

## Morphological and Molecular Analyses Reveal Separations among Spatiotemporal Populations of Anchovy (*Engraulis japonicus*) in the Southern East China Sea

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**Chih-Shin Chen, Chih-Hsiang Tzeng, and Tai-Sheng Chiu (2010)** Morphological and molecular analyses reveal separations among spatiotemporal populations of anchovy (*Engraulis japonicus*) in the southern East China Sea. *Zoological Studies* 49(2): 270-282. Coastal and oceanic populations of Japanese anchovy (*Engraulis japonicus*) occur in the southern East China Sea. The distributions of the larval stages of these populations provide a bases for subdividing anchovy into 3 spatiotemporal stocks for the management of local Taiwanese fisheries: spring coastal (sprW) and oceanic (sprE) stocks respectively in the western and eastern seas of Taiwan, and an autumn stock (autE) in Ilan Bay, northeastern Taiwan. Using larval morphological analyses to evaluate population similarities, we found subtle differences between the sprE and sprW geographic stocks, but strong intra-geographic differences between sprE and autE seasonal stocks. Molecular analyses, based on fragments of 740 bp (positions 200-939) of the mitochondrial DNA cytochrome *b* gene, revealed a substantial amount of genetic variations among populations. Analysis of molecular variance indicated significant differences among stocks, primarily due to the sympatric contrast between sprE and autE ( $F_{ST} = 0.073$ ,  $p < 0.001$ ), rather than due to geographic subdivisions, such as sprE vs. sprW ( $F_{ST} = 0.039$ ) or sprW vs. autE ( $F_{ST} = 0.007$ ). These results indicate the presence of a unique oceanic stock in eastern Taiwan in spring that is separated from the other populations. This spatiotemporal heterogeneity suggests a dynamic state of larval recruitment along the coast of northern Taiwan. We propose a dispersal route for larval anchovy from the Taiwan Strait to the northern coast, or active migration by adult anchovies in accordance with oceanographic patterns in the southern East China Sea. <http://zoolestud.sinica.edu.tw/Journals/49.2/270.pdf>

**Key words:** Anchovy, Morphometry, Mitochondrial DNA, Stock, Cohort migration.

Japanese anchovy (*Engraulis japonicus* Temminck and Schegell) is a small pelagic fish, commonly occurring in subtropical to temperate waters of the Indo-Pacific. This species is an important indicator of ecosystem changes in the western North Pacific (Takasuka et al. 2007 2008), and supports commercially important fisheries in China, Japan, Korea, and Taiwan (Chiu et al. 1997, Aoki and Miyashida 2000, Kim et al. 2005, Zhao et al. 2008). In the East China Sea (ECS), coastal populations of anchovy are distributed along the continental coast from Korea to southeastern

China, and an oceanic population occurs on the mid-shelf of the ECS near the boundary of the Kuroshio Current (compiled from Takeshita and Tsukahara 1972, Chen 1991, Chiu et al. 1997). The southern boundary of spawning migration for the anchovy may be in waters off northern Taiwan (Young and Chiu 1994), and thus several fishing grounds for larval anchovy in these regions have been developed since the 1970s (Yu and Chiu 1994). There are 2 geographic populations of larval anchovy in spring: one in the Taiwan Strait (coastal stock, on the western side of Taiwan) and

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the other on the Pacific (eastern) side of Taiwan (oceanic stock; Chiu et al. 1997, Yu et al. 2002). The coastal population might migrate along the Chinese coast and cross the Strait (Lan et al. 2008), and the oceanic one may migrate along the outer shelf from the ECS to Ilan Bay (Chen and Chiu 2003, Lee et al. 2009). An oceanic front lasting throughout the winter is located in northern Taiwan and may act as a barrier between the 2 populations (Wang and Chern 1990, Jan et al. 2002). However, the front recedes in autumn, and only 1 smaller population is found in Ilan Bay (Chiu and Chen 2001).

Anchovy larvae recruit into the waters around northern Taiwan at ages of < 2 mo (Chen and Chiu 2003), and became the target of fisheries (Huang and Chiu 1996). No data are available on the seasonal distribution of anchovy eggs; however, a compilation of ichthyoplankton surveys indicated that larvae are abundant in the waters off northern Taiwan during spring months of Apr.-May and autumn months of Aug.-Oct. (Chiu et al. 1997). During winter and summer, larvae are sparsely located and confined to near shore waters. Although these larval fish are difficult to identify, fishermen attempt to separate the various species of fish larvae to as great an extent as possible because the market value of the harvest depends on the species composition, the dominant species of which change on spatial and temporal scales. Annual catches of larval anchovy vary largely due to fluctuations in recruitment population sizes and shifts in environmental variables (Hsieh et al. 2009).

These fisheries are regulated by such measures as limited entry, seasonal closure, a minimal catch size, and total allowable catches. Basic information on the biology and ecology of anchovy is used to facilitate stringent management, including species composition of the catch (Chiu et al. 1997), age, growth (Chiu and Chen 2001), reproduction (Young and Chiu 1994), recruitment processes (Chen and Chiu 2003), population genetic structure (Yu et al. 2002), the shape of the fishing ground (Lee et al. 1995), and mechanisms affecting abundance (Tsai et al. 1996, Hsieh et al. 2009). However, applying a single regulatory measure island-wide is still problematic, because of dissimilar stocks, as evidenced by various fishing seasons and by the development of customized fishing gear at different localities (Yu and Chiu 1994). In spring, anchovy are apparently subdivided into 2 geographic stocks, one in the

Taiwan Strait (coastal, to the west of Taiwan) and the other in the Pacific Ocean (oceanic, to the east of Taiwan), and these show limited inter-stock gene flow (Yu et al. 2002). Within these geographic subdivisions, the spring and autumn cohorts were found to have distinct demographic characteristics (Chiu and Chen 2001).

To provide basic information to support the management of sustainable fisheries, we tested for differences among spatiotemporally subdivided populations in the southern ECS, and estimated their demographic histories. Two methods were used: 1) a study of morphological differences among stocks, an approach that was broadly used for stock identification before population markers became available, and 2) an examination of molecular markers, which are often recently used to identify species and to infer population structures (e.g., Kocher et al. 1989, Liu et al. 2006, Magoulas et al. 2006, Tzeng et al. 2007). To provide information for fisheries management and to determine whether current regulations are adequate, we determined the genetic basis of the population structure of larval anchovy in the southern ECS.

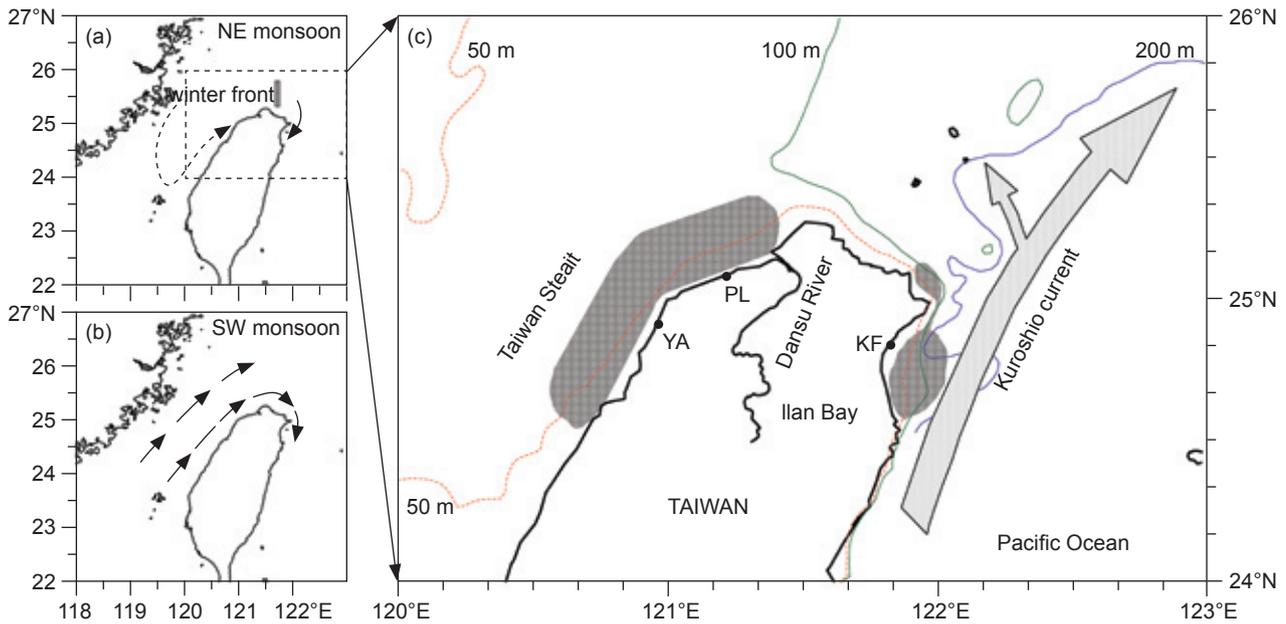
## MATERIALS AND METHODS

### Samples

In spring 2001, 7 samples of larval anchovy were collected at the peak of recruitment along the coasts of Taiwan (Table 1). In autumn, 6 additional samples were collected in Ilan Bay, where a particularly dense population had formed, to compare cohorts (i.e., intra-geographic comparison of spring and autumn larvae). Since no larval anchovy were found in the Taiwan Strait during autumn, we could not make a comparison between cohorts in that area. Samples were categorized into 3 groups: a spring oceanic stock (sprE), a spring coastal stock (sprW), and an autumn Ilan stock (autE). Samples from eastern Taiwan were obtained from catches of paired trawlers, and those from the Taiwan Strait were from drift-bag netters (Fig. 1). Fish were put on ice by the fishermen immediately after harvest, and were brought back to port for market auction. We sampled larval anchovy directly at the various wharfs, brought the samples back to the laboratory within 2 h, and preserved them in 90% ethanol until they could be identified to species. Fifty individuals, likely to be engraulids were selected from each batch

of samples, but only specimens confirmed to be Japanese anchovy (*Engraulis japonicus*) were ultimately analyzed. The use of larvae to represent a fish population may bias a sample because a

sample may include individuals of the same brood. However, our samples were likely to be random because several catches were mixed at sea in a large container, and the landings for each sea-trip



**Fig. 1.** Maps showing sampling localities and traditional fishing grounds (hatched areas, in c) for Japanese anchovy, and the prevailing monsoons during the sampling period (a, b). PL and YA are located in the Taiwan Strait, and KF is in the northwestern Pacific Ocean. Broken arrows in (a) indicate possible migration routes, and solid arrows in (b) indicate water movements driven by the southwesterly monsoon wind.

**Table 1.** Information on sampling localities, dates, and successful sample cases used in the morphological and genetic analyses of the Japanese anchovy

Category	Code	Sampling date	Sample size	SL range	Morphometry	Cytochrome <i>b</i>	Condition factor (K)
Spring, eastern Taiwan	sprE	1 Apr. 2001	28	24.9-30.9	17	22	0.323-0.524
		14 May 2001	28	24.0-32.0	26	18	0.333-0.575
		23 May 2001	38	25.0-31.1	15	4	0.373-0.571
Spring, Taiwan Strait	sprW	13 Mar. 2001	24	25.1-36.9	24	9	0.309-0.502
		3 Apr. 2001	27	28.8-34.8	21	7	0.349-0.554
		16 May 2001	32	25.4-32.2	32	19	0.401-0.561
		24 May 2001	19	24.1-29.8	19	9	0.328-0.523
Autumn, eastern Taiwan	autE	8 Aug. 2001	7	25.8-28.8	7	0	0.441-0.562
		17 Aug. 2001	29	19.7-31.5	27	9	0.336-0.668
		25 Aug. 2001	44	24.3-32.7	27	12	0.321-0.542
		1 Sept. 2001	28	22.8-30.3	26	13	0.389-0.569
		2 Sept. 2001	11	24.0-29.0	4	4	0.383-0.569
		15 Oct. 2001	38	23.6-30.2	34	7	0.462-0.551
Sum			353		279	133	

were large during the fishing season. In this study, we assumed that the cytochrome (Cyt) *b* gene was selectively neutral within each age group.

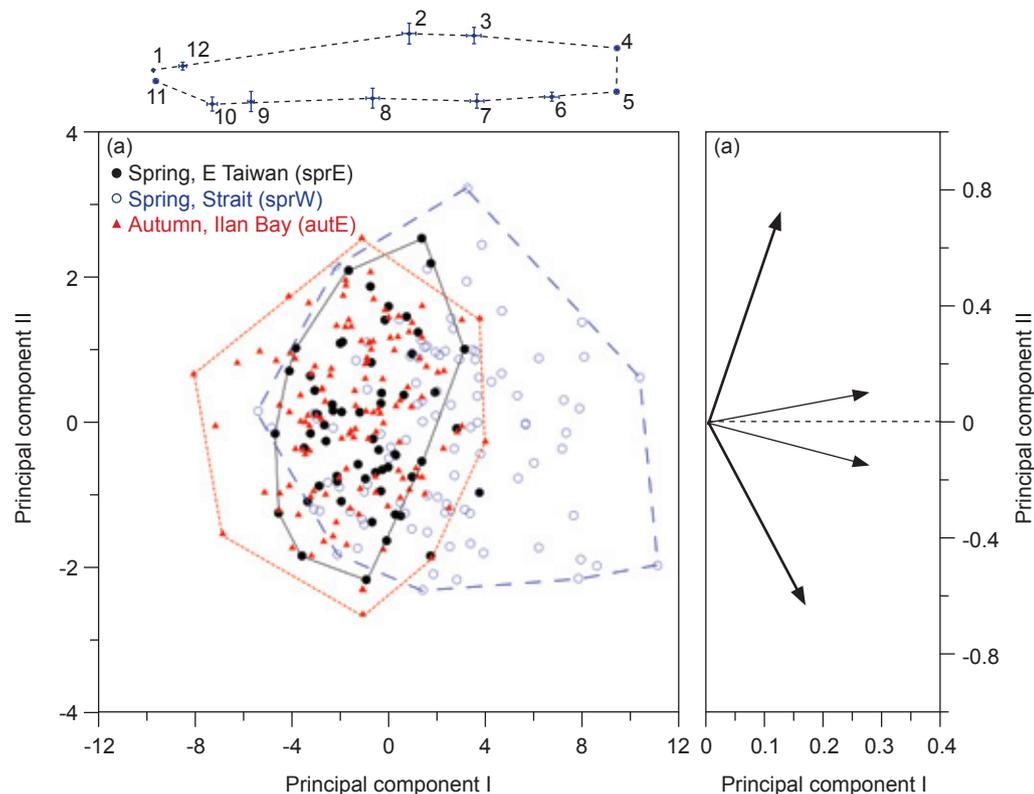
### Morphometric characters

Twelve external characters were measured on images of specimens using a digitizer (Mm1202 Data Tablets, Summagraphics, Fairfield, CT, USA) (see Fig. 2 upper inserts). To standardize the body size of each fish, the coordinates of the 12 body positions taken with a digitizer were adjusted by isometric dilation or expansion to make the standard length of each fish 25 mm, i.e., the length from point 1 to the midpoint of points 4 and 5 equal to 25 mm (see inserts in Fig. 2). The centroid of the 12 landmarks was calculated, and the following morphometric variables were taken on a series of measurements from the centroid to 1) the anterior point of the head, 2) the 1st insertion of the dorsal fin, 3) the last insertion of the dorsal fin, 4) the upper peduncle, 5) the lower peduncle, 6) the last insertion of the anal fin, 7) the 1st insertion of the anal fin, 8) the 1st insertion of the ventral fin, 9) the

lower tip of the suboperculum, 10) the tip of the angular bone, 11) the anterior of the submaxillary bone, and 12) the center of the left eye ball. Body mass (*W*) was measured to the nearest 0.001 g on an electronic balance, and standard length (SL) was measured to the nearest 0.1 mm with electronic vernier calipers.

### Mitochondrial Cyt *b* gene

Total DNA was extracted from a whole larva or a 20 mg muscle sample of a juvenile, digested by proteinase K overnight, and subjected to a standard phenol-chloroform procedure as described by Palumbi (1996). The concentration of the extracted DNA samples was estimated by ultraviolet spectrophotometry at 260/280 nm, then diluted to 100 ng/μl, and stored at -20°C until further treatments. A polymerase chain reaction (PCR) was employed to amplify a segment of 740 base pairs (bp) of the mitochondrial (mt)DNA Cyt *b*. Primers were designed according Kocher et al. (1989) and Inoue et al. (2001), and named Mcyt-*b* F: 5'-CCACCGTTGTTATCCAACCTAT-3',



**Fig. 2.** (A) Scatterplot for individual anchovy on the 1st 2 principal components; solid circles and line: spring Ilan Bay; open circles and dashed line: spring Strait; and solid triangles and dotted line: autumn Ilan Bay. (B) Loadings on the 1st 2 principal components of size-standardized morphometric traits measured on 279 Japanese anchovies. Upper insert: 12 body positions digitized for morphological analysis. The horizontal and vertical bars of each point indicate standard errors measured along the x- and y-axes, respectively.

and *Mcyt-b* R: 5'-GCTTTAAAACACTAAGC TACT-3'. PCRs were carried out in a total volume of 25  $\mu$ l of a solution containing 1  $\mu$ l of the DNA template (at a final concentration 4 ng/ $\mu$ l), 2.5  $\mu$ l 10x PCR buffer, 0.7  $\mu$ l  $Mg^{+2}$  (1 mM), 1  $\mu$ l *Mcyt b* F (0.4  $\mu$ M), 1  $\mu$ l *Mcyt b* R (0.4  $\mu$ M), 0.5 U Taq polymerase, and dNTP/1  $\mu$ l (2.5  $\mu$ M). Thermocycling was carried out in a PCR Express system (Thermo Hybaid, Middlesex, UK). The following PCR conditions were used: a denaturation step of 94°C for 5 min, followed by 45 cycles of 94°C for 30 s, 45°C for 30 s, and 72°C for 45 s; followed by a final extension at 72°C for 10 min. The reaction products of the PCR were analyzed on 7% polyacrylamide gels in TBE buffer, and were sequenced on an ABI 3730 sequencer (MegaBACE, Ramsey, MN, USA). The obtained sequence was compared to a sequence of Inoue et al. (2001) (NCBI accession no. AB040676), using DAMBE software (Xia and Xia 2001). All sequences were truncated to a segment of 740 bp equivalent to positions 200-939 of the *Cyt b* gene in AB040676. Sequences of *Cyt b* were deposited in GenBank (accession nos.: AY786295-7).

### Data analysis

We used log-transformed morphometric data to perform the principal component (PCA) and discriminant function analyses. Scatterplots of scores of each fish for the 1st 2 principal components were used to illustrate group differences. Standard classification rules were applied to construct a confusion matrix, indicating correct and incorrect classifications. Fulton's condition factor (*K*) was estimated from the body mass (*W*) and standard length (*SL*),  $K = 100 W/SL^3$ . An analysis of variance (ANOVA) was used to identify group differences. For *Cyt b*, the number of polymorphic sites (*S*), gene diversity (haplotype, *h*), nucleoside composition, transition/transversion rates (%), nucleotide diversity ( $\pi$ ), and mean number of pairwise differences (*D*) were calculated to estimate the genetic variability. The program MODELTEST (vers. 3.7; Posada and Buckley 2004.) was used to determine the best fit of the sequences to a mutation model. Population genetic differences of Japanese anchovy were detected by a hierarchical analysis of molecular variance (AMOVA) calculated by Arlequin vers. 2.000 (Schneider et al. 2000), in which temporal comparisons were first performed, and subsequently spatial differences were

calculated. The total variance was partitioned into interindividual and interpopulational differences, and anchovy populations differentiated into 2 geographic populations and 2 intra-geographic cohorts were examined by pairwise co-ancestry coefficients ( $F_{ST}$ ), and the corrected average differences  $D_{xy} = d_{xy} - (d_x + d_y)/2$ , where  $d_{xy}$  is the difference between populations, and  $d_x$  and  $d_y$  are differences within the respective populations (Weir and Cockerham 1984). Phylogenetic relationships among haplotypes were analyzed using the Neighbor-joining (NJ) method (Saitou and Nei 1987). Nucleotide mismatched distributions of intrapopulational pairwise comparisons of pairwise *Cyt b* haplotypes were compared with the expectation of a sudden-expansion model (Rogers and Harpending 1992). By assuming a large population size, DNASP (vers. 3.51, Rozas and Rozas 1997) was used to infer the demographic history, including the initial population size ( $\theta$ ) and timing ( $\tau$ ) of the beginning of the population expansion. MIGRATE software (Beerli and Felsenstein 2001) was used to estimate the number of migrants per generation (*Nm*) between populations.

## RESULTS

### Morphological variations

The 1st 2 principal components explained 91.9% (81.6% and 10.3%, respectively) of the total variance, indicating that the size was the most significant factor, as interpreted by Humphries et al. (1981). The 3rd component represented only 2.7% of the total variance, and hence the scatterplot of the 1st 2 principal components was used to illustrate individual differences (Fig. 2). The between-group variance in size indicated that larger larval anchovy were found in spring in the Taiwan Strait (sprW) compared to the other populations (sprE and autE), as indicated by larger scores on the 1st principal component. Further analysis indicated minor but significant morphological variations attributed to shape differences on the landmark pairs of (2, 8) and (3, 7). This implies that group contrasts were centered on the fish trunk area, and indicates that body height might be informative for group discrimination in addition to body size. However in comparisons of pairs of groups, such subtle variations led to considerable overlap, and the discriminant function could only correctly assign

71.68% of individuals (Table 2). Fulton's condition factors ( $K$ ) were  $0.45 \pm 0.015$  for sprE,  $0.46 \pm 0.012$  for sprW, and  $0.48 \pm 0.11$  for autE (see Table 1 for distribution ranges). The difference among groups was significant, as indicated by the ANOVA ( $F_{2, 277} = 8.083$ ,  $p = 0.0004$ ). The multiple-range tests yielded 2 homogeneous groups of sprE/sprW and autE based on 95% confidence intervals.

### Genetic variation

A substantial amount of the Cyt *b* variation was detected among populations (Table 3). An average of 91 (12.2%) polymorphic sites were found in 740 bp, in which transition rates were similar among samples (10.3%-11.2%), while transversion rates differed (1.3%-4.8%). Nucleotide diversity ( $\pi$ ) ranged 0.0130-0.0144. An average of 24 sites (3.2%) were singletons, and a common nucleotide frequency of < 95% was shown at 30 positions. Polymorphic sites involved synonymous substitutions at the 3rd codon, except for positions 889 (1st), 905 (2nd), and 908 (2nd).

Three of the most variable sites were positioned at 330 (G, 55.6% and A, 44.4%), 693 (G, 61.7% and A, 38.3%), and 900 (T, 57.1% and C, 42.9%). At position 900, the nucleotide of sprE was fixed for T, while this position in the sprW and autE sample was commonly C. The gene (haplotype) diversities ( $h$ ) were 0.995 (sprE), 1.00 (sprW), and 0.999 (sprE). The smallest average pairwise difference ( $D$ ) of 9.6 nucleotides was found in sprW; however, similar values of 10.4 and 10.5 were respectively found for sprE and autE.

The AMOVA detected a significant temporal difference between spring (sprE) and autumn (autE) populations in eastern Taiwan ( $F_{ST} = 0.074$ ,  $p < 0.001$ ); however, most of the variation occurred within populations (92.63%, Table 4a). Although spatial comparisons between sprW and sprE revealed 3.99% variation ( $F_{ST} = 0.040$ ,  $p < 0.001$ ; Table 4b), the spatial differences could not be considered since heterogeneity of the eastern Taiwan population was quite high (7.37%). Pairwise differences inferred from  $F_{ST}$  values and the corrected difference in the number of base pairs

**Table 2.** Confusion matrix resulting from the discriminant function analysis based on morphometric data from 3 spatiotemporally separated populations

Class	Percent correct	Predicted classifications		
		sprE	sprW	autE
Spring, eastern Taiwan (sprE)	46.6	27	8	23
Spring, Taiwan Strait (sprW)	69.8	5	67	24
Autumn, eastern Taiwan (autE)	84.8	7	12	106
Total	71.7	39	87	153

**Table 3.** Genetic parameters of Japanese anchovy populations estimated from 740 bp of the mitochondrial cytochrome *b* gene, showing intrapopulational variations

Genetic measure	Spring, eastern Taiwan (sprE)	Spring, Taiwan Strait (sprW)	Autumn, eastern Taiwan (autE)
Number of sequences	44	44	45
Polymorphic sites (S)	102	86	85
Haplotype diversity ( $h$ )	0.995	1.00	0.999
C: T: A: G	27.9: 32.0: 23.2: 17.9	27.0: 31.9: 23.2: 18.0	26.9: 31.8: 23.3: 18.0
Nucleotide diversity ( $\pi$ )	0.0141	0.0130	0.0144
Average difference ( $D$ , bases)	10.4	9.6	10.6
Theta initial	2.314	1.388	1.230
Tau ( $2\mu t$ )	8.093	8.203	9.398
Transitions (%)	10.3	11.1	11.2
Transversions (%)	1.3	2.3	4.8

indicated that the sprE sample significantly differed from the other 2 samples (Table 5). Surprisingly, the genetic distance of sympatric cohorts of spring and autumn (sprE vs. autE,  $F_{ST} = 0.039$  and  $D_{xy} = 0.409$ , both  $p < 0.001$ ) in Ilan Bay was greater than that of samples from allopatric populations in the Taiwan Strait and Ilan Bay in spring (sprE vs. sprW,  $F_{ST} = 0.073$  and  $D_{xy} = 0.836$ , both  $p < 0.001$ ). It is worth noting that the spatiotemporally separated populations (sprW and autE) were distanced with no statistical significance ( $F_{ST} = 0.007$ ,  $p = 0.1261$  and  $D_{xy} = 0.072$ ,  $p = 0.1546$ ). One parsimoniously informative consensus NJ tree was found with bootstrap support for branches ranging 0.02-0.96 (Fig. 3), indicating rather little structuring among populations and complicated sample relationships. However, 2 distinct branches were exclusively formed by samples from autE and sprW without the addition of sprE. Pairwise within-population differences of Cyt *b* well fit a model of sudden

population expansion (Fig. 4). The expected initial population size ( $\theta$ ) of the sprE population was double that of the other sprW and autE populations.

## DISCUSSION

### Morphological differences

The Japanese anchovy is a widely distributed pelagic fish species, and is the target of commercially important fisheries in the western North Pacific. Distinguishable morphological differences are also commonly found between geographically subdivided areas. For instance, using meristic and morphometric analyses, Takeshita and Tsukahara (1972) discerned distinct geographic races of Japanese anchovy in its northern ranges in the Japan Sea and Pacific

**Table 4.** Results of the analysis of molecular variance designed for partitioning of mitochondrial cytochrome *b* gene differences among temporal and spatial populations of the Japanese anchovy

(a) Temporal difference between spring (sprE) and autumn (autE) populations in eastern Taiwan

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Between populations	1	24.815	0.435	7.37
Within a population	87	475.709	5.468	92.63
Total	88	500.709	5.903	
Fixation index ( $F_{ST}$ ):	0.074	( $p = 0.000$ )		

(b) Spatial differences between populations in the Taiwan Strait (sprW) and in eastern Taiwan (sprE) in spring

Source of variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation
Between populations	1	14.692	0.216	3.99
Within a population	86	446.662	5.194	96.01
Total	87	461.354	5.409	
Fixation index ( $F_{ST}$ ):	0.040	( $p = 0.000$ )		

**Table 5.** Pairwise group fixation index ( $F_{ST}$ , above the diagonal) and corrected average difference ( $D_{xy}$ , below the diagonal) of Japanese anchovy populations estimated from cytochrome *b* gene variations

	sprE	sprW	autE
Spring, eastern Taiwan (sprE)	-	0.039*	0.073*
Spring, Taiwan Strait (sprW)	0.409*	-	0.007
Autumn, eastern Taiwan (autE)	0.826*	0.072	-

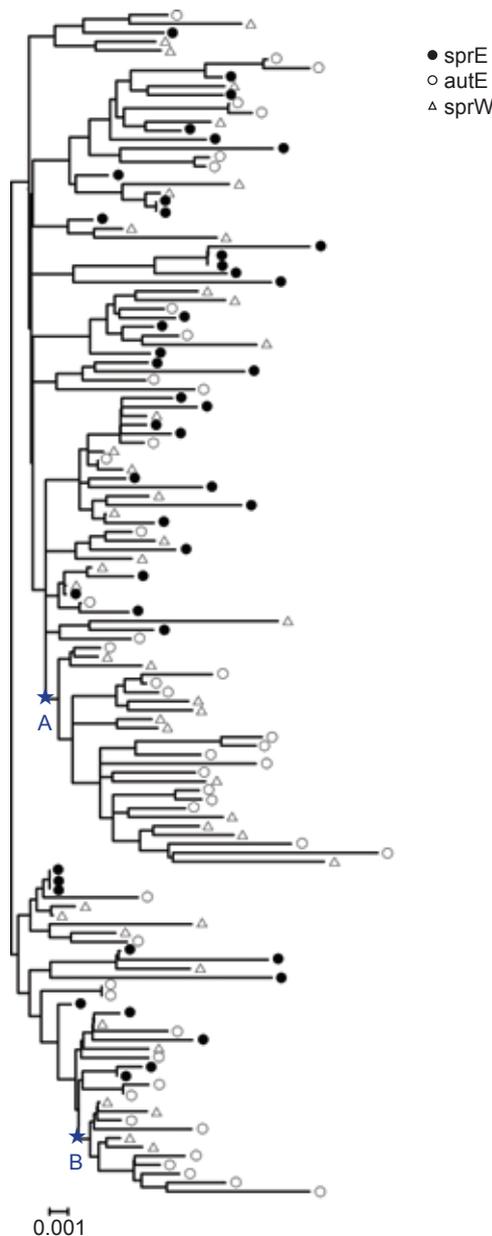
\*Significant at the 1% level;  $D_{xy} = d_{xy} - (d_x + d_y)/2$ , where  $d_x$  and  $d_y$  denote the average numbers of pairwise differences within populations, and  $d_{xy}$  denotes the average number of pairwise difference between populations.

Ocean. In its southern ranges, Young et al. (1994) described 2 color forms: one with a slender black body found mostly during the spring in northeastern Taiwan, and the other with a round pale body in the remaining ranges and seasons, but with no attestation of ecological races or taxonomic subspecies. At the specific locality of Ilan Bay, which opens onto the Pacific Ocean and is seasonally influenced by the Kuroshio Current, the anchovy showed discrete reproductive periods, and its larvae vary in nutritional condition, growth, and survival rates (Huang and Chiu 2000,

Chiu and Chen 2001). There are 3 major fishing grounds with different timings for recruitment of larval anchovy, which thus affects the local fishing seasons. By inspecting larvae with the naked eye, local fishermen can distinguish among anchovies harvested from different regions as well as seasons. In general, the product from the Pacific side has a higher economic value than that from the Strait. Nutrition in the larval stage affects the post-metamorphic survival and phenotype, such as size and shape (Saele et al. 2003). None of these morphological examinations bore out the probability that Japanese anchovy may consist of genetically distinct stocks, which would require different measures for sustainable fisheries. Contrarily, Tudela et al. (1999) documented morphological differences against genetic homogeneity, and suggested that the environment was the main factor shaping phenotypic differences between anchovy populations. However, our morphological findings of spatiotemporal differences among Japanese anchovy populations (Fig. 2) are seemingly genetically based (Fig. 3, Table 5).

#### Variations in the *Cyt b* gene

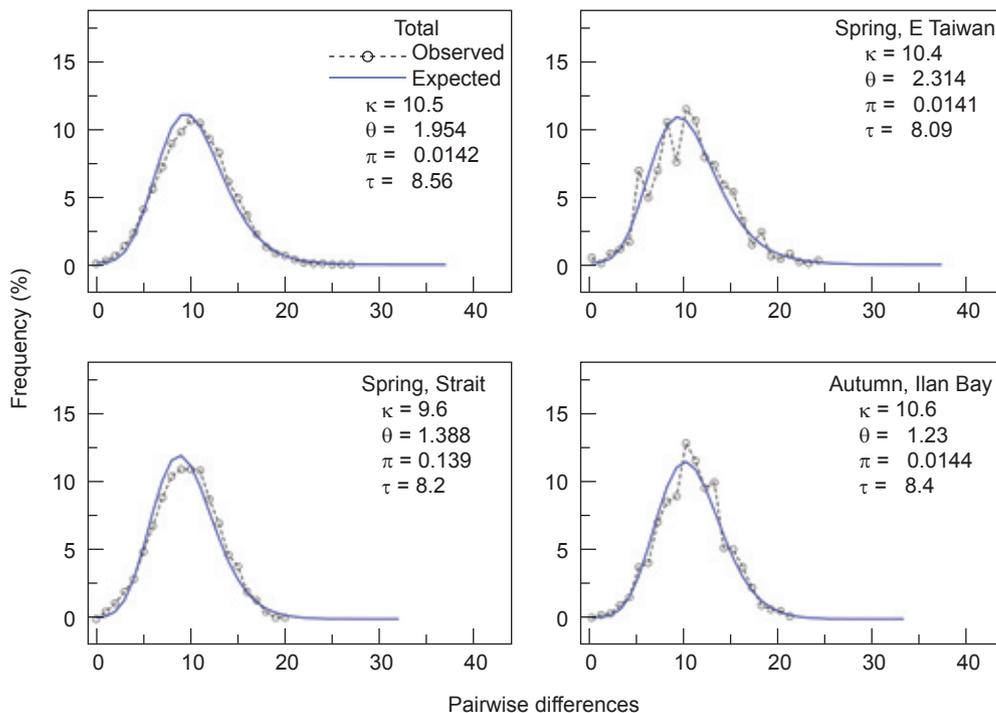
Based on the *Cyt b* gene, both haplotype diversities ( $h$ , 0.995-1.000) and nucleotide diversities ( $\pi$ , 0.013-0.014) were high for Japanese anchovy in the southern ECS (Table 3), compared to northern anchovy (*Engraulis mordax*) (0.743-0.949 and 0.004-0.006, respectively) and Pacific sardine (*Sardinops sagax*) in the East Pacific (0.826-0.929 and 0.003-0.005, respectively) (Lecomte et al. 2004). Many sites were singletons, and only 30 sites showed common alleles exhibiting a frequency of < 90%. The average percentage of polymorphic sites of *Cyt b* for the Japanese anchovy was 12.2%, which is higher than for marine small pelagic fish, such as the northern anchovy (9.83%), Pacific sardine (7.53%), and demersal Atlantic cod (*Gadus morhua*) (9.6%; Arnason et al. 2000), but comparable to marine panmictic fish, such as the European eel (*Anguilla anguilla*) (11.2%; Daeman et al. 2001). High genetic variations for *Engraulis* species were also shown by other markers of mtDNA in haplotypes of mtNADH dehydrogenase complex (ND5/6) genes (Bembo et al. 1995). There have been relatively few genetic studies on races or stocks of the Japanese anchovy compared to the European anchovy (*E. encrasicolus*; Grant et al. 2005, Magoulas et al. 2006). We found that most of the genetic variation of Japanese



**Fig. 3.** Neighbor-joining (NJ) tree for 133 cytochrome *b* gene samples taken from the southern East China Sea.

anchovy occurred within groups of temporally or spatially discrete populations rather than between populations (4.1%); nonetheless the genetic structuring of discrete populations was statistically detectable, in agreement with distinctive local fishing stocks. The stock distinctions are obvious for Japanese anchovy compared to the northern anchovy (0.33%) and Pacific sardine (1.21%), in which both species show undetectable differences among stocks due to latitudinal gradients in haplotype diversity (Lecomte et al. 2004). A similar partitioning but a higher genetic variation was found in Mediterranean populations of the European anchovy, in which 7.6% of mitochondrial ND5/6 variations (by restriction fragment length polymorphism, RFLP) were among samples from different water masses of the Adriatic, Ionian, and Aegean Seas (Bembo et al. 1995 1996). However, when measured by allozyme electrophoresis, the partitioning of variations into the between-population portion was relatively low in samples from the northwestern Mediterranean (Tudela et al. 1999); but Bembo et al. (1996) reported that 3.4% of the between-sea variation was found to support genetic structuring in the Adriatic, Ionian, and Aegean Seas of the eastern Mediterranean. In all European anchovy cases, the within-sea

variation was much greater than the between-sea variation, Spanakis et al. (1989) suggesting that the anchovy population well fit a dynamically structured model onto which hydrographic or biological (predation or behavior) factors are imposed. Borsa (2002) summarized that oceanic anchovy populations genetically differ from coastal-water populations as shown in the Adriatic Sea, but there were weak differences across broad geographic ranges among populations of the Gulf of Biscay, the western Mediterranean, and Ionian Sea. The relative high genetic diversity of the Japanese anchovy as revealed by *Cyt b* (Table 3) is probably related to a large effective population size maintained in the past to the present, and accordingly supports a sizable fishery on the coasts of Taiwan. Our AMOVA revealed a significant genetic structure among 3 spatiotemporally distinct groups (Table 4), and further examination exhibited that the genetic distance of the spring Ilan Bay population (sprE) with its sympatric but allochronous 'cohort' (autE) was longer than that to its synchronous but allopatric population (sprW) (Table 5). It is also worth noting that the smallest genetic distance was found with putative spatiotemporally separated populations of sprW and autE. Microsatellite data also supported



**Fig. 4.** Distribution of pairwise differences (observed, dotted line with open circles; expected, solid line) of the cytochrome *b* gene in Japanese anchovy populations.

a genetic difference between spring allopatric populations (Yu et al. 2002) corresponding to the coastal stock of the East China Shelf (sprW) and the oceanic stock of the Pacific Ocean (sprE), between which a marine barrier is established by substantial hydrographic differences and a thermal front which lasts throughout the winter (Wang and Chern 1990). The winter front begins to weaken as the northeasterly monsoon wanes during the early spring, and Strait water begins to move northeastward (Jan et al. 2002). Such a dynamic state of marine conditions might not allow the stocks to accumulate long-term genetic differences, as shown by barely unique characters for stocks in this study (Fig. 3); however our findings did show distinguishable local populations which should be taken into account in future management plans of anchovy resources in Taiwan.

### Stock patterns

In this study, we found subtle morphometric traits (Fig. 2, Table 2) and significant condition factor differences (Table 1) between seasonal cohorts (sprE and autE) in Ilan Bay, and these results are consistent with the findings of Young et al. (1994) for adults, and Chiu and Chen (2001) for larvae. These differences between “cohorts”, however, are actually leveled “stocks”, and can be ascribed to experiencing different hydrographic origins (Hsieh et al. 2005) as evidenced by genetic structuring of the Japanese anchovy in waters off northern Taiwan (Table 5). It is interesting that geographic differences in sprE and sprW recognized by fishermen in a morphological sense (which may be due to a size factor) and seemingly supported by a genetic analysis by Yu et al. (2002) could not be statistically confirmed in our morphometric analysis. Because the geographic comparison of anchovy populations conducted by Yu et al. (2002) used a batch sample of adults in spring, their results indicated a geographic

distinction similar to this study’s sprE and sprW comparison (Table 5); however, they did not take autumn samples to make comparisons across different seasons as we did with the sprE and autE ‘cohorts’. The potential explanations for such dynamic relations among populations are as follows: 1) in Ilan Bay, ‘cohort’ differences (sprE and autE) in nutrition condition, growth, and survival rates (but not in external morphometric measurements) and genetics may be attributed to the existence of 2 spawning stocks; 2) morphological similarities between geographic stocks of sprE and sprW may imply phenotypic plasticity due to similar environmental conditions, or a genetic shift due to natural selection or fishing impacts; and 3) both morphological and genetic similarities of the autumn Ilan Bay cohort (autE) to the allopatric spring Taiwan Strait population (sprW) support a dynamic process of larval recruitment along the coast of northern Taiwan.

Widely distributed marine fishes often have nearly panmictic populations with a minimum of gene flow due to migration of spawning adults or larval drift, and thus often exhibit barely detectable demographic differences. However, northern anchovy was found to contradict this generality as a sharp genetic subdivision occurs in the California central anchovy stock (Hedgecock et al. 1989), and further insights showed that microgeographic genetic heterogeneity was embedded within the stock (Hedgecock 1994). Two hypotheses were proposed by Hedgecock et al. (1994) to explain heterogeneity within broad regions of genetically similar populations: 1) immigration from neighbors, and 2) a consequence of variance in the reproductive success of spawning adults and subsequent sampling errors in the recruitment of larvae. Since multi-generational data for measuring random genetic drift do not allow us to test the 2nd hypothesis, we postulated that 2 geographical stocks are found in the waters off northern Taiwan, and the 2 seasonal ‘cohorts’

**Table 6.** Gene flow between populations of Japanese anchovy, estimated from a 740 bp fragment of the mitochondrial cytochrome *b* gene

Donor populations	Theta ( $N_e\mu$ )	$N_e^a$	Receiving populations ( $N_m$ )		
			sprE	sprW	autE
Spring, eastern Taiwan (sprE)	0.303	$3.03 \times 10^7$	-	103	43
Spring, Taiwan Strait (sprW)	2.534	$2.53 \times 10^8$	798	-	1603
Autumn, eastern Taiwan (autE)	0.077	$7.70 \times 10^6$	1	5	-

All values with bounds of the 95% confidence limit. <sup>a</sup>Using a mutation rate of 1% per million years.

in Ilan Bay are actually 2 stocks corresponding to coastal and oceanic stocks from their pre-spawning habitats in the ECS, as modeled by Yu et al. (2002). The demographic history of stocks revealed by pairwise differences (D) agrees with a sudden-expansion model (Rogers and Harpending 1992, Fig. 4). Under this model, the initial sizes of the autumn Ilan Bay population ( $\theta = 1.230$  for autE) and the spring Taiwan Strait population ( $\theta = 1.388$  for sprW) were comparable, but they are just about 1/2 that of the spring Ilan Bay population ( $\theta = 2.314$  for sprE). The geographically separated recruitment processes of Japanese anchovy well fit the hydrographic characteristics influenced by the prevailing monsoons (Chen and Chiu 2003). There is a frequent winter thermohaline front to the north of Taiwan, which forms a barrier between the Taiwan Strait and Pacific Ocean (Wang and Chern 1990). During early spring, anchovies migrate to Taiwan for spawning along 2 possible routes: along the coast of China to the Taiwan Strait (sprW) and from the oceanic ECS to outer Ilan Bay (sprE) (Chen and Chiu 2003). The front recesses with the outset of the southwesterly monsoon allowing water from the Taiwan Strait to move into Ilan Bay (Liang et al. 2003). These 2 features suggest an autumn migration of Strait anchovies into the Ilan Bay area: the above-described flow pattern together with a gradual northerly shift in the anchovy population found on the northwestern coast of Taiwan after summer (Chiu et al. 1997), and our demonstration of a close genetic link between the spring Taiwan Strait population and the autumn Ilan Bay population. Using our data, the MIGRATE software also estimated that on average more than 10 fold migrants moved from the Taiwan Strait to Ilan Bay compared to other populations (Table 6). Our data open up a window to elucidate the stock structure and migration patterns of Japanese anchovy in its southern ranges, and further genetic data are needed to make this larval drift pattern clearer.

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