* is due to the active movement of adult individuals and/or the passive dispersal of eggs and juveniles at frequencies sufficient to homogenize populations in the high Hawaiian Islands.

Evolutionary History and Demographics of *C. ignobilis* and *C. melampygyus* in the High Hawaiian Islands

The data presented here suggest *C. ignobilis* and *C. melampygyus* have experienced different evolutionary histories in Hawai'i. Previous work estimated the mutation rate of fish ATPase genes to be ~1.3% per million years (My) (Bermingham et al. 1997). Based on this approximation, the level of sequence divergence (i.e., 10.4–11.8%) between them suggests that they have been separated for 8–9.1 My. Currently, the geographic region where *C. ignobilis* and *C. melampygyus* evolved is unknown, but their range distribution across the Indian and Pacific Oceans suggests that they originated and spread from the Indo-West Pacific like many other coral reef fish species (Allen and Adrim 2003). In the high Hawaiian Islands, both species are comprised of 2 major groups or clades (Figure 2); however, the level of divergence between *C. ignobilis* clades is 3× higher than that of the *C. melampygyus* clades. One hypothesis is that these *C. ignobilis* clades diversified within the high Hawaiian Islands. However, the absence of population structuring reported here suggests a lack of sufficient isolating barriers in the islands to support this scenario. Instead, a more parsimonious explanation is that the *C. ignobilis* clades diversified in isolation elsewhere and the high Hawaiian Islands were independently colonized twice by the species, similar to what has been proposed for the Hawaiian grouper *Epinephelus quernus* (Rivera et al. 2004). Genetic analyses of

C. ignobilis from other geographic localities in its distribution range will prove useful toward testing this hypothesis.

Caranx ignobilis and *C. melampygyus* of the high Hawaiian Islands were also found to exhibit differing demographics. For example, distinct ratios in the frequencies of individuals per clade were recovered from each species (i.e., 2:1 for *C. ignobilis* and 1:1 for *C. melampygyus*). Thus, the genetic composition of *C. ignobilis* is biased toward members of a particular clade. Although the specific factors driving this biased ratio in *C. ignobilis* genetic diversity remains to be determined, competitive and/or reproductive differences between the clades could potentially produce such a pattern. Additionally, Fu's F_s and Hri tests suggest that *C. melampygyus* has undergone recent population expansion, whereas *C. ignobilis* has not (Table 1). At least 3 hypotheses can be proposed that might account for this difference. First, *C. melampygyus* may have colonized the high Hawaiian Islands more recently than *C. ignobilis*. Second, dietary differences and resource partitioning between these sympatric species (Meyer et al. 2001) imply each occupies a unique feeding niche. Under this scenario, *C. melampygyus* may be continuing to undergo expansion within its niche, whereas *C. ignobilis* has already reached demographic stability within its own. Third, high variance in the reproductive success of *C. melampygyus* relative to *C. ignobilis* could also produce a signal of expansion in the former species; such demographic peaks and contractions due to varying reproductive success have been observed in other marine fish species (e.g., Grant and Bowen 1998). Studies designed to distinguish between these (or additional) alternative hypotheses will provide valuable insight into the biology of *Caranx* species, both in Hawai'i and in general.

Hybridization between *C. ignobilis* and *C. melampygyus* of the High Hawaiian Islands

Murakami et al. (2007) previously reported on the occurrence of 2 *Caranx* hybrids from waters surrounding O'ahu, Hawai'i. The hybrid nature of these specimens was initially raised due to their intermediate phenotype between *C. ignobilis* or *C. sexfasciatus* and *C. melampygyus*. Subsequent morphological and molecular analyses confirmed this suspicion, and in both cases, mitochondrial data identified *C. melampygyus* as the maternal lineage (Murakami et al. 2007). However, missing from this analysis was an assessment of whether *C. melampygyus* could also serve as the paternal parent in hybridizations. The frequency of hybrids in the population and their distribution across the high Hawaiian Island were also not addressed.

Here, 2 of 33 *C. ignobilis* were apparent hybrids because they harbored mitochondrial haplotypes from *C. melampygyus* while possessing phenotypic traits identifiable with *C. ignobilis*. These individuals were caught, tagged, and sampled on the islands of Maui and Hawai'i (Supplementary Material S1), suggesting hybrids are not confined to O'ahu. In addition, assuming the *C. ignobilis* individuals included in this study are a random sampling of the species, *C. ignobilis* × *C. melampygyus* hybrids represent ~6% of the standing population. Although this value is relatively low, it does result in measurable gene

flow between the species, as indicated by the pairwise Φ_{ST} statistics (see Results). It also appears that mtDNA introgression is asymmetric as none of the 58 *C. melampygius* examined here possessed mitochondrial haplotypes from *C. ignobilis* or other *Caranx* species.

Members of the genus *Caranx* are just some of the many fish species capable of natural hybridization (reviewed by Campton 1987), but how hybrids are conceived remains to be determined because little information exists on the natural spawning behaviors of *C. ignobilis* and *C. melampygius* (reviewed by Murakami et al. 2007). In the Philippines, single male and female *C. ignobilis* pairs have been observed to simultaneously release gametes into the water column following a period of courtship (von Westernhagen 1974). If *C. melampygius* spawns in a similar fashion, time and place as that of *C. ignobilis*, hybridizations may happen accidentally as heterospecific gametes inadvertently encounter each other. However, although accidental cross-fertilization is a valid possibility, the finding that *C. melampygius* is consistently the maternal lineage in these hybridizations suggests alternative explanations. For example, *C. melampygius* may be incapable of being the paternal lineage due to gamete incompatibility and/or nonviability of hybrid offspring from such a cross. Another possibility is that *Caranx* species such as *C. ignobilis* and *C. sexfasciatus* possess behaviors similar to some salmonids, where males opportunistically “sneak” and raid spawning events of other closely related and numerically dominant species when they cannot find a conspecific mate (e.g., Garcia-Vazquez et al. 2002). As mentioned previously, catch data imply that *C. melampygius* is much more common than other jack species in Hawai'i (e.g., Meyer et al. 2001; Tagawa and Tam 2006), which is consistent with such a hypothesis. In either case, the potential importance of these hybrids to the evolution of the genus *Caranx* deserves further attention.

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