Vol. 460: 195–206, 2012
doi: 10.3354/meps09803

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 24

Mate selection in captive-breeding rockfishes *Sebastes* spp.: inference from parentage analysis and the major histocompatibility complex (MHC)

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ABSTRACT: Rockfish species of the genus *Sebastes* are notable for being numerous and diverse. Rockfishes are unusual among fish because they fertilize their eggs internally and release live, swimming larvae. They undergo complex courting behaviors, which may allow females to be selective about their mates. The major histocompatibility complex (MHC) is implicated as having an important influence on mate selection in other fishes, especially in sticklebacks and salmonids. Research suggests that females choose mates that optimize the MHC genotypes of their offspring. Previous research on rockfishes indicates that multiple functional MHC sequences may be found in each species, and that multiple mating is common in the genus, possibly as a bet-hedging strategy against uncertain or incomplete mate-selection information. In this project, we characterized the MHC genotypes of copper (*S. caurinus*) and quillback (*S. maliger*) rockfish parents, assessed parentage of 14 larval broods, and assessed the MHC genotypes of the parents to determine if MHC-mediated mate choice was occurring. As in previous studies, we found that rockfishes possess multiple, highly variable MHC genes, and that females may mate with multiple males. We also found evidence of female preference for particular males. However, we found no strong evidence of selection based on MHC genotype. Females were not consistently selective based on relatedness, allele count, proportion of shared alleles, or minimum, mean, or maximum DNA or amino acid genetic distance. Instead, it appears that females were selective based on other measures of mate quality not considered in this study, with some hedging of bets through multiple mating also occurring.

KEY WORDS: Major histocompatibility complex · MHC · Mate choice · Balancing selection · Mating system · Multiple paternity · Hybridization

INTRODUCTION

The genus *Sebastes* (the rockfishes) is notable for being speciose (approximately 110 species worldwide) and extremely diverse. Members of the genus are found in a variety of habitat types, from the intertidal zone to depths over 1000 m (Love et al. 2002). Morphology varies widely, and is correlated with life history and ecological role. Types range from streamlined, semi-pelagic species such as the bocaccio *S. paucispinis*, to deep-bodied, spiny, benthic forms, such as the cowcod *S. levis*. A full range of interme-

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diate morphologies also exists in the genus (Love et al. 2002). Rockfishes are long-lived, with some species, such as the rougheye rockfish *S. aleutianus* (205 yr) and shortraker rockfish *S. borealis* (156 yr) estimated to be among the longest-lived fishes in the world (Love et al. 2002). The group is particularly distinguished by the evolution of internal fertilization (Wourms 1991), which may afford them a greater ability to be selective about their mates than is typical of marine fishes.

In contrast to the majority of teleost fishes, rockfishes fertilize their eggs internally and release live, swimming larvae. Larvae are released at the pre-flexion yolk-sac stage, which allows them to avoid the high mortality associated with the egg and early larval stages for a relatively small maternal investment (Boehlert & Yoklavich 1984). Rockfishes are highly fecund, with broods containing thousands to millions of larvae per female (Love et al. 2002). In the unstable environmental conditions of the northeast Pacific, larval survival and recruitment are highly variable from year to year, and strong recruitment may occur only once in decades (Tolimieri & Levin 2005). Long lifespan coupled with high fecundity thus functions as a bet-hedging strategy to ensure population persistence between rare recruitment events in marine fishes such as rockfishes (Winemiller & Rose 1992).

Evidence suggests that rockfishes undergo complex courting behaviors, which may include pheromones and sight or sound cues, before mating can take place (Hallacher 1974, Helvey 1982, Shinomiya & Ezaki 1991, Gingras et al. 1998). Ultimately, the decision whether or not to mate appears to lie with the female (Hyde et al. 2008). Multiple mating, with up to 4 different fathers, has been demonstrated in 13 different rockfish species to date, and may be a common bet-hedging strategy in the genus when mate selection criteria are uncertain or incomplete (Yasui 2001, Hyde et al. 2008, Sogard et al. 2008, Van Doornik et al. 2008, Blanco Gonzalez et al. 2009). As consequences of selective mate choice by females and multiple paternity, a population of organisms that experiences sweepstakes recruitment, such as rockfishes, may experience enhanced genetic diversity and avoid the loss of rare alleles, may see diminished impacts of inbreeding depression, and may benefit from increased effective population size (Hedgecock 1994). Although chance may play the primary role in reproductive success for bet-hedging marine fishes, such as rockfishes (Winemiller & Rose 1992), another potential benefit of mate choice and multiple paternity may be improved pathogen or parasite resistance through optimization of major histocompatibility complex (MHC) genotype.

The MHC is a highly polymorphic multigene family involved in self-nonself recognition in the immune system of vertebrates. MHC genes encode receptors that bind fragments of local and foreign peptides, then present the combined fragments to T-cells, which may initiate a series of immune responses (Ploegh & Watts 1998). The amino acid (AA) sequence of the MHC protein determines antigen binding, and thus, which foreign peptides can be recognized (Brown et al. 1988). Therefore, greater MHC diversity would be expected to result in greater sensitivity to pathogens and a more effective immune response (Doherty & Zinkernagel 1975). Because self-reactivity can lower the number of T-cells in species that express several MHC loci, an intermediate number of alleles may result in better pathogen recognition in these cases (Woelfing et al. 2009). Given the high polymorphism and potential benefits associated with MHC-based mate preference, 2 main hypotheses have been proposed: (1) MHC genotypes could function to discriminate close kin, and might help females to avoid inbreeding (Penn & Potts 1999, Landry et al. 2001), or (2) negative assortative mating based on MHC genotype might improve the pathogen resistance of offspring (Doherty & Zinkernagel 1975, Landry et al. 2001). Mate selection based on MHC genotype has been demonstrated in several fish species to date, including three-spined sticklebacks *Gasterosteus aculeatus* (Reusch et al. 2001, Aeschlimann et al. 2003, Milinski et al. 2005, Eizaguirre et al. 2009, Kalbe et al. 2009, Lenz et al. 2009), a variety of salmonids (Atlantic salmon *Salmo salar*: Landry et al. 2001, Consuegra & de Leaniz 2008; brown trout *Salmo trutta*: Forsberg et al. 2007; Chinook salmon *Oncorhynchus tshawytscha*: Neff et al. 2008, Garner et al. 2010), and other freshwater fish species (e.g. rose bitterling *Rhodeus occelatus*: Casalini et al. 2009). This research suggests that MHC genes do influence mate choice decisions in fish, that both good genes (i.e. specific alleles that confer improved pathogen resistance) and overall MHC diversity may be considered by females, and that mating decisions may be complicated by other factors, such as body size and coloration or aggression between individuals (Landry et al. 2001, Reusch et al. 2001, Consuegra & de Leaniz 2008, Neff et al. 2008, Eizaguirre et al. 2009, Kalbe et al. 2009, Garner et al. 2010). However, there are very few studies on MHC-based mate selection in marine fishes. One reason for this may be the difficulty of performing laboratory-based experimental research on large, long-lived marine fishes. Another reason may be that marine fishes are much less likely to encounter close

kin, which would make the hypothesis that MHC-based mate selection partially functions to avoid inbreeding much less applicable (Landry et al. 2001). However, since marine fishes are exposed to numerous parasite threats, the potential improvement in immunocompetence associated with mate selection based on MHC genotype should apply equally to marine and freshwater fishes (Penn et al. 2002).

Copper (*Sebastes caurinus*) and quillback (*S. maliger*) rockfishes were chosen as model organisms to study MHC diversity and mate selection in nearshore rockfishes. The copper rockfish is found from the northern Gulf of Alaska to central Baja California, in waters from the subtidal zone to depths of ~180 m. The quillback rockfish ranges from the Gulf of Alaska to the northern California Bight, and is found from subtidal depths to ~275 m (Love et al. 2002). Both species prefer areas of high- to medium-relief rocks, although they may also be found over low-relief rock habitat. Both are long-lived, with coppers aged to 50 yr, and quillbacks aged to at least 95 yr (Love et al. 2002). Likewise, both mature around 7 yr (Lea et al. 1999). Larval release occurs between January and June in copper rockfish, and between March and June in quillback rockfish (Love et al. 2002). Both species probably only produce a single brood annually (Moser 1967). Copper and quillback rockfishes have been shown to hybridize in nature (Seeb 1998). An MHC class II B genotype has been described for a single copper rockfish (Aguilar & Garza 2005), but no data have yet been published on quillback rockfish. No research has been published on multiple paternity or mate selection in either species.

Our goals in this study were threefold. First, we characterized MHC diversity in captive-breeding populations consisting of 69 copper and 89 quillback rockfishes at the Oregon Coast Aquarium (Newport, OR). Then, we genotyped larvae of known mothers to assess parentage, including the prevalence of multiple paternity in these 2 previously unexamined species. Finally, we related the MHC genotypes of the parents of realized matings to the suite of possible matings to draw inferences about the role of MHC genotype in mate choice in rockfishes.

MATERIALS AND METHODS

Adult tissue collections

Copper and quillback rockfishes were collected as newly settled juveniles or as adults from the Pacific Ocean near Newport, Oregon to populate the Passages of the Deep section of the Oregon Coast Aquarium between 1997 and 1999 (J. Burke pers. comm.). In September and October of 2009, all the adult copper and quillback rockfishes were collected from the Orford Reef display tank in the Passages of the Deep section of the Oregon Coast Aquarium by divers using hand nets. Fish were placed into an adjacent medical isolation tank until all individuals had been captured. Fin-clip tissue samples were collected from each individual and stored in 95% ethanol for DNA analysis, and each fish was PIT-tagged for future identification before release back into the display tank.

Larvae collections

In 2009 and 2010, gravid female copper and quillback rockfishes were collected by SCUBA divers using hand nets from the Orford Reef tank, identified based on their PIT-tag number, and individually placed into 757 l plastic barrels with isolated inflow and filtered outflow until they released their larvae. Larval samples were collected by hand with dip nets and preserved in 50 ml Falcon tubes with 95% ethanol. A total of 4 broods were collected and genotyped in 2009 (additional broods were collected but failed to yield genotypes), and a total of 10 broods were collected and genotyped in 2010.

MHC genotyping

Total genomic DNA was extracted from adult fin-clip tissue samples using a standard glass-fiber plate protocol (Ivanova et al. 2006). Polymerase chain reaction (PCR), with primers XIS and MRS (Cohen 2002), was used to amplify the majority of exon 2 of the MHC B1 unit, along with the preceding intron and a segment of the leader peptide for all adult samples. PCR reactions were performed with GoTaq Flexi polymerase (Promega) and optimized for high fidelity. PCR cycling conditions were as follows: initial denaturation at 95°C for 2 min, then 35 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min, followed by a final extension at 72°C for 5 min. These PCR products were cloned using the TOPO TA cloning kit (Invitrogen), and 12 colonies were sequenced per adult sample. Clones were sequenced on an ABI 3730XL (Applied Biosystems) capillary sequencer using

M13 forward and reverse primers. All sequences were aligned and edited in Sequencher v4.7 (Gene Codes), and trimmed to include only exon sequences (based on sequences from Aguilar & Garza 2005) for analysis. All exon sequences have been deposited in Genbank (Accession nos. JQ620251–JQ621832). Only unique exon sequences were used in MHC diversity analyses. Although the total number of MHC genes in rockfishes is unknown, we hereafter refer to distinct sequences as alleles. Synonymous (d_s) and non-synonymous (d_n) substitution rates were calculated for the complete exon, and for putative antigen binding site (ABS) and non-ABS codons (inferred from the human HLA-DRB1 locus; Brown et al. 1993; and following Aguilar & Garza 2005 and Cohen 2002) using DnaSP v5.0 (Librado & Rozas 2009). Significance of d_n:d_s ratios was tested using a paired Student's *t*-test in S-Plus (Insightful).

Parentage analysis

Genomic DNA was extracted from adult fin-clip tissue samples or whole larvae using a standard glass-fiber-plate protocol (Ivanova et al. 2006). For all adults, 22 previously published microsatellite markers (Table 1; Roques et al. 1999, Wimberger et al. 1999, Miller et al. 2000, Gomez-Uchida et al. 2003, Westerman et al. 2005) were genotyped and tested for utility in parentage analysis. Locus-by-locus deviations from Hardy-Weinberg equilibrium and inbreeding coefficients were assessed using Genetix v4.05.2 (Table 1; Belkhir et al. 2004). Larval genotypes utilizing 6 markers (*Spi4*, *Spi6*, *Spi10* [Gomez-Uchida et al. 2003]; *Sma3*, *Sma10*, *Sma11* [Wimberger et al. 1999]) were adequate to unambiguously identify parents. Microsatellite markers were PCR amplified with fluorescently-labeled forward primers (MWG Biotech). PCR conditions were as follows: initial denaturation at 94°C for 2 min, then 25 cycles of 94°C for 1 min, 45–62°C for 30 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. PCR products were visualized alongside internal molecular weight standards using an ABI 3730XL DNA analyzer and sized using Genemapper (ABI) software. Parentage was assessed using GERUD2.0 (Jones 2005) with known maternal genotypes. Putative paternal genotypes reconstructed from larval genotypes in GERUD2.0 were matched to the data set of true parental genotypes to identify actual fathers.

Mate selection

To assess whether females mated non-randomly with the available males in our captive population, we conducted hypothesis tests on 5 different parameters. In each case, we calculated the parameter for all possible pairs in the population using custom scripts in R (R Development Core Team 2011), and calculated critical values (2.5 and 97.5% quantiles for 2-sided tests, 95% quantile for 1-sided tests) for the population. Then, we calculated each parameter for all the pairings we observed in our matings. We also calculated the mean value for each parameter for the following 5 groupings of our observed matings: all broods, Male 2592 alone, Male 2653 alone, all broods excluding Male 2592, and all broods excluding Males 2592 and 2653. We tested the hypothesis that females choose mates based on their level of relatedness, by calculating maximum-likelihood estimates of relatedness (r) in ML-Relate (Kalinowski et al. 2006) using all 22 genotyped microsatellite markers. Because the level of pairwise relatedness was low on average in the population, we tested the hypothesis that females mated with more closely related males than if they had been choosing randomly (1-tailed test). We also performed several hypothesis tests to assess the association between MHC genotype and mate choice in rockfishes.

In all MHC tests we assumed that MHC genotypes were unbiased and were a random sampling of the loci present in the individual, since the primers used (XIS and MRS; Cohen 2002) are highly degenerate. To test whether females choose mates based on differences in allele counts, we calculated pairwise absolute differences between the number of distinct MHC alleles found in the mother versus number of distinct alleles found in the father. The hypothesis being tested here was that females chose males with significantly greater or fewer distinct alleles (2-tailed test). To test the hypothesis that females select mates based on shared alleles, we calculated the proportion of shared MHC alleles (Wetton et al. 1987). The proportion of shared alleles (D) was calculated as $D = 2F_{ab}/(F_a + F_b)$, where F_{ab} is the number of alleles shared by the individuals in the pair, and F_a and F_b are the numbers of alleles found in each individual. The hypothesis here was that mated pairs share significantly more alleles than random pairs drawn from the population (1-tailed test). To test the hypothesis that females choose mates based on genetic distances between their own and potential mates' alleles, we calculated the mean, minimum, and maximum DNA distance and AA distance between all

Table 1. *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish). Genotyping primers, locus-by-locus number of alleles, expected (H_e) and observed (H_o) heterozygosities with significance (p), and inbreeding coefficient (F_{IS}) with significance (p), characterized for all possible parents in Orford Reef display tank, Oregon Coast Aquarium. Markers used in parentage analysis are highlighted in grey. Temperature profile: 94°C / 2 min, 25 × (94°C / 1 min, anneal / 30 s, 72°C / 10 s), 72°C / 10 min, 10°C hold

Locus	Species	Number of alleles	H_e	H_o	p	F_{IS}	p	Annealing temperature (°C)	Source
Spi4	Copper	15	0.88	0.84	0.342	0.042	0.187	58	Gomez-Uchida et al. (2003)
	Quillback	16	0.88	0.88	0.739	0.010	0.472		
Spi6	Copper	20	0.90	0.88	0.340	0.022	0.321	58	
	Quillback	19	0.88	0.88	0.359	0.007	0.481		
Spi10	Copper	5	0.64	0.75	0.980	−0.178	0.992	58	
	Quillback	4	0.61	0.60	0.214	0.020	0.437		
Spi12	Copper	4	0.40	0.39	0.137	0.027	0.444	58	
	Quillback	7	0.43	0.36	0.002	0.156	0.031		
Spi18	Copper	24	0.89	0.89	0.044	0.000	0.565	58	
	Quillback	21	0.87	0.84	0.005	0.034	0.217		
Sma1	Copper	13	0.80	0.87	0.956	−0.093	0.988	48	Wimberger et al. (1999)
	Quillback	12	0.67	0.63	0.077	0.061	0.171		
Sma2	Copper	6	0.32	0.31	0.021	0.012	0.510	58	
	Quillback	5	0.47	0.38	0.047	0.182	0.030		
Sma3	Copper	7	0.58	0.61	0.728	−0.055	0.790	58	
	Quillback	5	0.72	0.76	0.916	−0.067	0.899		
Sma4	Copper	7	0.60	0.54	0.017	0.109	0.129	58	
	Quillback	6	0.71	0.66	0.001	0.078	0.135		
Sma5	Copper	3	0.12	0.10	0.004	0.186	0.149	58	
	Quillback	4	0.44	0.46	0.615	−0.045	0.710		
Sma10	Copper	16	0.70	0.64	0.153	0.084	0.123	58	
	Quillback	15	0.81	0.72	0.001	0.116	0.013		
Sma11	Copper	6	0.66	0.73	0.856	−0.107	0.936	58	
	Quillback	10	0.62	0.55	0.057	0.112	0.039		
Seb9	Copper	5	0.59	0.66	0.269	−0.117	0.900	62	Roques et al. (1999)
	Quillback	5	0.67	0.66	0.280	0.017	0.443		
Sal1	Copper	11	0.81	0.71	0.069	0.122	0.033	45	Miller et al. (2000)
	Quillback	8	0.77	0.72	0.125	0.076	0.115		
Sal2	Copper	6	0.61	0.64	0.586	−0.052	0.794	48	
	Quillback	9	0.72	0.67	0.037	0.065	0.143		
Sal3	Copper	10	0.68	0.64	0.241	0.054	0.255	48	
	Quillback	8	0.63	0.43	<0.001	0.324	<0.001		
Sal4	Copper	10	0.82	0.81	0.085	0.011	0.475	52	
	Quillback	8	0.79	0.77	0.468	0.022	0.401		
Sra7-7	Copper	11	0.70	0.77	0.865	−0.107	0.959	57	Westerman et al. (2005)
	Quillback	13	0.77	0.81	0.552	−0.045	0.834		
Sra7-25	Copper	13	0.83	0.83	0.507	0.001	0.548	57	
	Quillback	11	0.81	0.73	0.018	0.098	0.040		
Sra11-103	Copper	3	0.43	0.40	0.277	0.068	0.338	57	
	Quillback	3	0.28	0.25	0.181	0.129	0.169		
Sra15-8	Copper	12	0.81	0.79	0.162	0.033	0.311	52	
	Quillback	7	0.80	0.79	0.364	0.013	0.476		
Sra16-5	Copper	30	0.95	0.97	0.666	−0.020	0.858	52	
	Quillback	22	0.86	0.79	0.098	0.081	0.030		

possible pairings in our population, and between our actual matings. DNA distances were calculated using an F84 (Felsenstein 1984) substitution model in the DNADIST module of Phylip v3.67 (Felsenstein 1989). Amino acid distances were calculated using the Dayhoff PAM substitution matrix (Dayhoff 1979) as implemented in the PROTDIST module of Phylip v3.67 (Felsenstein 1989). For mean and maximum distances, we tested the hypothesis that our realized matings had significantly higher or lower distances

than random pairs drawn from the population (2-tailed test). For the minimum distance, we tested the hypothesis that actual matings had significantly higher distances than random population draws (1-tailed test).

RESULTS

MHC genotyping

Sequences between 400 and 800 base pairs (bp) in length were recovered from both study species. Genbank nucleotide BLAST (NCBI) searches utilizing these sequences as the query invariably returned previously published (Aguilar & Garza 2005) *Sebastes* MHC sequences as the highest-likelihood match. In accordance with the findings of Aguilar & Garza (2005), sequences were composed of a long intron, containing a variably-repeated minisatellite sequence with no species-specific pattern, and a 255 bp exon sequence. We found between 2 and 9 unique sequences per individual copper rockfish, and between 2 and 10 sequences in quillback rockfish. The mean ± SD number of unique sequences per individual (standardized to sequences per 10 clones) was 5.31 ± 1.62 for copper rockfish and 5.58 ± 1.63 for quillback rockfish (Fig. 1). We identified a total of 166 alleles in 69 copper rockfish and 315 alleles in 89 quillback rockfish. Forty-one sequences were found in both species. Allele sequences varied widely, with genetic distances (General time reversible + Invariant sites + Gamma distributed model) between sequences ranging from a minimum of 0.00395 to a maximum of 0.38749 across both species. The ratio of

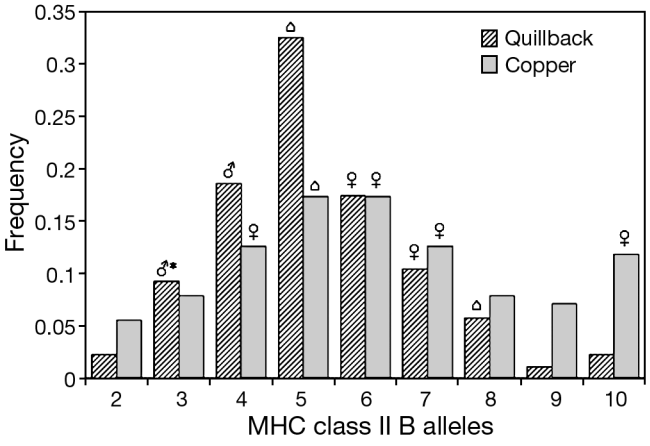

Fig. 1. *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish). Frequency distribution of the number of major histocompatibility complex (MHC) class II B alleles (exon 2 sequences) in 69 adult copper (striped bars) and 87 quillback (grey bars) rockfishes from the Oregon Coast Aquarium. Mean ± SD number of MHC alleles per 10 clones sequenced was 5.31 ± 1.62 for copper rockfish and 5.58 ± 1.63 for quillback rockfish. (♀) Presence of a female parent, (♂) presence of a male parent, and (♁) both parents in the category; (♂*) dominant male (ID no. 2592) in our broods

Table 2. *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish). Brood, mother's ID, mother's species, number of larvae successfully genotyped, number of unique paternal alleles detected at each locus, identified sires, and the number of larvae fathered by each sire. * indicates hybridization

Brood	Mother's ID	Species	No. of larvae	*Spi4*	*Spi6*	*Spi10*	*Sma3*	*Sma10*	*Sma11*	ID of sires (number of larvae)
1	2501 (2009 brood)	Copper	93	2	1	1	0	0	1	2592* (92), 2653* (1)
2	2501 (2010 brood)	Copper	91	2	1	1	0	0	1	2592* (91)
3	2502	Quillback	94	2	1	0	1	0	2	2592 (94)
4	2504 (2009 brood)	Copper	92	2	3	2	0	2	2	2575 (87), 2529* (5)
5	2504 (2010 brood)	Copper	93	2	2	0	1	0	1	2592* (93)
6	2506	Quillback	87	2	1	0	1	1	0	2523 (87)
7	2566	Quillback	94	2	2	0	1	2	0	2585 (94)
8	2571	Copper	93	1	1	1	0	1	0	2653* (93)
9	2574	Copper	94	4	2	1	2	1	2	2638 (76), 2592* (18)
10	2590	Copper	183	1	1	1	0	1	1	2653* (183)
11	2598	Copper	86	2	1	0	1	0	2	2592* (86)
12	2608	Quillback	93	1	2	0	1	0	1	2592 (93)
13	2628	Copper	93	2	1	1	0	0	1	2582 (93)
14	2670	Copper	93	1	2	0	1	0	1	2592* (93)

Table 3. *Sebastes caurinus* (copper rockfish) and *S. maliger* (quillback rockfish). Mean (± SD) non-synonymous (d_n) and synonymous (d_s) substitution rates for comparisons within and between species, d_n:d_s ratios, and results of paired Student's *t*-tests testing the hypothesis that $d_n = d_s$. ABS = antigen binding site

Species	Gene region	d_n	d_s	d_n:d_s	*t*	df	p
Both	ABS	0.348 (±0.114)	0.146 (±0.100)	2.381	926.0	144452	<0.001
	Non-ABS	0.156 (±0.050)	0.078 (±0.049)	2.008	635.1		<0.001
	Overall	0.209 (±0.060)	0.096 (±0.052)	2.174	705.6		<0.001
Copper	ABS	0.351 (±0.116)	0.153 (±0.106)	2.289	278.9	29645	<0.001
	Non-ABS	0.153 (±0.052)	0.078 (±0.051)	1.971	299.9		<0.001
	Overall	0.208 (±0.062)	0.098 (±0.055)	2.120	392.0		<0.001
Quillback	ABS	0.347 (±0.116)	0.143 (±0.096)	2.427	381.8	50085	<0.001
	Non-ABS	0.159 (±0.052)	0.078 (±0.049)	2.038	434.1		<0.001
	Overall	0.211 (±0.062)	0.096 (±0.051)	2.204	561.9		<0.001

non-synonymous (d_n) to synonymous (d_s) substitution rates was significantly >1 in all comparisons (Table 2). This higher rate of non-synonymous versus synonymous substitutions was found in both species (combined, and considered separately), and irrespective of whether all codons were considered; solely putative ABS codons, or solely non-ABS codons.

Parentage analysis

We identified paternity of a total of 1379 larvae in 14 broods (Table 3). In 13 of 14 cases, GERUD2.0 reconstructed a single putative paternal genotype. In one case (Mother 2501, 2009 brood), GERUD2.0 returned 40 potential paternal genotype pairs. In this case, only 1 of the top 5 most likely paternal genotypes matched actual fathers in the parental data set. Twelve different mothers produced larvae, and 8 different fathers were matched to GERUD reconstructions in the sample set. Two mothers produced larvae in both 2009 and 2010. Three broods showed evidence of multiple paternity (2 sires), although the contribution of the second father was always a small fraction of the total (no more than 20%). One sire, 2592, was involved in producing 8 of the 14 broods in the sample set. This individual, a quillback rockfish, mated with females of both species and produced viable larvae with both (data not shown). Other hybrid matings were also observed in the sample set.

Mate selection

Only a single observed mate pair (out of 17 mated pairs in 14 broods) had a relatedness value that exceeded the 95% critical value (Table 4). None of the group means exceeded the 95% value for relatedness. Likewise, no individual or mean absolute allele number difference was greater than the 95% critical value. Three mate pairs shared a significantly greater proportion of their alleles than expected due to chance. Considering DNA distances, 3 pairs had mean values that fell below the 2.5% critical value and 1 pair had a mean value that exceeded the 97.5% value. Two pairs had maximum distance values that fell below the 2.5% critical value. No mate pairs had maximum distance values that exceeded the 97.5% critical value and none of the 5 group means diverged significantly from the population values for mean or maximum DNA distance. Four pairs had significantly greater minimum DNA distances than would be expected due to chance, but none of the minimum DNA distance group mean values were significantly different from the population mean. Amino acid distances showed the same pattern as DNA distances. Three pairs had significantly smaller mean AA distances than expected by chance, while 1 pair had a significantly larger mean distance. Two pairs had significantly smaller maximum AA distances. Finally, 2 pairs had significantly greater minimum AA distances than expected by chance, and a further 2 pairs were nearly significant (these near-significant pairs were significant in the DNA analysis). None of the AA distance group means diverged significantly from the underlying population.

DISCUSSION

MHC genotyping

Both copper and quillback rockfishes show evidence of multiple MHC class II B genes. Aguilar &

Table 4. *Sebastes caurinus* and *S. maliger*. Significance testing results for individual broods and all group mean comparisons. Values below the 2.5% critical value are **bold**, values above the 97.5% critical value are *italicized*, and values above the 95% (1-tailed) critical value are ***bold italicized.*** AA: amino acid

Brood	Mother's ID	Father's ID	Relatedness	Absolute difference	Proportion of shared alleles	DNA distance			AA distance		
						Mean	Max.	Min.	Mean	Max.	Min.
1	2501	2592	0.122	1.667	0.000	0.201	0.212	***0.185***	0.398	0.428	0.351
1	2501	2653	0.122	0.455	***0.222***	0.153	0.297	0.000	0.312	0.616	0.000
2	2501	2592	0.122	1.667	0.000	0.201	0.212	***0.185***	0.398	0.428	0.351
3	2502	2592	0.000	0.000	0.000	**0.116**	**0.132**	0.094	**0.230**	**0.261**	0.194
4	2504	2529	0.052	0.159	0.000	0.166	0.240	0.004	0.323	0.462	0.000
4	2504	2575	0.250	0.000	0.000	0.185	0.249	0.058	0.361	0.538	0.103
5	2504	2592	0.033	2.222	0.000	0.176	0.225	0.138	0.358	0.440	0.282
6	2506	2523	0.000	3.333	0.000	0.216	0.321	0.107	0.430	0.681	0.243
7	2566	2585	***0.297***	0.500	***0.286***	0.151	0.337	0.000	0.298	0.646	0.000
8	2571	2653	0.190	1.705	0.182	0.157	0.232	0.000	0.298	0.454	0.000
9	2574	2592	0.177	3.810	***0.250***	**0.030**	**0.132**	0.000	**0.057**	**0.249**	0.000
9	2574	2638	0.017	1.310	0.000	0.201	0.280	0.113	0.415	0.608	0.217
10	2590	2653	0.060	1.688	0.000	*0.257*	0.347	***0.178***	*0.533*	0.693	***0.404***
11	2598	2592	0.050	3.667	0.000	0.188	0.247	0.148	0.408	0.603	0.301
12	2608	2592	0.118	3.333	0.000	0.185	0.217	0.137	0.383	0.447	0.264
13	2628	2582	0.231	1.818	0.000	0.251	0.350	***0.196***	0.496	0.687	***0.381***
14	2670	2592	0.000	1.212	0.000	**0.087**	0.247	0.000	**0.199**	0.603	0.000
Means:	All matings		0.108	1.679	0.055	0.172	0.252	0.091	0.347	0.520	0.182
	2592		0.078	2.197	0.031	0.148	0.203	0.111	0.304	0.432	0.218
	2653		0.124	1.282	0.141	0.189	0.292	0.059	0.381	0.587	0.135
	Excluding 2592		0.135	1.219	0.077	0.193	0.295	0.073	0.385	0.598	0.150
	Excluding 2592 and 2653		0.141	1.187	0.044	0.195	0.296	0.080	0.387	0.604	0.157
	Population mean		0.073	1.828	0.016	0.200	0.303	0.093	0.411	0.633	0.189
	Quantiles:	**2.50%**	–	–	–	0.140	0.201	–	0.287	0.408	–
		97.50%	–	–	–	0.256	0.379	–	0.530	0.797	–
		95%	0.269	4.550	0.222	–	–	0.174	–	–	0.352

Garza (2005) found between 2 and 7 unique sequences in single individuals of the 12 *Sebastes* species they considered, including copper rockfish. This compares closely with the average of 5.31 and 5.58 unique sequences per 10 clones sequenced for copper and quillback rockfishes, respectively, in our sample set. Given the high number of alleles per individual in both species, it is likely that sequencing 12 clones per individual underestimates the true intra-individual MHC diversity in both species. However, we believe that this is unlikely to bias our analysis of the role of MHC in mate selection. It was not possible to assess the paralogous versus allelic status of our different sequences, because intron sequences varied widely in repeat number and thus could not be aligned. Both species were very diverse, both in terms of number of unique sequences, and in genetic distances between sequences. We also found strong evidence of balancing selection, both in ABS and non-ABS codons. This agrees with previous findings, and likely indicates recent functionality of these genes. Our finding of a relatively high degree of trans-species allelism is also in line with previously published findings on MHC in rockfishes (Garrigan & Hedrick 2003, Aguilar & Garza 2005).

Parentage analysis

We found evidence of multiple paternity in 3 of 14 broods examined, all of which were produced by copper rockfish mothers. No evidence of multiple paternity was found in broods from quillback rockfish. Multiple paternity has been described in 13 of 21 rockfish species examined to date (Yoshida et al. 2001, Hyde et al. 2008, Sogard et al. 2008, Van Doornik et al. 2008, Blanco Gonzalez et al. 2009), and seems to be common in the genus. Considering the close relationship of copper and quillback rockfishes (Hyde & Vetter 2007), the absence of evidence of multiple paternity in quillback rockfish in the present study may be a consequence of low sample size, rather than evidence that it does not occur. Multiple paternity may be a bet-hedging strategy for rockfishes when a female's knowledge of mate selection criteria is imperfect (Yasui 2001). Such a strategy may improve the genetic diversity of offspring from a single female, thereby improving the odds that some fraction of her larvae encounter favorable environmental conditions and survive (Cushing 1990, Hyde et al. 2008, Van Doornik et al. 2008). Another potential benefit of multiple mating for individual females is the potential reduction in the probability of incomplete fertilization of eggs in a brood (Gunderson 1977, Sogard et al. 2008). As a consequence of the benefits to individual females, the population may benefit from reduced inbreeding depression, and an increase in the effective population size (N_E; Hyde et al. 2008). Evidence for hybrid matings was surprisingly common in our data (9 of 14 broods). Although this may be related to effects of long-term holding of parents in captivity, hybridization has previously been described in wild populations of these 2 species (Seeb 1998), and may be a potential source of genetic diversity in the genus *Sebastes*. Finally, a single male quillback rockfish sired part or all of 8 of the 14 broods in the sample set, and mated with females of both species. Although no previous data exists for mating preferences in rockfishes, results from another study of the related *Sebastiscus marmoratus* suggest that females may select the largest male available, when direct comparisons are possible (Ng et al. 2003). Thus, the dominance of 1 male in our samples may simply result from him being the largest available male. Without matched size and sex data for our samples, however, we can only conjecture.

Mate selection

Our results suggest that rockfish females pay little attention to overall relatedness when selecting possible mates. We likewise found no evidence for mate selection based on difference in number of MHC alleles. Several pairings shared significantly more alleles than would be expected due to chance, although none of the mating means diverged significantly from null expectations. Similarly, several individual pairings had mean, maximum, or minimum AA or DNA distances that fell outside the critical values. Significant mean and maximum DNA and AA distance values mostly fell below the lower critical value, although 1 mating had mean DNA and AA distances that exceeded the upper critical value. Four matings had significantly or near-significantly greater minimum DNA and AA distances than would be expected due to chance. None of the combined means fell outside the critical values of any of the measured parameters. Significant mean and maximum distances tended to fall below the lower critical value. However, a number of minimum distances fell above the critical value, suggesting that females may prefer males that are moderately divergent in their MHC genotype. Our results are

consistent with previous findings in sticklebacks that females choose mates with intermediate MHC diversity (Lenz et al. 2009), in this case with respect to genetic distances. However, the overall pattern in our data is of random mating with respect to relatedness and MHC genotype.

CONCLUSIONS

Rockfishes are characterized by low overall survivorship through the larval stage, coupled with a very high degree of stochasticity in larval survival between years (Love et al. 2002). In most years, few young fish may survive to recruit to the adult population, and strong year classes may only occur once in a decade (Love et al. 2002, Tolimieri & Levin 2005). The importance of chance in deciding early survivorship may severely discount the importance of selecting mates to specifically optimize larval MHC genotype. Our research suggests that some females may use MHC genotype information to choose mates, but that there is no consistent pattern of preference in the study population. Instead, evidence from the current study suggests that females select mates based on some other measure of quality, such as size, as has previously been shown in the sister genus to *Sebastes*, the genus *Sebastiscus* (Ng et al. 2003). In addition, some females mated with multiple males, potentially to avoid unfertilized eggs, or as a bet-hedging strategy when males displayed similar quality measures (Yasui 2001). The current research strongly suggests that female copper and quillback rockfishes are selective about which males they allow to mate. However, further research will be required to properly isolate the male characteristics that the females use to assess quality.

Acknowledgements. The authors gratefully acknowledge all those who assisted in sample collection and PIT-tagging: E. Mochon-Collura, B. Brady, Z. Steele, R. Hildebrand, D. Wyatt, O. Jaff, J. Fischer, B. Johnson, M. Akers, P. Rankin, T. Frierson, I. Chun, S. Clausen, K. Ravenscroft-Ligman, V. Hodges, J. Burke, and countless Oregon Coast Aquarium volunteer divers. We thank F. Alberto for assistance with data analysis, and S. Heppell, S. Palumbi, V. Buonaccorsi, and 3 anonymous reviewers for comments that greatly improved the manuscript. This work was partially funded by the National Science Foundation REU Site program under Grant No. NSF OCE-0648515.

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Editorial responsibility: Philippe Borsa, Montpellier, France

Submitted: October 13, 2011; Accepted: May 4, 2012
Proofs received from author(s): July 6, 2012