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Original article

Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mitochondrial DNA control region markers

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ABSTRACT: Genetic polymorphism in kingfish, collected from coastal waters of Japan, Australia and New Zealand, were examined using microsatellite (MS) DNA and mitochondrial DNA (mtDNA) control region markers. Sixteen to 25.7 alleles per locus were observed in three MS markers, while the average observed (and expected) heterozygosities were 0.782 (0.918), 0.750 (0.809) and 0.650 (0.888) for Australian, Japanese and New Zealand kingfish, respectively. Twelve mtDNA haplotypes were detected by the digestion of control region sequences with five endonucleases: *Hae*III, *Hin*fI *Mbo*I, *Rsa*I and *Taq*I. Significant genetic divergence was observed between the kingfish population from Japan and those from Australia–New Zealand. There was no significant differentiation among the Australian and New Zealand population samples.

KEY WORDS: **genetic divergence, kingfish, microsatellite DNA, mitochondrial DNA control region, *Seriola lalandi*.**

INTRODUCTION

The yellowtail kingfish (*Seriola lalandi*) is an economically important fish, and is widely distributed throughout the Pacific and Atlantic oceans. These large pelagic carnivores are the targets of both commercial and recreational fishing activities in several Pacific countries. Commercial kingfish, *Seriola lalandi lalandi*, catch averaged approximately 300 tons during the 1990s in New South Wales.[1] More than 50 000 tons of the yellowtail (including kingfish) were caught in Japanese waters in 1999.[2] In New Zealand waters, the kingfish *S. lalandi lalandi* supports significant recreational fishery with an estimated catch of 300–550 tons; the species is also taken by catch in coastal fisheries (Annala JH *et al.*, unpubl. data, 1998).

Increasing fishing activity will reduce the chance of successful reproduction of a fish species, and give rise to changes in genetic variability and population structure. Greater awareness and efforts to conserve the genetic resources in the natural population are needed. Because genetic variability seems to be an important feature of a population for evaluating the short-term fitness of individuals and for the long-term survival of the population,[3] an alternative may be pursuant to the eventual determination of the genetic variability of natural population.[4]

Genetic variability can be evaluated by two parameters: (i) allelic diversity; and (ii) heterozygosity. Various techniques are used to estimate levels of genetic variability, including isozymes, mitochondrial DNA (mtDNA), minisatellite and microsatellite DNA. As part of a program to develop easily assayed, highly polymorphic genetic markers for population studies, we cloned and sequenced microsatellite (MS) DNA regions for the genus *Seriola* from the greater amberjack genome.[5] Three of these loci were applied to examine the genetic variability of kingfish collected from Japan, Australia and New Zealand. We also assayed genetic variability using an Restriction Fragment Length Polymorphism (RFLP)-mtDNA control

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region marker. Assessing the baseline genetic variation at MS loci and mtDNA is a prerequisite for determining population structure in kingfish in order to develop policies for the management and/or conservation of its natural genetic resources.

MATERIALS AND METHODS

Samples

Muscle tissue samples were removed from 325 kingfish collected from Japan (J-1), Australia (A-1–3) and New Zealand (N-1). Australian kingfish were collected from three regions along the New South Wales (NSW) coast, separated by a distance of approximately 200 km. Collection locations and sample sizes are shown in Table 1. DNA was extracted by a standard phenol–chloroform method according to Harris *et al.*[6]

Microsatellite polymorphism

Three MS loci were amplified by polymerization chain reaction (PCR), as modified from McConnel *et al.*,[7] using three primers developed in a previous study.[5] Genetic polymorphism was measured as the number of alleles, and by the observed and expected heterozygosities. Probability analysis was used to test for Hardy–Weinberg expectation (HWE) using ARLEQUIN version 1.0.[8] The AMOVA (Analysis of Molecular Variance) procedure in ARLEQUIN was used to measure population differentiation. Pair-wise F_{st} tests were used to test the significance of MS allele frequency homogeneity among populations. Nei's unbiased genetic distances[9] of kingfish populations were calculated using the PHYLIP computer package,[10] and used to construct an Unweighted Pair-Grouping Method using Arithmetric Average dendrogram.

Mitochondrial DNA control region polymorphism

The mtDNA control region and parts of cytochrome *b* and 12S rRNA genes were amplified using PCR as described by Martin *et al.*[11] Polymerization chain reaction products were digested by using the four-base recognition enzymes *Taq*I, *Mbo*I, *Hae*III and *Rsa*I, and one five-base recognition enzyme *Hin*fI. Fragment patterns generated by each of the restriction endonucleases was compiled for each individual as a composite haplotype. Genetic variation between localities was evaluated as the number of haplotype and as haplotype diversity,[12] and tested using AMOVA. Pair-wise F_{st} was used to test the significance of mtDNA haplotype frequency homogeneity among populations. The significance of geographic heterogeneity in mtDNA haplotype frequencies was tested using Monte Carlo χ^2 tests.[13]

RESULTS

Microsatellite markers

A high level of polymorphism was observed among kingfish collected from Japan (J-1), Australia (A-1–3) and New Zealand (N-1). The allele frequencies for the three loci are listed in Table 2. The average number of alleles ranged from 16.0 to 25.7 alleles per locus in N-1 and A-3 populations, respectively. The observed and expected heterozygosity ranged from 0.656 (N-1) to 0.782 (A-2) and 0.809 (N-1) to 0.922 (A-3), respectively. Allelic variation was found at the locus *Sdn-03**, which has been reported previously as being monomorphic in greater amberjack.[5] An example of an allele pattern is shown in Fig. 1. The genotype proportions in each population for each locus were tested for goodness-of-fit to the HWE. Significant deviation from the HWE was observed in all of the Australian

Table 1 Sample lots of king fish collected from natural waters

Sample	Locality	No. sample	Length size (cm)	Time of collection	Remarks
J-1	Japan	60	48.0–84.0 (FL)	October '96	Collected from Kochi pref.
A-1	Australia	80	52.0–101.0 (FL)	May–June '99	Approx. 35° south, NSW coast
A-2	Australia	80	52.0–95.0 (FL)	May–June '99	Approx. 33.30° south, NSW coast
A-3	Australia	80	51.0–98.0 (FL)	May–June '99	Between 30–32° south, NSW coast
N-1	New Zealand	25	62.0–121.0 (FL)	June–November '99	Collected North Island including Cape Brett, Poor Knight and White Island

FL, Fork length.

Table 2 Allele frequency distribution of kingfish collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (N-1) at loci *Sdn-03**, *Sdn-06** and *Sdn-09**

Locus	Allele	J-1	A-1	A-2	A-3	N-1
*Sdn-03**						
	**179*	–	–	–	0.013	–
	**183*	–	–	0.014	–	–
	**185*	–	–	–	0.019	–
	**187*	–	0.013	0.007	0.007	–
	**191*	0.009	0.013	–	–	–
	**193*	0.009	–	–	–	–
	**195*	–	0.006	–	–	–
	**197*	–	0.031	–	0.019	–
	**199*	–	0.019	–	–	0.02
	**201*	–	0.025	0.029	0.033	0.02
	**203*	0.026	0.019	0.029	0.046	0.06
	**205*	–	0.038	–	0.013	–
	**207*	–	0.013	–	0.033	–
	**209*	–	0.019	–	–	0.02
	**211*	–	0.019	0.014	0.013	0.04
	**213*	–	0.013	0.014	0.013	0.04
	**215*	0.026	0.031	0.022	0.013	–
	**217*	0.034	0.019	0.007	0.013	0.02
	**219*	–	0.056	0.058	0.019	0.02
	**221*	0.017	0.056	0.072	0.066	0.06
	**223*	–	0.038	0.029	0.033	–
	**225*	0.009	0.031	0.072	0.033	0.02
	**227*	0.026	0.019	0.051	0.053	0.14
	**229*	0.026	0.05	0.043	0.053	0.06
	**231*	0.034	0.05	0.094	0.053	0.12
	**233*	0.017	0.044	0.058	0.072	0.08
	**235*	0.043	0.05	0.043	0.013	0.08
	**237*	0.026	0.063	0.022	0.033	0.06
	**239*	0.069	0.019	0.043	0.019	0.04
	**241*	0.034	0.031	0.036	0.033	–
	**243*	0.086	0.038	0.087	0.039	0.04
	**245*	0.009	0.025	0.029	0.033	–
	**247*	0.009	0.019	0.007	0.046	–
	**249*	0.043	0.006	0.022	0.019	–
	**251*	0.026	0.038	0.014	0.039	0.02
	**253*	0.026	0.013	0.007	–	–
	**255*	0.017	0.019	0.014	0.046	–
	**257*	0.043	0.038	0.022	0.007	0.02
	**259*	0.043	0.006	–	–	–
	**261*	0.017	–	0.029	0.013	–
	**263*	0.043	–	–	0.007	0.02
	**265*	0.026	0.019	–	0.007	–
	**267*	0.052	–	–	–	–
	**269*	0.034	–	–	0.007	–
	**271*	0.052	–	–	0.013	–
	**273*	0.009	–	0.007	–	–
	**275*	0.009	–	–	–	–
	**277*	0.017	–	–	–	–
	**279*	0.017	–	–	–	–
	**285*	0.009	–	–	–	–
	**287*	0.009	–	–	0.007	–
*Sdn-06**						
	**270*	–	–	–	0.013	–
	**272*	–	–	–	0.007	–
	**274*	–	–	0.007	–	0.042
	**276*	–	0.056	–	0.007	0.021
	**278*	–	–	0.021	0.013	0.104

Table 2 Continued

Locus	Allele	J-1	A-1	A-2	A-3	N-1
	*280	–	–	0.014	0.007	–
	*282	0.042	0.019	0.007	0.026	–
	*284	0.008	0.013	–	–	–
	*286	0.333	0.006	–	–	–
	*288	0.008	0.006	0.014	0.013	–
	*290	0.35	0.056	0.021	0.053	0.042
	*292	0.108	0.013	0.014	0.013	0.021
	*294	0.025	0.025	–	0.007	0.021
	*296	0.058	0.05	0.042	0.092	0.063
	*298	0.008	0.069	0.049	0.046	0.021
	*300	0.025	0.15	0.035	0.086	–
	*302	–	0.025	0.063	0.066	0.083
	*304	–	0.088	0.07	0.059	0.042
	*306	–	0.138	0.141	0.125	0.271
	*308	–	0.025	0.056	0.079	0.021
	*310	–	0.156	0.204	0.171	0.125
	*312	–	0	0.028	0.007	0.063
	*314	–	0.063	0.07	0.046	0.063
	*316	0.008	0.006	0.021	0.026	–
	*318	0.017	0.019	0.042	0.013	–
	*320	–	0.019	0.049	0.026	–
	*322	–	–	0.028	–	–
*Sdn-09**						
	*152	0.05	–	–	–	–
	*154	0.008	–	–	–	–
	*156	0.341	–	0.019	–	–
	*158	–	0.05	0.006	0.057	0.063
	*160	0.375	0.013	0.019	0.006	0.021
	*162	0.092	–	–	0.006	–
	*164	0.033	0.038	0.026	0.069	0.125
	*166	0.042	0.069	0.038	0.063	–
	*168	0.025	0.075	0.064	0.057	0.063
	*170	0.033	0.05	0.045	0.051	0.083
	*172	–	0.088	0.064	0.101	0.063
	*174	–	0.175	0.167	0.139	0.25
	*176	–	0.05	0.096	0.051	0.021
	*178	–	0.213	0.205	0.228	0.167
	*180	–	0.006	0.045	0.006	0.021
	*182	–	0.081	0.083	0.019	0.104
	*184	–	0.044	0.038	0.019	–
	*186	–	0.013	0.013	0.019	–
	*188	–	0.031	0.032	0.069	0.021
	*190	–	0.006	0.038	0.038	–

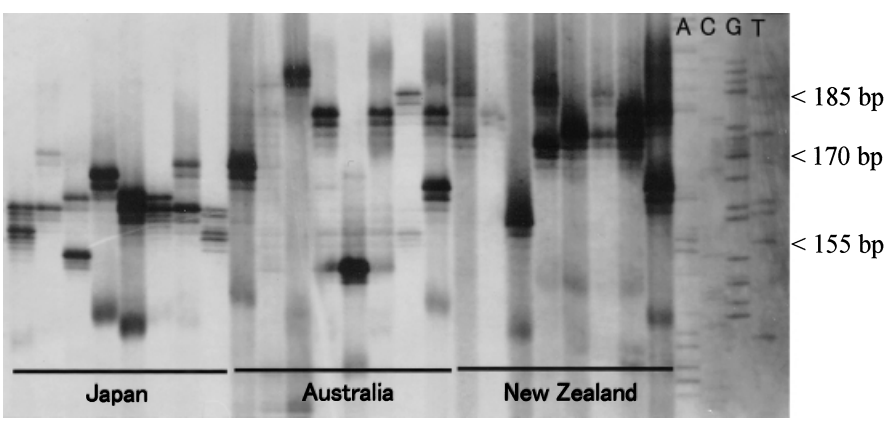

Fig. 1 Example patterns of microsatellite polymorphism.

Table 3 Genetic variability of kingfish collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (N-1) as revealed by microsatellite markers

Sample		Locus			Average
		Sdn-03	*Sdn-06*	*Sdn-09*	
J-1	No. samples	58	60	60	
	No. alleles	36	13	9	19.3
	Observed heterozygosity (Ho)	0.7	0.8	0.75	0.75
	Expected heterozygosity (He)	0.961	0.748	0.723	0.809
	Ho/He	0.728*	1.069	1.037	0.945
A-1	No. samples	80	80	80	
	No. alleles	36	20	16	24
	Observed heterozygosity (Ho)	0.825	0.75	0.75	0.775
	Expected heterozygosity (He)	0.963	0.905	0.887	0.918
	Ho/He	0.857*	0.829*	0.846*	0.844
A-2	No. samples	69	71	78	
	No. alleles	30	21	17	22.7
	Observed heterozygosity (Ho)	0.884	0.718	0.744	0.782
	Expected heterozygosity (He)	0.95	0.908	0.754	0.871
	Ho/He	0.931	0.791*	0.986	0.903
A-3	No. samples	76	76	79	
	No. alleles	37	23	17	25.7
	Observed heterozygosity (Ho)	0.763	0.671	0.62	0.685
	Expected heterozygosity (He)	0.961	0.915	0.891	0.922
	Ho/He	0.794*	0.733*	0.696*	0.741
N-1	No. samples	25	24	24	
	No. alleles	21	15	12	16
	Observed heterozygosity (Ho)	0.72	0.583	0.666	0.656
	Expected heterozygosity (He)	0.929	0.874	0.863	0.888
	Ho/He	0.755	0.667	0.772	0.738

*Departure from Hardy–Weinberg Equilibrium at Boferroni value, $\alpha = 0.05/5$.

Table 4 *P* values of F_{st} pair-wise comparison test of samples collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (N-1) based on the microsatellite DNA (above diagonal) and mitochondrial DNA D-loop (below diagonal)

	J-1	A-1	A-2	A-3	N-1
J-1	//////////////	0.000*	0.000*	0.000*	0.000*
A-1	0.000*	//////////////	0.069	0.485	0.02
A-2	0.000*	0.614	//////////////	0.178	0.149
A-3	0.000*	0.406	0.485	//////////////	0.069
N-1	0.000*	0.881	0.941	0.901	//////////////

*Significant at Bonferroni corrected level $P < 0.01$.

samples, except population A-2 at loci *Sdn-03** and *Sdn-09**. Departure from the HWE was also found in Japanese kingfish at locus *Sdn-03** (Table 3).

The AMOVA revealed significant genetic differentiation among the kingfish samples, with an overall F_{st} value of 0.046 ($P < 0.001$). Pair-wise F_{st} values revealed highly significant differences between the samples from Japan and Australia, and between Japan and New Zealand, but no significant differences between the samples from Australia and New Zealand (Table 4).

Genetic distances estimated among the five populations were relatively high; the average Nei pair-wise distance was 0.066. A dendrogram of the kingfish population relationship was produced using UPGMA. The Japanese kingfish were clearly separated from the Australian and New Zealand ones (Fig. 2).

Mitochondrial DNA marker

Polymerization chain reaction products of the control region were approximately 1.5–1.7 kilobase (kb) in length. Polymorphism of restriction fragment patterns was observed with four restriction enzymes, *Hae*III, *Hin*fI, *Mbo*I and *Taq*I; an example of a restriction digestion is shown in Fig. 3.

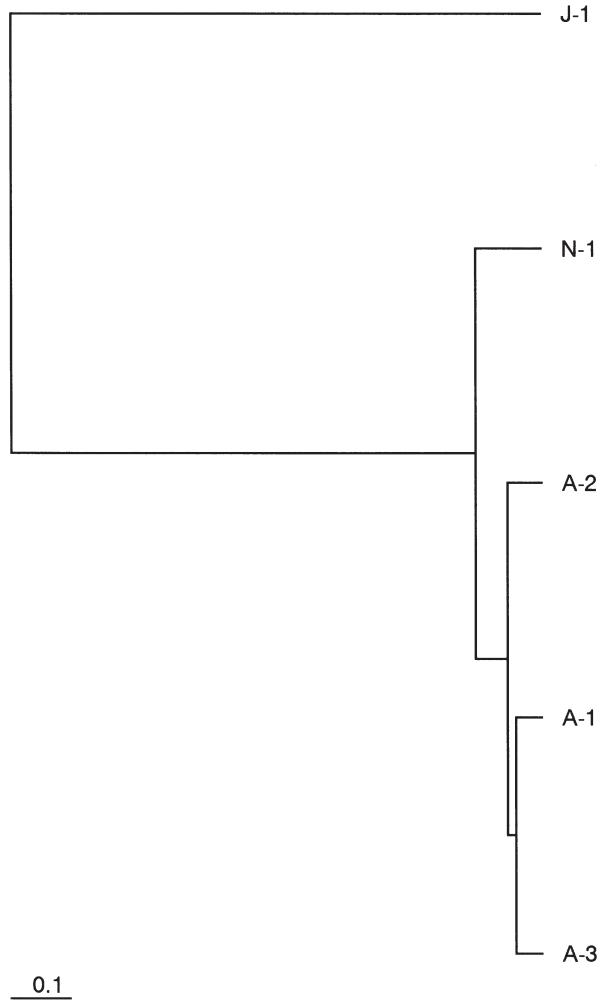

Fig. 2 Unweighted Pair-Grouping Method using Arithmetric Average dendrogram of kingfish collected from Japan, Australia and New Zealand based on the Nei unweighted genetic distance of allele frequency.

Digestion of the mtDNA D-loop fragment with *Hin*fI resulted in five patterns, whereas enzymes *Taq*I, *Mbo*I, and *Hae*III produced three to four fragment patterns. Twelve mtDNA composite haplotypes were found in the total samples; five haplotypes were found in Japanese samples and the remaining seven haplotypes were restricted to samples from Australia and New Zealand (Table 5). There was a highly significant heterogeneity in haplotype frequencies among all samples ($P < 0.0001$), but there was no significant heterogeneity among the Australian and New Zealand samples ($P = 0.19$) or among the Australian samples ($P = 0.38$).

Haplotype diversity ranged from 0.509 (N-1) to 0.660 (J-1). The highest number of haplotypes was observed in A-1 and A-3 (six haplotypes), and the lowest in N-1 and A-2 samples (four haplotypes) (Table 4). Genetic differentiation was observed among kingfish samples of the present study. Test significance of pair-wise F_{st} showed a significant difference between kingfish collected from Japan compared with the Australian or New Zealand samples. Significant differentiation was not observed between the Australian and New Zealand samples (Table 4).

DISCUSSION

High polymorphism was observed at the MS loci in the kingfish samples, with an average per locus per population observed heterozygosity of 0.729 and an average per locus per population expected heterozygosity of 0.882. The average number of alleles per locus per population was 21.5. The polymorphism level of kingfish was higher than that observed in the greater amberjack,[14] but com-

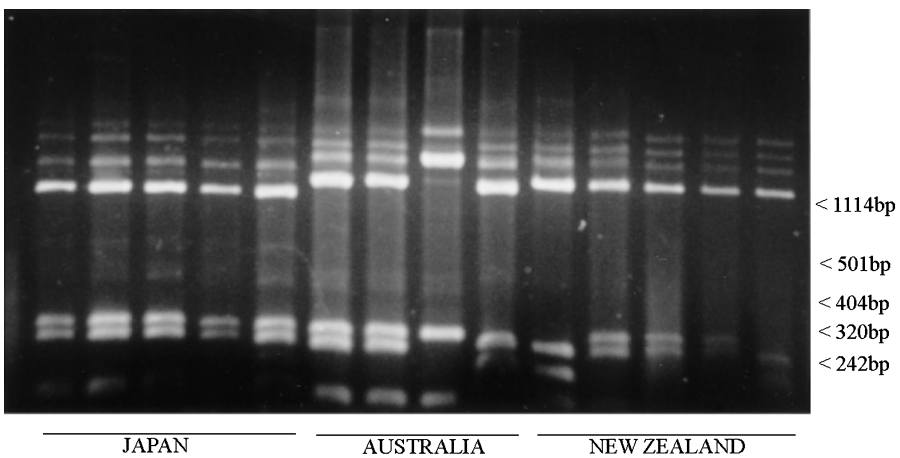

Fig. 3 Example patterns of mitochondrial DNA control region polymorphism.

Table 5 Genetic divergence of kingfish collected from Japan (J-1), Australia (A-1 to 3) and New Zealand (Z-1) as revealed by mitochondrial DNA D-loop analysis

Haplotype*	J-1	A-1	A-2	A-3	N-1
1 AAEFD	0.182	0.0	0.0	0.0	0.0
2 OAEFD	0.382	0.0	0.0	0.0	0.0
3 OAFFD	0.4	0.0	0.0	0.0	0.0
4 ABEFD	0.018	0.0	0.0	0.0	0.0
5 OAFFE	0.018	0.0	0.0	0.0	0.0
6 ABGFE	0.0	0.013	0.063	0.052	0.04
7 ABGFF	0.0	0.313	0.313	0.257	0.28
8 ABGFG	0.0	0.613	0.6	0.597	0.64
9 ABHFF	0.0	0.0	0.0	0.0	0.04
10 ABHFG	0.0	0.025	0.0	0.039	0.0
11 ACGFF	0.0	0.038	0.013	0.026	0.0
12 ACGFG	0.0	0.0	0.0	0.039	0.0
No. samples	55	80	80	77	25
No. haplotypes	5	6	4	6	4
Haplotype diversity	0.66	0.525	0.538	0.576	0.509

*Generated by *TaqI, HaeIII, MboI, RsaI* and *HinfI* endonucleases.

parable with other migratory species such as tuna,[15] red sea bream[16] and Atlantic salmon.[17] A relatively high variability was also observed in the mtDNA control region marker.

Genotype proportions in each population for each locus were tested for goodness-of-fit to the HWE. Significant deviation from the HWE was observed in all of the Australian samples, except A-2 at locus *Sdn-03** and *Sdn-09**, as well as in Japanese kingfish at locus *Sdn-03**. Homozygous excesses might be produced by the mixing of genetically different subpopulations (Wahlund effect) and/or mutation in the priming site. In the present study, there is no evidence of genetic differentiation among Australian kingfish, as shown by the F_{st} test (Table 4); therefore, the Wahlund effect seems unlikely. Furthermore, studies on the movement of kingfish using tags showed that the Australian kingfish population is unlikely to consist of more than two different stocks. [18] Non-amplifying alleles or null alleles, have been inferred quite frequently from fish population analysis for MS loci,[19] perhaps because of the relatively high mutation rates of this class of marker. Alleles at low frequency may cause the homozygous excess observed in the present study.

Based on the two markers, there are unclear differences in the polymorphism level among kingfish samples. A relative big difference was observed only in the average number of alleles. The allelic number for Australian kingfish was higher than those observed for the Japanese and New Zealand samples. This may be because of the limited samples examined in New Zealand, and perhaps because the Australian population consisted of the larger, more effective population size than the Japanese populations. The long distance of their movements observed in the waters of NSW[1] may cause the likelihood of the Australian kingfish to be well mixed, and lead to it making up a single Mendelian population.

The distribution of allele and haplotype frequencies of the Japanese samples was very different to the Australian and New Zealand samples. There are area-specific alleles and haplotypes among kingfish samples. Alleles with high frequencies that were observed in the Japanese samples were found in low frequency in the Australian and New Zealand samples. The Japanese kingfish are characterized by unique haplotypes that are not found in the Australian and New Zealand populations (Tables 2,4). Furthermore, the F_{st} test of the allele and haplotype frequencies showed significant genetic differentiation between Australian–New Zealand and Japanese kingfish (Table 5). We estimated that N_em between Japanese and Australian populations, and between Japanese and New Zealand populations was 2.1 and 1.8, respectively; whereas N_em between Australian and New Zealand populations, and within Australian populations was 42.9 and 951.7, respectively. These results indicate that the migration rate (m) between Japanese and Australian–New Zealand kingfish may be very small because the N_e value estimated from the average *He* was very large (average, 23 466).

Gillanders *et al.* mentioned that the Australian species of yellowtail kingfish is thought to be one of three physically similar subspecies, but of geographically separate populations.[1] The Australian

and New Zealand species are known as *Seriola lalandi lalandi*, whereas the Japanese speices is known as *S. lalandi aureovittata*. This result is contrary with that observed in greater amberjack. Gushiken noted that there was no differentiation between greater amberjack from Japan and the Pacific Oceanic region, suggesting that there may be gene flow among the greater amberjack populations.[20] Given the greater mobility of kingfish, the large genetic divergence among Northern and Southern Hemisphere populations is surprising, even though differing genetic structures were also observed in the Southern and Northern Hemisphere populations of red sea bream.[21] Therefore, ideally, additional population samples of kingfish should be tested to clarify the genetic structure of kingfish from geographically intermediate areas.

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