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GENETIC DIVERSITY IN U.S. HATCHERY STOCKS OF *CRASSOSTREA ARIAKENSIS* (FUJITA, 1913) AND COMPARISON WITH NATURAL POPULATIONS IN ASIA

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ABSTRACT Although several different U.S. hatchery stocks of the Asian Suminoe oyster *Crassostrea ariakensis* were used in laboratory and field trials assessing performance, and in comparative studies with the native oyster *Crassostrea virginica*, the genetic composition of these hatchery stocks has not yet been examined comprehensively. Using eight microsatellite markers we investigated the genetic variability among five hatchery stocks and compared the genetic makeup of these stocks with 8 wild populations from Asia. Results showed significant genetic differentiation among the 5 hatchery stocks that was 6-fold larger than that observed among wild populations. A significant reduction in genetic diversity was observed in hatchery stocks compared with wild source populations, indicating a genetic bottleneck. Two long-established stocks showed significant decreases in both allelic diversity and heterozygosity compared with the wild Japanese source population, whereas three recently established stocks showed less severe reductions in allelic diversity and a nonsignificant change in levels of heterozygosity compared with their source Chinese populations. These microsatellite markers also proved useful for assignment of hatchery individuals back to their source stocks with a high degree of confidence. Although assignment of wild individuals back to their population of origin proved less reliable, approximately 70% of wild individuals could be assigned either to their source population or to geographically proximal populations. Our results suggest that results obtained from experiments that used hatchery animals of a single *C. ariakensis* stock for biological and ecological studies should be interpreted cautiously, because they may not always accurately reflect the behavior of wild populations or of other hatchery stocks.

KEY WORDS: Suminoe oyster, *Crassostrea ariakensis*, microsatellites, hatchery stocks, genetic diversity, genetic bottleneck

INTRODUCTION

The Suminoe oyster *Crassostrea ariakensis* naturally occurs along the coast of the northwest Pacific, and is a commercially important oyster species for aquaculture in both China and Japan (Langdon & Robinson 1996, Guo et al. 1999, Zhou & Allen 2003). In the 1970s, it was accidentally introduced to the west coast of the United States along with a shipment of *Crassostrea gigas* and *Crassostrea sikamea* oyster seed from the Kumamoto Prefecture in Japan (Breese & Malouf 1977). The introduced stocks of *C. ariakensis* have been spawned in hatcheries and raised in culture in the coastal waters of Oregon and Washington, but to date no wild populations are known to have established in the region, possibly because of the low water temperatures in the northeast Pacific (Breese & Malouf 1977, Perdue & Erickson 1984, Langdon & Robinson 1996). Recently, proposals to introduce a nonnative oyster species to the Chesapeake Bay region of the U.S. east coast led to research on nonnative oyster species, including *C. gigas* and *C. ariakensis* (Mann et al. 1991). Based on preliminary studies, *C. ariakensis* appeared to grow faster and be more tolerant to local oyster parasites in some locations of Chesapeake Bay compared with the native eastern oyster *Crassostrea virginica* and the other nonnative species tested, *C. gigas* (Calvo et al. 1999, Calvo et al. 2001, Grabowski et al. 2004). Therefore, focus shifted to *C. ariakensis* and, during the late 1990s, *C. ariakensis* broodstocks were imported from Oregon to Rutgers University and to the Virginia Institute of Marine Science (VIMS). Two other stocks of oysters, imported from the Yellow River basin, Shandong Province in northern China, and Beihai, Guangxi Province in southern China, have been maintained at VIMS since 1999 (NRC 2004, Zhang et al. 2005). Although no reproductively capable *C. ariakensis* were ever approved for in-water testing along the U.S. east coast, sterile triploid *C. ariakensis* derived from these hatchery stocks (Calvo et al. 2000, Allen et al. 2003) were used in field trials, and both triploid and diploid hatchery animals were used for laboratory experiments conducted under quarantine conditions for comparative taste (Grabowski et al. 2003), biological and ecological studies (Calvo et al. 2001, Grabowski et al. 2004, Bishop & Hooper 2005, Hudson et al. 2005, Alexander et al. 2008, Kingsley-Smith & Luckenbach 2008, McGhee et al. 2008, Paynter et al. 2008, Tamburri et al. 2008), as well as for testing disease tolerance (Calvo et al. 2001, Grabowski et al. 2004, Moss et al. 2006).

Little is known, however, about the genetic makeup of the hatchery stocks and their genetic differentiation from wild populations, which might be associated with differences in biology, behavior, and performance under various environmental conditions. Studies on some marine bivalves show that allelic reduction is quite common in hatchery lines, and it is often associated with deviations in allelic or genotypic frequencies compared with the natural source populations (Hedgecock & Sly 1990, Gaffney et al. 1996, Yu & Guo 2005, Carlsson et al. 2006). This drift is thought to arise as a result of bottleneck effects from the small effective number of parents typically contributing to spawns in hatcheries, and nonrandom selection that often occurs during breeding and larval recruitment in the hatchery (Hedgecock & Sly 1990, Gaffney et al. 1996, Boudry et al. 2002, Yu & Guo 2005). Consequently, inbreeding is common in hatchery stocks, and sometimes a concomitant decrease in various performance measures can occur (Hedgecock et al. 1995, Bierne et al. 1998, Ernande et al. 2003). Studies have shown that there is a small but significant genetic heterogeneity among wild *C. ariakensis* populations in Asia, and the genetic differentiation increases with geographic distance among populations, indicating a genetic pattern of isolation by distance

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(Xiao et al. 2010). Although an initial study showed reduced genetic diversity in 5 VIMS hatchery stocks compared with wild populations (Zhang et al. 2005), this previous study did not provide detailed information on the genetic structuring within and among *C. ariakensis* hatchery stocks and wild populations.

Because of concerns over the social, economic, and ecological risks of introducing the nonnative *C. ariakensis* into the Chesapeake Bay ecosystem, the proposed introduction of *C. ariakensis* for helping restore the Eastern oyster population in Chesapeake Bay is no longer being considered (http://www.nao.usace.army.mil/OysterEIS/FINAL_PEIS/homepage.asp). However, accidental release or intentional, illegal introduction of reproductively capable *C. ariakensis* is still possible through various means (Simberloff 2005). Some triploid *C. ariakensis* have been found by local farmers in the waters of North Carolina and Chesapeake Bay (K. Reece unpubl. data). The highly polymorphic microsatellite markers used here have proved useful for differentiating among natural populations of *C. ariakensis* in Asia (Xiao et al. 2010). The current study tested further the utility of these markers to differentiate among U.S. hatchery stocks, to assess the relationship among these stocks and their natural source populations, and to assign *C. ariakensis* of unknown origin back to their source domestic stocks or wild populations.

MATERIALS AND METHODS

Samples

A total of 245 individuals from 5 hatchery stocks were collected in 2002 (Table 1) and confirmed to be *C. ariakensis* by PCR-RFLP analysis (Zhang et al. 2005, Cordes et al. 2008). Forty-eight *C. ariakensis* oysters were shipped from Taylor Shellfish Farms, Inc. (TUI), and genomic DNA was extracted directly from the tissues of these oysters for genotyping. TUI was derived from the ''Oregon strain,'' which was inadvertently introduced to the west coast of the United States in the 1970s from Japan, and has been spawned and reared in hatcheries for several generations (Breese & Malouf 1977). In comparison with TUI, a west coast *C. ariakensis* (WCA) sample, also derived from the ''Oregon Strain,'' was comprised of the offspring of a *C. ariakensis* stock imported from the coastal waters of Washington state to VIMS in 1999. A north China *C. ariakensis* stock (NCA) was derived directly from wild broodstock brought to VIMS from the Yellow River Basin, Shandong Province, northern China, and spawned in 1999 at the VIMS Aquaculture and Breeding Technology Center (ABC). Similarly, two south China *C. ariakensis* stocks (SCA99 and SCA00) were derived from a wild broodstock collected in Beihai, Guangxi Province, southern China, and spawned by ABC in 1999 and 2000, respectively. For the purpose of this study, TUI and WCA were considered to be long-established stocks because of the long separation time (around 30 y) from their natural source populations in Japan (Breese & Malouf 1977). NCA, SCA99, and SCA00 were considered recent stocks because the broodstocks were transported into the United States quite recently, and each stock had undergone only 1 generation of hatchery propagation at the time of sampling.

As inferred by the population genetic structure analysis of Xiao et al. (2010), samples from eight genetically heterogeneous natural populations in Asia were also included in the analyses (Table 1). They were used for comparisons of genetic variability and diversity among hatchery stocks and wild populations, and as reference populations for assignment tests. Two additional wild samples, which were not included in the wild population genetic structure analysis, were genotyped and used as test

TABLE 1.

Sample information for 5 *C. ariakensis* hatchery stocks, 8 natural populations from Asia used for comparisons of genetic variability, and 2 test samples comprised of additional individuals from 2 wild samples (IR05a, YR05a) used for validating the assignment tests.

Sample Code	Collecting Date	Sample Type	Sample Size	Source
TUI	2002	Hatchery	48	Taylor Shellfish Farms, Inc., derived from the 'Oregon Strain' on west coast of United States
WCA	2002	Hatchery	50	ABC, derived from the 'Oregon Strain' on west coast of United States
NCA	2002	Hatchery	50	ABC, F1 generation of Yellow River stock (YR) from northern China, spawned in 1999
SCA99	2002	Hatchery	50	ABC, F1 generation of Beihai stock (BH) from southern China, spawned in 1999
SCA00	2002	Hatchery	47	ABC, F1 generation of Beihai stock (BH) from southern China, spawned in 2000
IR*	1999, 2005	Wild	99	Ariake Sea, Saga Prefecture, Kyushu, Japan
KR*	2004	Wild	33	Kawha River, South Korea
SR*	2004	Wild	20	Sumjin River, South Korea
KI*	2004	Wild	20	Kanghwa Island, Incheon, South Korea
YR*	1999, 2006, 2005, 2006	Wild	132	Yellow River basin, Shandong Province, China
YzR*	2005, 2006, 2006	Wild	137	Yangzi River estuary, China
HC*	2005, 2006	Wild	95	Haicheng, Fujian Province, China
BH*	1999, 2005	Wild	69	Dafeng River, Beihai, Guangxi Zhuang, China
IR05a	2005	Wild	50	Ariake Sea, Saga Prefecture, Kyushu, Japan
YR05a	2005	Wild	36	Yellow River, Weifang, Shandong Province, China

* The eight wild populations were from Xiao et al. (2010). Sixteen wild samples originally used for the study in Xiao et al. (2010) were grouped into 8 genetically distinguished populations based on the results of the genetic analysis in that study.

samples for the assignment analyses conducted here. These test samples were composed of 50 individuals collected from the Itoki River in Japan (IR05a) and 36 individuals from the Yellow River in China (YR05a) in 2005. These additional samples were confirmed to be *C. ariakensis* using the PCR-RFLP molecular identification key of Cordes et al. (2008).

DNA Extraction, Microsatellite Amplification, and Data Analysis

Genomic DNA extraction, PCR amplifications of 8 microsatellite markers (*Car11-70, Car115-a0, Car119-6a, Car130-08, CarG1-0b, CarG4-60, CarG110, CarG122;* GenBank accession nos.: EU241319-EU241322, EU241324-EU241327), and separation of PCR products on a PRISM ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA) followed the protocols described in Xiao et al. (2008). Allele sizes in base pairs (bp) were called based on comparisons with the panels generated by scoring 605 wild individuals of *C. ariakensis* from Japan, South Korea, and China (Xiao et al. 2010), using the software package GeneMarker (SoftGenetics, State College, PA).

Based on multilocus genotype data, inter- and intrapopulation variation were measured by various parameters. Observed and expected heterozygosity (H_O and H_E, respectively) were calculated using the program GENETIX (Belkhir et al. 1996–2004). Unbiased F statistics (F_{IS} and θ_{ST} (Weir & Cockerham 1984)) were calculated using GENEPOP 3.4 (Raymond & Rousset 1995), and the significance levels of F_{IS} and θ_{ST} values were assessed by bootstrapping with 10,000 iterations at each locus and over all loci in GENETIX. Allelic richness (A) was computed by FSTAT 2.9.3 (Goudet 2001) and adjusted to a sample size of 47 diploid individuals by a rarefaction method (Petit et al. 1998). The genetic relationship between hatchery stocks and natural populations, as well as among all samples was visualized through construction of a neighbor-joining (NJ) tree using the software package PHYLIP 3.67 (Felsenstein 1989) based on Cavalli-Sforza and Edwards' (1967) genetic distances.

In the assignment analyses, 8 wild populations (IR, KR, SR, KI, YR, YzR, HC, and BH) and the 5 hatchery stocks were used as the reference samples. To assess the ability of the markers to assign individuals correctly back to their source populations, self-assignment tests were first conducted (during which the population of origin for a sampled individual was considered the source) for the eight natural populations and 5 hatchery populations by a ''leave-one-out'' procedure that excluded the individual to be assigned from the population during computation (Piry et al. 2004). To assign ''unknown'' individuals to a natural population or hatchery stock, 10 individuals from each of the 5 hatchery samples (named TUIa, WCAa, NCAa, SCA99a, and SCA00a) were drawn randomly and removed from the ''reference'' database. These 50 hatchery individuals, along with 86 test individuals (total $n = 136$) from two additional natural samples (IR05a and YR05a), were assigned to a population or stock based on their multilocus genotype profiles. A Bayesian method (Rannala & Mountain 1997) implemented in the program GeneClass 2 (Piry et al. 2004) was used to compute the probability of an individual being classified to each reference population. The reference population with the highest assignment probability was chosen as the assigned source for each individual, and compared with the known information from sampling.

RESULTS

Genetic Diversities Within the Hatchery Stocks and Comparisons with Natural Populations

A total of 152 different alleles were amplified at 8 microsatellite loci in 246 individuals from 5 hatchery stocks. Only 52.6% of the alleles that amplified in wild samples (Xiao et al. 2010) were observed in the hatchery stocks. In addition, 3 alleles (from loci *CarG4-60*, *Car130-08,* and *Car115-a0*) that were not observed in the wild samples were amplified from hatchery stocks. One of these unique alleles was only found in the Japanese-derived hatchery stocks TUI and WCA; the other two were found only in stocks SCA99 and SCA00, which were derived from southern Chinese populations.

Adjusted allelic richness (A) across all loci in hatchery stocks ranged from an average of 3.8 (TUI)–12.2 (SCA99) alleles per locus per stock (Table 2). There was a significant difference in A values among these 5 hatchery stocks, and the long-established stocks (TUI and WCA) had much lower A values compared with the recently derived stocks ($P < 0.001$, 2-tailed Mann-Whitney test).

Multilocus analysis of the hatchery stocks indicated that the TUI stock showed a significant negative F_{IS} (–0.226), which is an indication of an excess of observed heterozygotes compared with expected. However, individual tests of HWE for each stock at every locus (Table 2) revealed that 15 of 40 tests were significantly out of HWE after a sequential Bonferroni correction ($P < 0.05$, $K = 5$, initial $\alpha = 0.01$), and 11 (69%) of them were the result of an excess of heterozygotes. TUI had the highest number (5 of 8) of deviations from HWE, all resulting from $H_O > H_E$.

We compared further the genetic diversity in terms of allelic richness (A) and heterozygosity (H_O, H_E) of the hatchery stocks with their wild source populations. For the purpose of comparison, A values of hatchery stocks were adjusted to a sample size of $n = 14$, which was the adjusted sample size for the wild samples in the previous study (Xiao et al. 2010). The values for all three parameters were decreased significantly (all P values < 0.05) in the two long-established stocks TUI and WCA compared with those parameters in their source population (IR; Figs. 1 and 2); whereas the three recent stocks (NCA, SCA99, and SCA00) had reduced values of A ($P = 0.009$; Fig. 1), but not of H_O and H_E ($P = 0.615$ and 0.105, respectively; Fig. 2) compared with their source populations (YR and BH, respectively). The average A, H_O, and H_E values of the two long-established stocks (TUI and WCA) were decreased by 60.4%, 10.9%, and 26.4%, respectively, compared with their wild source population (IR). In comparison, the average reduction in A in the three recent hatchery stocks, although significantly lower than in the wild populations, was much smaller, and H_E and H_O values did not decrease significantly. Allelic richness was 17.7% lower for NCA compared with its source population YR, and 29.5% lower for the SCA99 and SCA00 stocks compared with the wild BH population.

Genetic Differentiation Among Hatchery Stocks and Wild Populations

Population pairwise θ_{ST} values among hatchery samples (Table 3) ranged from 0.054 (SCA99 vs. SCA00)–0.238 (TUI vs. SCA00; (global $\theta_{ST} = 0.132$), and were clearly larger than those previously observed among 8 natural populations

TABLE 2.

Microsatellite diversity in 5 hatchery stocks of *C. ariakensis*.

	CarG110	*CarG4-60*	*Car119-6a*	*Car11-70*	*Car130-08*	*CarG122*	*CarG1-0b*	*Car115-a0*	**Multilocus**
TUI									
A	3.0	6.0	3.9	4.9	4.0	2.0	2.0	4.9	3.8
H_O	0.750	0.771	1.000	0.854	0.302	0.521	0.229	0.787	0.652
H_E	0.508	0.697	0.615	0.658	0.462	0.385	0.203	0.690	0.527
P	**<0.001**	0.089	**<0.001**	**0.001**	**0.002**	**<0.001**	**<0.001**	0.052	**<0.001**
F_{IS}	–0.469	–0.096	–0.620	–0.289	0.356	–0.343	–0.119	–0.131	–0.226
WCA									
A	4.0	12.3	7.0	7.8	6.0	2.9	2.0	8.0	6.3
H_O	0.840	0.900	0.841	0.680	0.378	0.300	0.435	0.860	0.654
H_E	0.655	0.848	0.721	0.809	0.690	0.285	0.386	0.820	0.652
P	**0.001**	0.121	0.017	**0.005**	**<0.001**	0.240	0.112	0.207	0.405
F_{IS}	–0.273	–0.052	–0.155	0.169	0.462	–0.041	–0.117	–0.039	0.007
NCA									
A	7.0	19.5	8.7	12.9	15.7	2.0	9.4	19.5	11.8
H_O	0.640	0.900	0.840	0.960	0.860	0.340	0.820	0.940	0.788
H_E	0.621	0.911	0.810	0.848	0.895	0.282	0.759	0.925	0.756
P	0.327	0.192	0.271	**0.003**	0.094	**<0.001**	0.112	0.341	0.055
F_{IS}	–0.021	0.022	–0.027	–0.122	0.049	–0.195	–0.071	–0.007	–0.031
SCA99									
A	9.0	15.9	10.9	11.8	16.5	3.9	7.9	21.7	12.2
H_O	0.860	1.000	0.816	0.920	0.837	0.440	0.745	0.717	0.792
H_E	0.836	0.900	0.861	0.864	0.883	0.426	0.722	0.911	0.800
P	0.316	**<0.001**	0.093	0.087	0.063	0.350	0.313	**<0.001**	0.150
F_{IS}	–0.018	–0.102	0.062	–0.055	0.063	–0.023	–0.021	0.223	0.021
SCA00									
A	10.0	13.8	8.0	10.9	11.7	4.0	8.9	13.8	10.1
H_O	0.766	0.957	0.936	0.915	0.447	0.298	0.783	0.848	0.744
H_E	0.730	0.890	0.809	0.850	0.807	0.268	0.687	0.870	0.739
P	0.227	0.041	**0.003**	0.079	**<0.001**	**<0.001**	0.025	0.175	0.436
F_{IS}	–0.039	–0.065	–0.147	–0.065	0.455	–0.100	–0.129	0.037	0.004
Overall									
A	6.6	13.5	7.7	9.7	10.8	3.0	9.0	13.6	8.8
H_O	0.771	0.906	0.887	0.866	0.565	0.380	0.603	0.831	0.726
H_E	0.670	0.849	0.763	0.806	0.747	0.329	0.551	0.843	0.695

A is allelic richness adjusted to a sample size of $n = 47$ by the rarefaction method (Petit et al. 1998); H_O and H_E are observed and expected heterozygosity, respectively; F_{IS} is Weir and Cockerham's (1984) inbreeding coefficient; *P* is the probability that F_{IS} is null. Numbers in bold type are significantly different from 0 after a sequential Bonferroni correction at the $P < 0.05$ level (K = 5, initial adjusted $\alpha = 0.01$). See Table 1 for sample descriptions.

(global $\theta_{ST} = 0.020$[1]). All pairwise θ_{ST} values among hatchery populations were highly significant ($P < 0.001$), and those comparisons that involved the long-established stocks (TUI and WCA) all had θ_{ST} values greater than 0.100, and were larger than those observed among recent stocks (<0.065). Genetic relationships among the hatchery stocks and the 8 wild populations were visualized by an unrooted NJ tree (Fig. 3). Hatchery stocks derived from the same natural sources, such as TUI and WCA (Japan), and SCA99 and SCA00 (southern China), grouped together with high bootstrap support (>90%). TUI and WCA were genetically closest to their wild source population (IR), with a moderate bootstrap support value (75%). SCA99 and SCA00 also grouped with their wild source population (BH), but with lower bootstrap support (<50%). Similarly, the NCA stock (derived from broodstock collected from the Yellow River region in China) was genetically closest to the northern Chinese (including YR and YzR) and Korean populations (KR, SR, and KI), but without strong bootstrap support values (<50%). Long branch lengths indicated significant drift of the hatchery stocks away from their parental sources.

Assignment Tests

The results of all assignment tests are shown in Table 4, including two groups of self-assignments and the assignments of some additional and/or random samples to natural and/or hatchery origins. The self-assignment tests correctly assigned 84.0%–97.9% of hatchery individuals back to their hatchery stocks, whereas variable percentages (20.0%–72.7%) of wild individuals were assigned back to their specific wild source populations. The three Korean populations, which had small sample sizes (20–33), had quite a low incidence of correct assignments (20.0%–30.0%). Nevertheless, results (Table 4) indicate that a large portion of wild individuals were assigned either correctly to their source or to a geographically proximal population. For example, 15.0%–40.0% of individual oysters from the 3 Korean populations (KR, SR, and KI) were

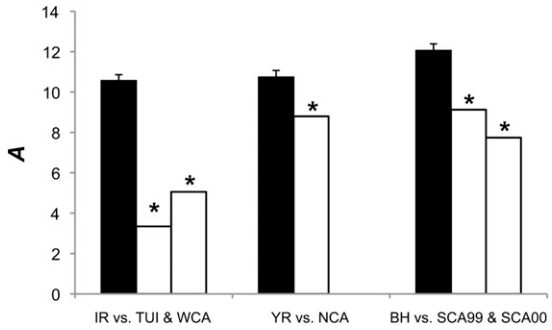

Figure 1. Comparisons of adjusted allelic richness (*A*) between the 3 wild source populations from Asia (IR, YR, and BH; black columns) and their derived hatchery stocks (TUI and WCA, NCA, SCA99, and SCA00, respectively; white columns). Error bars are standard deviations of mean values for wild populations. *A* values were adjusted to a sample size of $n = 14$ for both hatchery stocks and wild populations for the purpose of comparison because 14 was the adjusted sample size used for wild samples (Xiao et al. 2010). See Table 1 for sample abbreviations and source locations. *Significant ($P < 0.05$) difference between the hatchery stock and its wild source population. Both long-established hatchery stocks (TUI and WCA) and recently derived hatchery stocks (NCA, SCA99, and SCA00) have significantly lower values of *A* compared with their source populations (IR, YR, and BH).

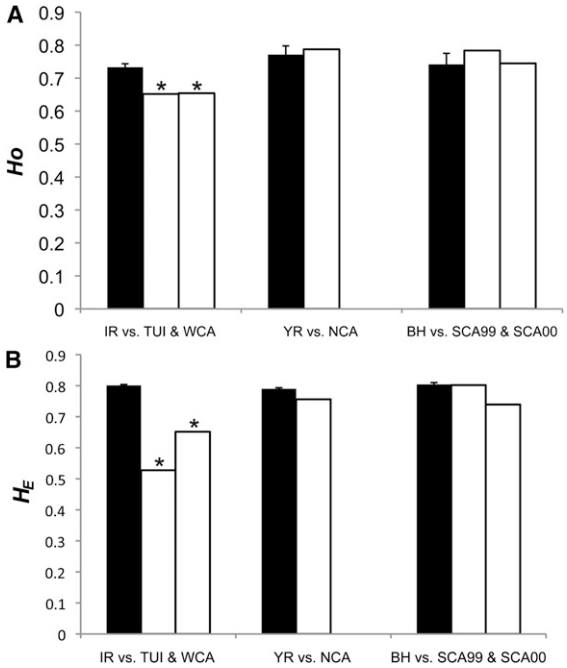

Figure 2. (A, B) Multilocus observed (H_O) (A) and expected (H_E) (B) heterozygosities for the three wild source populations from Asia (IR, YR, and BH; black columns) and their derived hatchery stocks (TUI and WCA, NCA, SCA99 and SCA00, respectively; white columns). Error bars are standard deviations of mean values for wild populations. *Significant ($P < 0.05$) difference between the hatchery stock and its wild source population. Only long-established hatchery stocks (TUI and WCA) show significantly reduced H_O, and H_E compared with their source population (IR).

assigned to the Yellow River basin population (YR), and an additional 20.0% and 35.0% of individuals from the SR and KI populations, respectively, were assigned to the Yangzi River population (YzR). Similarly, a substantial proportion of oysters (14.7%) from Haicheng (HC) were classified to the other southern Chinese population (BH), and vice versa (i.e., 29.0% from BH assigned to HC).

Tests using both hatchery stocks and natural populations as references for 136 putatively "unknown" individuals from 7 different samples resulted in relatively accurate assignment of the hatchery animals to either the correct stock, a closely related stock, or to the source populations, and likewise, assignment of the wild animals to either the correct population or to geographically proximal populations. Interestingly, although 70% of the animals from the NCAa hatchery sample were assigned to the NCA stock, the other 30% were assigned to wild populations (YR and YzR) in northern China, which was either the source population for the NCA stock or near the source. In addition, although 90% of the animals from the hatchery SCA99a sample were assigned to the SCA99 stock, one animal (10%) was assigned to a wild population from southern China (HC). Ninety percent of the individuals in the TUIa sample were assigned to the TUI stock, and 10% were assigned to the WCA stock, which was derived from the same Japanese source population. Although 90% of the animals from SCA00a were assigned correctly to SCA00, 10% were assigned to the other hatchery stock from the same southern Chinese broodstock (SCA99). Only one individual (2%) from the wild Japanese IR05a sample was assigned to an unrelated hatchery stock (SCA99), whereas 68% were assigned to the natural IR population and 22% were assigned to Korean or northern Chinese populations. Approximately 64% of the wild YR05a sample were correctly assigned to the YR population, and another 22% of the individuals were assigned to the geographically proximal YzR population.

DISCUSSION

Genetic Makeup of the Hatchery Stocks

In a hatchery setting, reductions in allelic diversity are believed to be caused by small effective population sizes (N_e) resulting from few animals being used as broodstock, as well as nonequal gametic viability and/or differential spawning conditions of the potential parents (Hedgecock & Sly 1990, Hedgecock 1994). Reductions in heterozygosity, however, do not always respond immediately to these conditions, depending on the number and severity of the bottlenecks, the original

TABLE 3.

Pairwise θ_{ST} (above diagonal) and *P* values (below diagonal) among 5 hatchery stocks of *C. ariakensis*.

	NCA	WCA	SCA99	SCA00
TUI	0.228	0.123	0.194	0.238
NCA	—	0.149	0.062	0.055
WCA	<0.001	—	0.101	0.150
SCA99	<0.001	<0.001	—	0.054
SCA00	<0.001	<0.001	<0.001	—

All comparisons were highly significant after a sequential Bonferroni correction ($P < 0.05$, K = 10, initial adjusted $\alpha = 0.005$). See Table 1 for sample abbreviations.

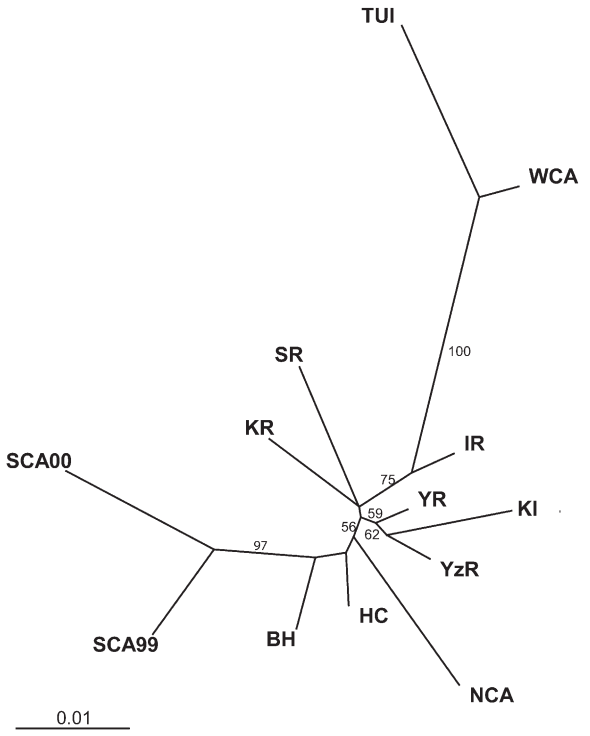

Figure 3. Unrooted neighbor-joining tree based on Cavalli-Sforza and Edwards' (1967) genetic distances among 5 hatchery stocks (TUI, WCA, NCA, SCA99, and SCA00) and eight wild populations (IR, KR, SR, KI, YR, YzR, HC, and BH) of *C. ariakensis*. See Table 1 for sample abbreviations and source locations. Numbers on internal branches are bootstrap support values greater than 50% after 10,000 iterations.

N_e, and the evenness of allelic frequencies after the bottleneck (Hedgecock & Sly 1990, Leberg 1992). Reductions in both types of diversity are commonly observed in cultivated fishes (Allendorf & Phelps 1980, Ryman & Ståhl 1980), whereas hatchery-propagated marine bivalves have been typically observed to lose only allelic diversity (Hedgecock & Sly 1990, Yu & Guo 2005, Carlsson et al. 2006). In the current study, reductions in genetic diversity were greatest in the long-established hatchery stocks (TUI and WCA) that had been isolated from their source populations for the longest times. This is not surprising because these stocks have been domesticated for more than 30 y and have undergone multiple generations of hatchery spawning. In contrast, the more recently domesticated stocks, which were analyzed after only one generation of hatchery propagation, showed reductions in allelic diversity but not in heterozygosity, consistent with previous reports that allelic diversity is more sensitive to bottleneck events and that a time lag exists before measurable decreases in heterozygosity are observed (Nei et al. 1975, Hedgecock & Sly 1990, Leberg 1992, Petit et al. 1998,). A relation curve based on studies of 78 animal species (DeWoody & Avise 2000) showed that heterozygosity changed little after the effective number of alleles reached 10. However, if inbreeding is continued over several generations so that allelic diversity continues to decrease, heterozygosity eventually decreases correspondingly, as indicated by the current study in which the recent stocks (NCA, SCA99, and SCA00) only demonstrated significantly reduced allelic richness, and allelic richness, as well as the observed and expected heterozygosity values, were all significantly lower in the long-established hatchery stocks (TUI and WCA) compared with their wild source population (Figs. 1 and 2).

Unlike natural populations in which deviations from HWE (mostly at locus *Car115-a0*) were primarily the result of heterozygote deficiencies likely resulting from null alleles (Xiao et al. 2010), an overall high proportion (68.8%) of the significant F_{IS} values in these hatchery stocks were the result of heterozygote excess. In fact, 83.3% of the deviations observed in the TUI stock were the result of an excess of observed heterozygotes. A closer look at the genotype frequencies showed that a few genotypes were highly prevalent in this hatchery stock (data not shown), which is not an uncommon observation in hatchery-propagated inbred oyster families (Saavedra & Guerra 1996, McGoldrick & Hedgecock 1997, Bierne et al. 1998, Marsic-Lucic & David 2003). Uneven parental contribution and selection against deleterious homozygotes resulting from identical-by-descent markers might contribute to the distorted genotypic frequencies in such hatchery stocks, particularly in the short term (McGoldrick & Hedgecock 1997, Bierne et al. 1998, Launey & Hedgecock 2001). Furthermore, the distorted genotypic frequencies observed here indicate nonequal contributions to the progeny genotypes from the parents, and there were probably only a few successful breeders at each spawning. This ''founder effect'' can cause shifts in allelic frequencies at individual loci by random chance (i.e., drift). Loss of rare alleles and a high prevalence of a few common alleles can result in very different allelic distributions compared with the parental population. Relatively rapid divergence from source populations and from other stocks derived from the same source is often observed in highly fecund species like oysters (Hedgecock 1994, Saavedra & Guerra 1996, Vercaemer et al. 2006). This likely explains the large genetic distance among hatchery stocks and between the hatchery stocks and wild populations in this study.

Implications for C. ariakensis *Stock Management*

Although *C. ariakensis* was seriously considered for introduction into the Chesapeake Bay region for the purpose of helping to restore the Chesapeake Bay oyster populations and the oyster industry, an alternative that involves only restoration of the native Eastern oyster was put forth as the preferred option in the final programmatic environmental impact statement (http://www.nao.usace.army.mil/OysterEIS/FINAL_PEIS/homepage.asp). As an economically important species in Asia, however, *C. ariakensis* has been considered for potential additional aquaculture development on the west coast of the United States (Breese & Malouf 1977, Perdue & Erickson 1984, Langdon & Robinson 1996). In France there has been some interest in using *C. ariakensis* as an alternative to, or as additional oyster species for, the *C. gigas* aquaculture industry (Cochennec et al. 1998). Understanding the genetic makeup of these hatchery stocks and managing them based on the genetic information is important for any oyster industry planning to raise *C. ariakensis* in culture.

Based on the results described here, there are three possible effects of genetic bottlenecks on the current U.S. hatchery stocks tested. First, loss of genetic diversity over the long term may cause inbreeding depression. Although the overall and long-term effects of inbreeding depression and its converse, heterosis (hybrid vigor), are not completely understood in

TABLE 4.

Number and percentage of individuals assigned to various hatchery and wild reference samples using Rannala and Mountain's (1997) Bayesian method.

Sample	Sample Size	Sample												
		IR	KR	SR	KI	YR	YzR	HC	BH	TUI	WCA	NCA	SCA99	SCA00
(A) Eight wild populations*														
IR	99	**72 (72.7)**	5 (5.1)	2 (2.0)	3 (3.0)	4 (4.0)	6 (6.1)	4 (4.0)	3 (3.0)					
KR	33	2 (6.1)	**8 (24.2)**	3 (9.1)	1 (3.0)	8 (24.2)	3 (9.1)	5 (15.2)	3 (9.1)					
SR	20	2 (10.0)	2 (10.0)	**6 (30.0)**	1 (5.0)	3 (15.0)	4 (20.0)	2 (10.0)	0 (0.0)					
KI	20	0 (0.0)	1 (5.0)	0 (0.0)	**4 (20.0)**	8 (40.0)	7 (35.0)	0 (0.0)	0 (0.0)					
YR	132	4 (3.0)	7 (5.3)	2 (1.5)	12 (9.1)	**83 (62.9)**	15 (11.4)	9 (6.8)	0 (0.0)					
YzR	137	4 (2.9)	4 (2.9)	3 (2.2)	7 (5.1)	15 (10.9)	**87 (63.5)**	14 (10.2)	3 (2.2)					
HC	95	7 (7.4)	9 (9.5)	0 (0.0)	3 (3.2)	6 (6.3)	9 (9.5)	**47 (49.5)**	14 (14.7)					
BH	69	1 (1.4)	10 (14.5)	1 (1.4)	0 (0.0)	1 (1.4)	1 (1.4)	20 (29.0)	**35 (50.7)**					
(B) Five hatchery populations*														
TUI	48									**47 (97.9)**	1 (2.1)	0 (0.0)	0 (0.0)	0 (0.0)
WCA	50									3 (6.0)	**46 (92.0)**	0 (0.0)	1 (2.0)	0 (0.0)
NCA	50									0 (0.0)	0 (0.0)	**42 (84.0)**	0 (0.0)	8 (16.0)
SCA99	50									0 (0.0)	0 (0.0)	1 (2.0)	**47 (94.0)**	2 (4.0)
SCA00	47									0 (0.0)	0 (0.0)	1 (2.1)	2 (4.3)	**44 (93.6)**
(C) Seven putatively unknown samples														
IR05a	50	**34 (68.0)**	3 (6.0)	1 (2.0)	0 (0.0)	3 (6.0)	4 (8.0)	0 (0.0)	4 (8.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)
YR05a	36	0 (0.0)	1 (2.8)	0 (0.0)	2 (5.6)	**23 (63.9)**	8 (22.2)	1 (2.8)	1 (2.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TUIa	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**9 (90.0)**	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
WCAa	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**9 (90.0)**	0 (0.0)	1 (10.0)	0 (0.0)
NCAa	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (20.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**7 (70.0)**	0 (0.0)	0 (0.0)
SCA99a	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**9 (90.0)**	0 (0.0)
SCA00a	10	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)	**9 (90.0)**

(A) Number (percent) of correct self- assignments for 8 wild populations. (B) Number (percent) of correct self-assignments for 5 hatchery stocks. (C) Number (percent) of assignments for 7 putatively "unknown" samples to each of the reference samples from (A) and (B).

* The eight wild populations were from Xiao et al. (2010).

See Table 1 for sample abbreviations. Numbers in bold type indicate the number of oysters that were correctly classified to sample of origin.

marine bivalves, positive correlations between multilocus heterozygosity and fitness-related traits were often observed in highly inbred families and populations of bivalves (Gaffney et al. 1990, Hedgecock et al. 1995, McGoldrick & Hedgecock 1997, Bierne et al. 1998, David 1998, Naciri-Graven et al. 2000, Launey & Hedgecock 2001, Hedgecock et al. 2007). Reduction in heterozygosity, although undetectable in the first several generations of inbreeding (Hedgecock & Sly 1990), could become significant after continued use of a small numbers of individuals for spawning, which are drawn from stocks that are already showing reduced allelic diversity. Therefore, successive introduction of broodstock from wild populations is recommended to enrich and maintain healthy genetic pools in existing hatchery stocks, especially for those stocks isolated from their source for the longest times (TUI and WCA). Second, given the high genetic differentiation among the hatchery stocks derived from different source populations, there might be phenotypic divergence among stocks. As the practical basis of selective breeding, phenotypic differences are often intentionally selected to produce broodstock with specific traits; however, this process can be unintentionally associated with a decrease in genetic diversity, and particularly in allelic diversity (Yu & Guo 2005, Carlsson et al. 2006). Third, inadvertent artificial selection pressure resulting from time and energy constraints in the laboratory or hatchery has been shown to cause trait differences from what is observed in wild animals, and to reduce variance in growth and development of domesticated oysters. For example, Taris et al. (2006) reported that by culling *C. gigas* larvae in early stages, the stocks resulted in individuals that were an average of 10% larger and had a 12% shorter time to settlement than control groups, and the variance in the parameter means was reduced by 30%–40% and 55%, respectively. It is still unclear whether the genetic shifts in the *C. ariakensis* hatchery stocks compared with their wild source populations is linked to any changes in growth performance, early recruitment, or development, because the results of side-by-side performance trials comparing within and among hatchery stocks and wild populations have not been reported, and there is little information on the basic biology of *C. ariakensis* in its native region. Differences in larval settlement and swimming, however, were observed among different strains of *C. ariakensis* (Luckenbach 2004, Tamburri et al. 2008). Numerous comparative studies between *C. ariakensis* and *C. virginica* on biological traits such as growth, early development, and disease tolerance were conducted using stocks of *C. ariakensis* derived from the so-called "Oregon Strain" from the west coast of the United States (Calvo et al. 2001, Hudson et al. 2005, Kingsley-Smith & Luckenbach 2008, Paynter et al. 2008), which correspond to the TUI and WCA stocks used in this study. These stocks showed the greatest genetic bottleneck effects and genetic drift from their natural source populations and the other hatchery stocks. Therefore, interpretations of comparisons among studies conducted in the United States during the past decade might be compromised as a result of the use of stocks that might differ from each other and

from wild populations not only genetically, but also in performance and fitness parameters.

Genetic Tracking

Assignment tests based on an individual's genotypic profile have been widely used to detect immigrants, identify hybrids, trace the origins of animals and plants, and detect dispersal patterns (Paetkau et al. 1997, Maudet et al. 2002, Castric & Bernatchez 2003, Manel et al. 2005, Vercaemer et al. 2006). Although an introduction of fertile *C. ariakensis* oysters to Chesapeake Bay was not approved, accidental release or intentional, illegal introduction of reproductively capable *C. ariakensis* has been a concern (Simberloff 2005). Some loose triploid *C. ariakensis* have been found in the waters of North Carolina and Chesapeake Bay (K. S. Reece unpubl. data), although biosecurity regulations for the EIS trials required that all *C. ariakensis* animals be contained during, and retrieved at the conclusion of, the scientific studies. Therefore, the ability to trace such introductions of *C. ariakensis* could be important for controlling these nonnative oysters.

In the current study, domesticated oysters could be tracked back to their specific hatchery stock origins with very high accuracy (≥70%), and most of the observed "incorrect assignments" were assigned to stocks derived from the same source population or to the source population itself. The ability to assign wild oysters accurately back to their specific source population, however, was rather low to moderate (20%–73%), probably as a result of reduced genetic differentiation among these natural populations compared with the relatively high differentiation among the hatchery stocks. However, in an analysis including only the larger sample sizes (i.e., excluding the Korean populations with samples sizes, $n \leq 33$), more than 70% of the wild animals in this study were assigned either to their specific source population or to that from a geographically proximal location. This is not surprising given a genetic pattern of isolation by distance observed in the wild populations (Xiao et al. 2010). Because the probability of correct assignments largely depends on population genetic differentiations (Cornuet et al. 1999, Manel et al. 2005), relatively smaller genetic differentiation and higher gene flow among populations with shorter geographic distances, compared with populations farther apart, probably resulted in a relatively high number of individuals being misassigned to a population geographically proximate to their sampled populations. It has been reported that a 100% correct assignment could be achieved by scoring 10 microsatellite loci on 30–50 individuals from each of 10 populations with F_{ST} values around 0.1 (Cornuet et al. 1999), which is in the range observed among the hatchery stocks in the current study ($\theta_{ST} = 0.132$). However, for those populations with F_{ST} values approximately 10-fold lower, (e.g., ≈ 0.01, comparable with the θ_{ST} value of 0.020 that we observed among the 8 natural populations), the percentage of correct assignments seldom reached 50%, even using 20 loci and 90 individuals per population (Cornuet et al. 1999). Therefore, the low sample sizes of some wild populations in this study, particularly the Korean samples, probably affected the accuracy of these assignment tests. Overall, assignment test results suggest that these microsatellite markers are very powerful for genetic identification of domesticated stocks, and that bolstering the samples sizes and adding more markers could further improve the utility of these markers for identifying animals from wild populations.

NOTE

1. Global θ_{ST} was 0.018 among 16 wild *C. ariakensis* samples originally analyzed. The 16 wild samples were then grouped into eight genetically differentiated populations based on their genetic relationship and after grouping the global θ_{ST} was 0.020 (Xiao et al. 2010).

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