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Fixed allele frequency differences among Palauan and Okinawan populations of the damselfishes *Chrysiptera cyanea* and *Pomacentrus coelestis*

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Abstract. A survey of intraspecific allozymic variation among samples of the damselfishes *Chrysiptera cyanea* and *Pomacentrus coelestis* collected from Palau and Okinawa revealed levels of genetic divergence far in excess of estimates reported previously for populations of coral reef fishes. Absolute or nearly fixed differences in allele frequencies were detected at *ADA**, *sMDH-2**, *MEP-1**, *PEPB**, *PEP-LT** and *sSOD**, and at *sAAT**, *GPI-A**, *LDH-1**, and *PEPB**, among the geographic samples of *C. cyanea* and *P. coelestis*, respectively. Examination of allele frequencies at most other loci (26 for *C. cyanea*; 24 for *P. coelestis*) revealed slight differentiation or identical fixation among the geographic samples. The observed patterns of allele frequency distribution suggest the existence of localized demes of these fishes: these demes may be cryptic and/or incipient species. Whether or not speciation is incipient, the observed patterns of allozymic and isozymic variability suggest that natural selection is a factor in the maintenance of population substructuring of the study species. Pronounced allelic differences were highly concentrated at a small number of loci: strongly bimodal frequency distributions of loci relative to their genetic identities (*I*) were observed for among-population comparisons in both study species. Allozymes encoded by the *PEPB** locus in samples of the noncongeners from Palau exhibited identical electrophoretic mobilities. In Palauan *P. coelestis*, *mSOD** is expressed but *sSOD** apparently is not, whereas in Okinawan *P. coelestis*, both *mSOD** and *sSOD** are expressed.

Introduction

Within the coral reef fish family Pomacentridae, examples of species known to exhibit color polymorphism abound (Allen 1975, 1991). At one extreme, variation may be evident among relatively distant populations of a species, as in the case of the spiny chromis *Acanthochromis polyacanthus* from the Great Barrier Reef and other localities in the Indo-western Pacific, which exhibits substantial differences in relative amounts and localization of black and white body coloration (Allen 1975). At the other extreme, variation may be exhibited by adult conspecifics occupying territories within a few meters of one another on the same reef, as in the case of the surge demoiselle *Chrysiptera leucopoma*, which may be yellow-bodied and blue dorsally, drab gray with a white midbody dorsoventral line (the "*amibilis*" phase), or intermediate (yellow-gray with a slight hint of the dorsoventral line) (Allen 1975, 1991). In the latter example, ecological and physiological factors contributing to the color polymorphism remain to be elucidated, but the color variation cannot be attributed to population genetic substructuring: a yellow-phase *C. leucopoma* will fade into the gray phase after a single week of confinement in an aquarium (own personal observations). In the former example, the absence of a pelagic larval stage in *A. polyacanthus* (Robertson 1973) suggests that the species may have relatively poor long-range dispersal capability; hence, geographically isolated subpopulations may have been less constrained by the homogenizing effect of gene flow from evolving into various morphotypes [Allen 1975; see also Ehrlich's (1975) comments on M. Soule's unpublished allozymic data for *A. polyacanthus*].

Only the work of Bell et al. (1982) has carefully addressed the issue of whether intraspecific morphologic variation correlates with levels of genetic substructuring of damselfish populations. Bell et al.'s survey of Clark's anemonefish, *Amphiprion clarkii*, sampled along a southwest–northeast transect (spanning ≈1600 km) corresponding roughly to the Pacific coastlines of major islands in the Japanese Archipelago (Okinawa, Kakeroma, Kagoshima, Sukumo, Miyakejima, and the geographically isolated Bonin Islands), indicated that size and body darkness increase with increasing latitude. A concomitant electrophoretic survey revealed levels of genetic substructuring sufficient for those authors to suggest that, despite the likely dispersive effect of the Kurushio Current, gene flow within the archipelago was low and no patterns of clinal variation were evident. Bell et al. concluded that no evidence was provided for parallel trends in patterns of morphologic and genetic variation.

The present paper examines the population genetics of the blue devil *Chrysiptera cyanea*, which exhibits conspicuous morphologic variation at the northernmost outskirt of its range, and of the neon damsel, *Pomacentrus coelestis*, which does not. Throughout most of its range in the western Pacific, *C. cyanea* exhibits sexual dimorphism: purportedly, males have a bright yellow-orange tail and ventral area (usually from lower jaw to anal fin), whereas females and juveniles are solid blue with a small black spot at the base of the hindmost dorsal rays (Allen 1975, Myers 1989). Philippine and Ryukyu Islands populations of *C. cyanea* lack both the yellow coloration and the black spot (Masuda et al. 1984, Myers 1989). *C. cyanea* and *P. coelestis* were collected from Sesoko Island, Okinawa, which represents the northern outskirts of their respective ranges, and from the Republic of Palau. Under the hypothesis that morphologically variable populations represent separate demes, it was predicted that levels of among-sample genetic divergence would be greater for *C. cyanea* than for *P. coelestis*.

Materials and methods

Sample sizes and collection sites for each of the study species were as follows: *Crysiptera cyanea*: Republic of Palau (Short Drop-Off), $n = 27$; Okinawa (Sesoko Island), $n = 19$; *Pomacentrus coelestis*: Republic of Palau (Blue Corner), $n = 20$; Okinawa (Sesoko Isand), $n = 14$. Individuals of each species at each collection site were collected within a roughly circular area (approximate diameter of 10 m). Specimens were stored at −80C until dissected, thawed, measured for standard lenght (SL = tip of snout to caudal peduncle), photographed, sexed (by examination of gonads) and then tagged and refrozen as voucher specimens: a strip of skeletal muscle was removed from each individual. Tissue homogenization, starch gel electrophoresis, preparation of histochemical stains, gel photography, and gel preservation were performed as described by Morizot and Schmidt (1990).

Products of the following loci [given with corresponding fish nomenclatural abbreviation, enzyme commission number (Nomenclature Committee of the International Union of Biochemistry 1984) and electrophoretic buffer (TEB: Tris-EDTA-borate, pH 8.0; TC: Tris-citrate, pH 7.0; NADP: addition of 30 mg nicotinamide adenine dinucleotide phosphate to gel; ME: addition of mercaptoethanol to gel)] used for optimal resolution were resolved, after examination for cryptic variability on both buffer systems, in each of the study species: aconitase [*sAH** (cytosolic) and *mAH** (mitochondrial); 4.2.1.3; TEBME], adenosine deaminase (*ADA**; 3.5.4.4; TEBME), glucose-6-phosphate dehydrogenase [*G6PDH**; 1.1.1.49; TEB-NADPME (not resolved in *Pomacentrus coelestis*)], glucose-6-phosphate isomerase (*GPI-A** and *GPI-B**; 5.3.1.9; TCME), aspartate aminotransferase [*sAAT** (cytosolic) and *mAAT** (mitochondrial); 2.6.11; TEB], isocitrate dehydrogenase (*IDHP-1** and *IDHP-2**; 1.1.1.42; TCME), malate dehydrogenase (*sMDH-1** and *sMDH-2**; 1.1.1.37; TCME), malic enzyme (*MEP-1** and *MEP-2**; 1.1.1.40; TEBME), mannose-6-phosphate isomerase (*MPI**; 5.3.1.8; TCME), unidentified muscle proteins (*PROT-1**, *PROT-2**, and *PROT-3**; TEBME), peptidase [*PEPB** (leucine-glycine-glycine); 3.4.11; TCME], peptidase [*PEP-LT** (leucine-tyrosine); 3.4.11; TEBME], phosphoglucomutase (*PGM**; 5.4.2.2; TCME), and superoxide dismutase (*mSOD** and *sSOD**; 1.15.1.1.; *mSOD** better on TCME and *sSOD** on either TEBME or TC). In the present study, three distinct zones of lactate dehydrogenase activity in skeletal muscle tissue (presumably *LDH-A**) are referred to as *LDH-1**, *LDH-2**, and *LDH-3** (1.1.1.27; TCME). Allozymic banding patterns consistent with simple models of Mendelian inheritance and the subunit structure of the enzyme were recorded as genotypes. Locus designations follow established nomenclature for fish genes

Table 1. *Chrysiptera cyanea*. Allele frequencies and single-locus genetic identities (I), fixation indices (F_{ST}) and inbreeding coefficients (F_{IS}) for polymorphic loci in samples collected from Palau and Okinawa. Numbers in parentheses show number of individuals genotyped for each locus

Locus and allele	Palau	Okinawa	I	F_{ST}	F_{IS}
*sAAT**	(27)	(19)			
**119*	0.02	0.00	0.995	0.049	0.213
**100*	0.87	1.00			
**78*	0.04	0.00			
**74*	0.07	0.00			
*sAH**	(25)	(18)			
**100*	0.96	1.00	0.999	0.150	−0.031
**89*	0.02	0.00			
**81*	0.02	0.00			
*ADA**	(27)	(19)			
**100*	0.00	1.00	0.000	0.931	−0.038
**86*	0.96	0.00			
**69*	0.04	0.00			
*GPI-A**	(27)	(19)			
**117*	0.02	0.08	0.998	0.020	−0.072
**100*	0.98	0.92			
*GPI-B**	(27)	(19)			
**−161*	0.00	0.24	0.954	0.134	−0.019
**−100*	1.00	0.76			
*sMDH-1**	(27)	(19)			
**112*	0.04	0.00	0.999	0.019	−0.038
**100*	0.96	1.00			
*sMDH-2**	(27)	(19)			
**100*	0.13	1.00	0.148	0.770	−0.149
**84*	0.87	0.00			
*MEP-1**	(26)	(13)			
**112*	0.88	0.04	0.176	0.720	−0.106
**100*	0.12	0.96			
*MEP-2**	(27)	(19)			
**100*	0.98	1.00	0.999	0.009	−0.019
**86*	0.02	0.00			
*MPI**	(27)	(19)			
**100*	1.00	0.97	0.999	0.013	−0.027
**91*	0.00	0.03			
*PEPB**	(27)	(19)			
**100*	0.98	0.00	0.000	0.964	−0.019
**94*	0.00	1.00			
**91*	0.02	0.00			
*PEP-LT**	(25)	(15)			
**107*	0.04	0.00	0.000	0.926	−0.042
**100*	0.96	0.00			
**91*	0.00	1.00			
*PGM**	(27)	(19)			
**122*	0.22	0.00	0.955	0.087	0.030
**116*	0.02	0.00			
**100*	0.72	0.94			
**75*	0.00	0.03			
**72*	0.04	0.03			
*mSOD**	(27)	(19)			
**147*	0.02	0.00	0.999	0.009	−0.019
**100*	0.98	1.00			
*sSOD**	(27)	(19)			
**100*	1.00	0.00	0.000	1.000	–
**63*	0.00	1.00			

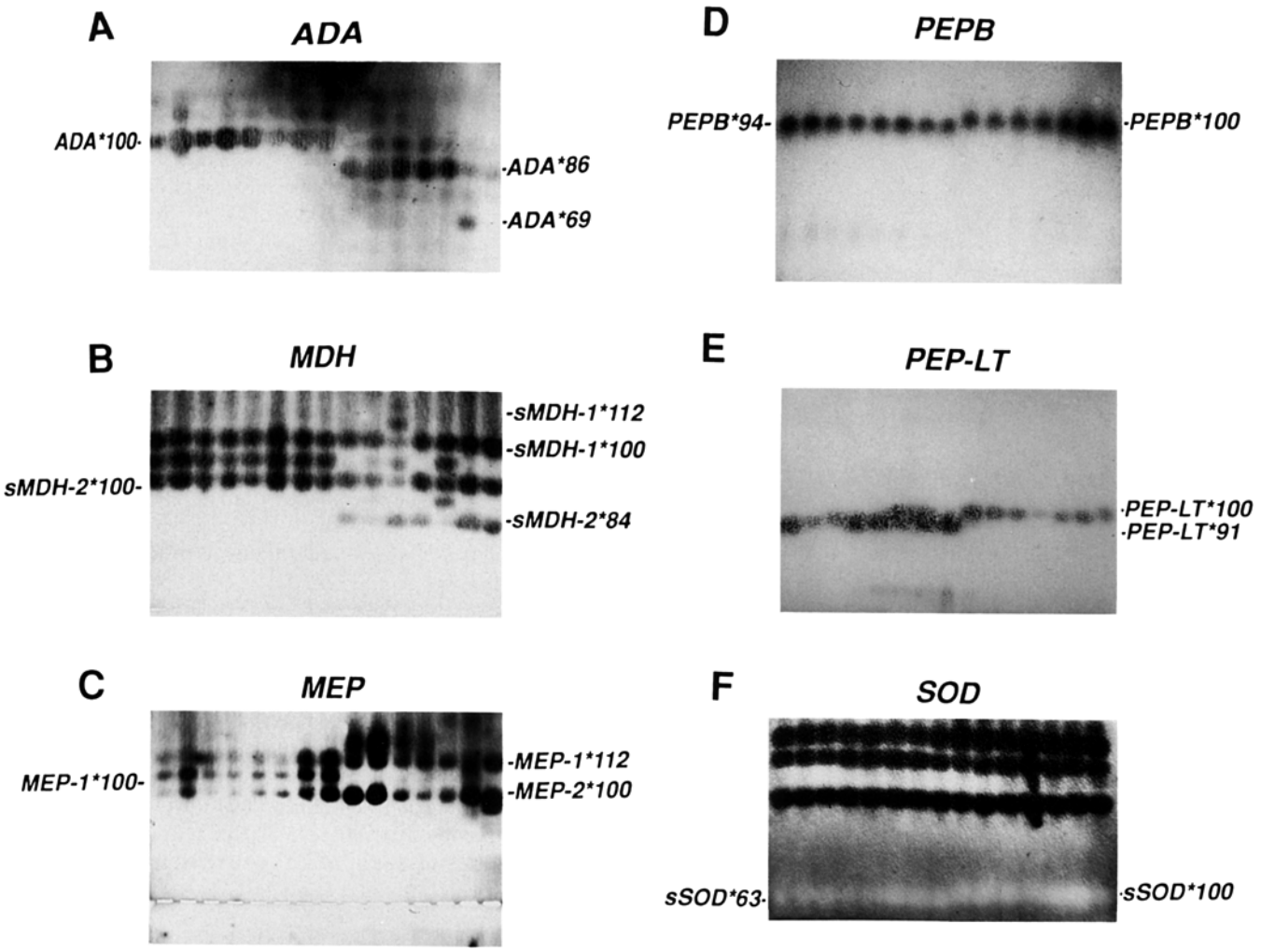

Fig. 1. *Chrysiptera cyanea*. Electrophoretic banding patterns for *ADA* (A), *MDH* (B), *MEP* (C), *PEPB* (D), *PEP-LT* (E) and *SOD* (F) (latter visualized late on a TC pH 7.0 gel stained for lactate dehydrogenase). From left to right, Lanes 1–8 represent individuals from Sesoko Island, Okinawa, Lanes 9–15 individuals from Short Drop-Off Reef, Palau

(Shaklee et al. 1990). For multilocus enzyme systems, loci were assigned numerical designations, with the most anodally migrating isozyme designated "1" and less anodal locus products receiving progressively greater numerical designations.

Loci were considered polymorphic if the frequency of the most common allele was ≤0.99 in either of the samples of a species. Alleles at polymorphic loci were assigned numerical designations expressing the mobility of their respective protein products relative to the mobility of the most common allozyme (designated "100") among samples of each study species. For loci exhibiting alternate fixation of two alleles, the allozyme exhibiting the most anodal mobility was designated "100". Allozyme mobility was determined by measuring the distance separating the loading well and the center of each visualized band of allozymic activity with a vernier caliper. An individual homozygous for the most common allele at each locus was loaded in each gel as a standard for comparison with conspecifics. When the presumptive genotype of a heterozygous individual was in question due to minor allozymic mobility differences, the individual was rerun side-by-side with heterozygous or homozygous individuals whose genotypes had been ascertained.

Genotypic frequencies at polymorphic loci were tested for conformance to Hardy–Weinberg equilibrium (chi-square goodness of fit; Sokal and Rohlf 1969). Expected genotypic ratios were computed from observed allele frequencies using Levene's (1949) unbiased method for small samples. Chi-square tests for conformance to Hardy–Weinberg expectations and calculations of Wright's (1978) fixation indices (F_{ST}) and inbreeding coefficients (F_{IS}) were performed with BIOSYS-1 (Swofford and Selander 1981). Estimates of genetic divergence among samples were obtained by computation of single locus genetic identities (I) and mean genetic distances (D_H) according to the method of Hillis (1984).

Results

Products of the following loci were monomorphic in samples of *Chrysiptera cyanea*: *mAH**, *G6PDH**, *mAAT**, *IDHP-1**, *IDHP-2**, *LDH-1**, *LDH-2**, *LDH-3**, *PROT-1**, *PROT-2**, and *PROT-3**. Presumptive allele frequencies and corresponding genetic identities (I), fixation indices (F_{ST}) and inbreeding coefficients (F_{IS}) for polymorphic loci [57.7% (15 of 26) loci polymorphic at 0.99 criterion] among samples of *C. cyanea* are reported in Table 1. No significant deviations of observed genotypic frequencies from expected Hardy–Weinberg frequencies were detected. Absolute or nearly fixed allele-frequency differences were detected at *ADA**, *sMDH-2**, *MEP-1**, *PEPB**, *PEP-LT** and *sSOD** (Fig. 1). The genetic dis-

Table 2. *Pomacentrus coelestis*. Allele frequencies and single-locus genetic identities (I), fixation indices (F_{ST}) and inbreeding coefficients (F_{IS}) for polymorphic loci in samples collected from Palau and Okinawa. Numbers in parentheses show number of individuals genotyped for each locus

Locus and allele	Palau	Okinawa	I	F_{ST}	F_{IS}
*sAAT**	(20)	(14)			
**100*	1.00	0.00	0.000	1.000	–
**82*	0.00	1.00			
*sAH**	(19)	(13)			
**100*	1.00	0.69	0.908	0.182	0.278
**89*	0.00	0.31			
*ADA**	(20)	(14)			
**106*	0.03	0.00	0.865	0.064	−0.004
**100*	0.64	0.35			
**93*	0.33	0.29			
**79*	0.00	0.25			
**69*	0.00	0.11			
*GPI-A**	(20)	(14)			
**100*	0.95	0.00	0.000	0.909	−0.503
**91*	0.05	0.00			
**83*	0.00	1.00			
*GPI-B**	(20)	(14)			
**–100*	0.97	1.00	0.999	0.013	−0.026
**36*	0.03	0.00			
*LDH-1**	(20)	(14)			
**100*	1.00	0.00	0.000	1.000	–
**99*	0.00	1.00			
*MPI**	(20)	(14)			
**107*	0.03	0.00	0.975	0.060	0.322
**100*	0.94	0.82			
**97*	0.03	0.00			
**84*	0.00	0.18			
*PEPB**	(20)	(14)			
**100*	0.95	0.00	0.000	0.750	0.401
**91*	0.05	0.00			
**83*	0.00	0.89			
**66*	0.00	0.11			
*PGM**	(20)	(14)			
**171*	0.00	0.04	0.999	0.008	−0.032
**100*	0.97	0.96			
**82*	0.03	0.00			
*mSOD**	(20)	(14)			
**100*	1.00	0.96	0.999	0.018	−0.037
**60*	0.00	0.04			

tance (D_H) between Palauan and Okinawan populations of *C. cyanea* was 0.251 (26 loci). The mean fixation index was 0.708 and the mean inbreeding coefficient was −0.014. Inspection of the specimens revealed an absence of yellow ventral and tail coloration in all specimens collected from Sesoko Island, Okinawa. Of the 23 sexually mature individuals (i.e., individuals with mature testes or ovaries) collected from the Short Drop-Off Reef in Palau, 22 males exhibited the dorsocaudal spot (or a vestige of it) and yellow coloration of the tail and ventral area: the single mature female exhibited the dorsocaudal spot and lacked the yellow ventral coloration. Five of the 19 individuals collected from Sesoko Island exhibited vestiges of a small dorsocaudal spot; no individuals from Sesoko exhibited yellow coloration.

Products of the following loci were monomorphic in samples of *Pomacentrus coelestis*: *mAH**, *mAAT**, *IDHP-1**, *IDHP-2**, *LDH-2**, *LDH-3**, *sMDH-1**, *sMDH-2**, *MEP-1**, *MEP-2**, *PROT-1**, *PROT-2**, *PROT-3**, and *PEP-LT**. Presumptive allele frequencies and corresponding genetic identities (I), fixation indices (F_{ST}), and inbreeding coefficients (F_{IS}) for polymorphic loci [44% (11 of 25) loci polymorphic at 0.99 criterion] among samples of *P. coelestis* are reported in Table 2. Significant deviations of observed genotypic frequencies from expected frequencies were detected at *MPI** in the Palauan sample ($\chi^2 = 39.03, df = 3, p = 0.000$) and PEPB* in the Okinawan sample ($\chi^2 = 8.32, df = 1, p = 0.004$). Absolute or nearly fixed allele-frequency differences were detected at *sAAT**, *GPI-A**, *LDH-1**, and *PEPB** (Fig. 2). The genetic distance (D_H) between Palauan and Okinawan populations of P. coelestis was 0.195 (24 loci). The mean fixation index was 0.605 and the mean inbreeding coefficient was 0.130.

In addition to the detection of fixed differences at several loci among populations of both study species, three findings are noteworthy. Allozymes encoded by the *PEPB** locus in samples of *Chrysiptera cyanea* and *Pomacentrus coelestis* from Palau exhibited identical electrophoretic mobilities. In Palauan *P. coelestis*, *mSOD** is expressed but *sSOD** is apparently not, whereas in Okinawan *P. coelestis*, both *mSOD** and *sSOD** are expressed. Bimodal frequency distributions of loci relative to their genetic identities (I) were observed for among-population comparisons in both study species.

Discussion

The salient results of this study are two-fold: (1) levels of genetic divergence among geographic populations of both *Chrysiptera cyanea* and *Pomacentrus coelestis* are greater than levels documented for confamilials studied to date; (2) in both study species, population genetic substructuring is evident from pronounced allele frequency differences concentrated at a few loci. Latitudinal differences (≃ 19° latitude separate Palau and Okinawa) in collection localities for the species in the present study far exceed among-sample distances utilized in previous studies of damselfishes, which revealed substantially lower levels of genetic subpopulation differentiation. The geographic breadth of sample pairwise comparisons utilized by Vawter et al. (1980) for Atlantic damselfishes and Bell et al. (1982) and Shaklee (1984) for Pacific damselfishes did not exceed ≃ 3000 km (either east to west or north to south; one exception to this is the Atlantic side of Panama vs Bermuda comparison for *Abudefduf saxatilis* performed by Vawter et al. 1980). Hence, the magnitude of intraspecific genetic differences reported for *C. cyanea* and *P. coelestis* is perhaps not surprising.

However, the documentation of striking differences at few loci coupled with virtual genetic homogeneity at most other loci is unprecedented for intraspecific surveys of damselfishes. Only the unpublished work of M. Soule (as

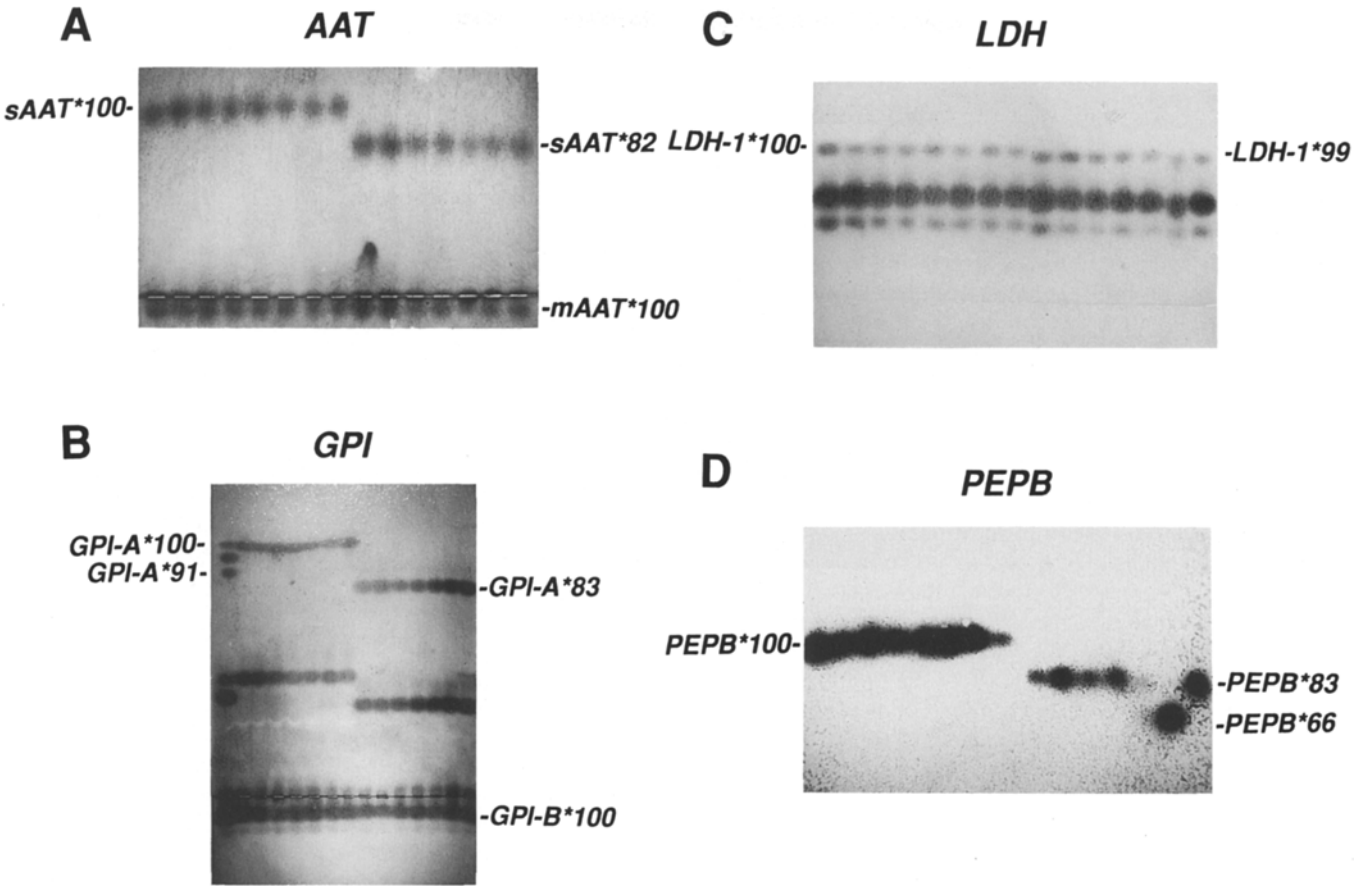

Fig. 2. *Pomacentrus coelestis*. Electrophoretic banding patterns for *AAT* (A), *GPI* (B), *LDH* (C), and *PEPB* (D). From left to right, Lanes 1–8 represent individuals from Short Drop-Off Reef, Palau, Lanes 9–15 individuals from Sesoko Island, Okinawa

cited in Ehrlich 1975) has reported similar levels of genetic divergence among populations of *Acanthochromis polyacanthus* scattered along the Great Barrier Reef. Several authors (Ehrlich 1975, Shaklee 1984, Thresher et al. 1989) have alluded to Soule's results and concurred that they are consistent with expectations for populations among which limited gene flow occurs, possibly as a consequence of the poor dispersal capability, i.e., lack of a planktonic larval stage, of *A. polyacanthus* (Robertson 1973). Based on studies of otolith pretransition-increment counts, estimates for planktonic larval duration within the family Pomacentridae range from 0.0 d (*A. polyacanthus*) to 37.4 d (*Stegastes fasciolatus*): estimates for *Chrysiptera cyanea* and *Pomacentrus coelestis* are 17.4 d and 19.5 d, respectively (Thresher et al. 1989). Interestingly, Thresher et al. have suggested that a planktonic duration of 15 to 20 d may be a threshold below which pomacentrid fishes have numerous local populations and above which little or no population substructuring seems evident. Those authors have also suggested that *C. cyanea* is morphologically divisible into six geographic subpopulations, whereas *P. coelestis* is divisible into four.

Detection of absolute or nearly fixed differences in gene frequencies among operational taxonomic units of sympatric marine fish is generally acknowledged to indicate species identity (reviewed by Shaklee et al. 1982). For example, Smith and Robertson (1981) concluded that deep- and slender-bodied forms of the New Zealand sprat *Sprattus antipodum* are separate species on the basis of two absolute and three nearly fixed differences (out of 13 loci) between the two types. Waples (1981) confirmed the presence of three lizardfishes in the genus *Sauridia* (*S. gracilis*, *S. nebulosa*, and *S. flamma*) from Hawaii, where only *S. gracilis* has previously been recognized: fixed differences were detected between pairs of species at 10 or more of the 29 loci examined. Shaklee and Tamaru (1981) distinguished Hawaiian bonefishes of the genus *Albula* as either *A. neoguinaica* or *A. glossodonta* on the basis of fixed differences at 58 of 84 loci. Gorman and Kim (1977) detected absolute differences at 7 of 28 enzyme loci between the geminate damselfish species *Abudefduf troschelii* and *A. saxatilis* [Pacific vs Atlantic sides of Panamá (in allopatry)]. Most recently, Lacson and Bassler (1992) examined the biochemical systematics of three damselfishes of the genus *Pomacentrus* and four of the genus *Stegastes* and found fixed differences at 3 of 14 loci between the closely related species *S. nigricans* and *S. albifasciatus*. In the light of these studies, the present data indicate the presence of genetically distinct demes of *Chrysiptera cyanea* and *Pomacentrus coelestis*. However, it is acknowledged that thorough tests of speciation must include sampling from one or more sites where both biochemical

types of each study species may occur in sympatry: tests in allopatry are acknowledged to be weaker because of the potential for geographic and/or temporal variation in allele frequency distributions (reviewed by Shaklee et al. 1982).

A parallel explanation for the observed patterns of allelic variability among the geographic samples of *Chrysiptera cyanea* and *Pomacentrus coelestis* is that the demes are incipient species and genetic substructuring is being maintained by natural selection. It is widely accepted that limited gene flow and concomitant genetic drift in potentially subdivided populations affects all loci (reviewed by Ayala 1974). This is clearly not the pattern evinced by the strongly bimodal frequency distribution of loci relative to genetic identities among geographic samples of the species in the present study (Tables 1 and 2). A plausible scenario is that certain allozyme genotypes are favored at certain latitudes (the Japanese Archipelago represents the northernmost outskirts of both species' ranges) where environmental parameters, such as temperature regimes, vary. Biochemical evidence for this scenario is: (1) *C. cyanea* and *P. coelestis* are subdivided intraspecifically with respect to allele frequencies at *PEPB**, yet Palauan populations of both species (which are not congeners) are fixed for the same presumptive allele; (2) in Palauan *P. coelestis*, *mSOD** is expressed but *sSOD** apparently is not, whereas in Okinawan *P. coelestis*, both *mSOD** and *sSOD** are expressed. In other words, patterns of variability at certrain loci such as *PEPB** and *sSOD** are similar in noncongeners (note the fixed difference at *sSOD** in *C. cyanea*). Ultimately, verification of a model incorporating selection requires evidence that certain allozyme genotypes at loci such as *PEPB** and *sSOD** are indeed more fit in their respective environments (e.g. Koehn et al. 1976, 1980, 1983, Koehn 1978, Vrijenhoek et al. 1992). Additional support for this hypothesis would derive from studies which examine allozymic electrophoretic and functional variability at suspect loci (i.e., those which exhibited fixed or nearly fixed allelic differences in the present study) across a broader range of fish taxa (e.g. Graves et al. 1983) collected from Palau, Okinawa, and one or more sites in between.

In summary, the results of the present study indicate that *Chrysiptera cyanea* and *Pomacentrus coelestis* are each divisible into at least two demes. It is emphasized that unequivocal biochemical tests of cryptic and/or incipient speciation must await identification of localities (if they exist) where representatives of these biochemically-defined demes reside in sympatry. Nonetheless, detection of fixed allelic differences between Micronesian and northwest Pacific populations of additional damselfish species which have "Okinawan" morphs, such as *C. rex* (own personal observations) and *Amphiprion clarkii* (Bell et al. 1982), and additional species which do not, would implicate the Ryukyu Islands as a focal point for studies of incipient speciation. A more pressing issue, it seems, is whether selection for allozyme genotypes plays more of a role in reef-fish speciation than is commonly thought.

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