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Population Genetic Structuring in *Acanthopagrus butcheri* (Pisces: Sparidae): Does Low Gene Flow Among Estuaries Apply to Both Sexes?

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Abstract

Acanthopagrus butcheri completes its entire life history within estuaries and coastal lakes of southern Australia, although adults occasionally move between estuaries via the sea. Consequently, it is expected that populations of *A. butcheri* in different estuaries will be genetically distinct, with the magnitude of genetic divergence increasing with geographic isolation. However, previous genetic studies of *A. butcheri* from southeast Australia yielded conflicting results; allozyme variation exhibited minimal spatial structuring (θ = 0.012), whereas mitochondrial DNA distinguished the majority of populations analyzed (θ = 0.263) and genetic divergence was positively correlated with geographic isolation. This discrepancy could reflect high male gene flow, which impacts nuclear but not mitochondrial markers. Here we estimated allele frequencies at five nuclear microsatellite loci across 11 southeast Australian populations (595 individuals). Overall structuring of microsatellite variation was weaker (θ = 0.088) than that observed for mitochondrial DNA, but was able to distinguish a greater number of populations and was positively correlated with geographic distance. Therefore, we reject high male gene flow and invoke a stepping-stone model of infrequent gene flow among estuaries for both sexes. Likewise, management of *A. butcheri* within the study range should be conducted at the scale of individual or geographically proximate estuaries for both sexes. The lack of allozyme structuring in southeast Australia reflects either the large variance in structuring expected among loci under neutral conditions and the low number of allozymes surveyed or a recent colonization of estuaries such that some but not all nuclear loci have approached migration-drift equilibrium.

Keywords: Isolation by distance — microsatellite — migration-drift equilibrium — sea level — selection — stepping stone

Introduction

Patterns of molecular variation among groups of individuals separated spatially or temporally are extensively employed to test for contemporary gene flow, as this knowledge is beneficial for studies of ecology and evolution, as well as for the assessment of conservation priorities (e.g., Garber et al., 2004; Beacham et al., 2005). However, different classes of molecular markers may provide conflicting interpretations of gene flow, owing to differences in their underlying characteristics (mutations rates, mode of inheritance, function, effective population size). Consequently, it is desirable to apply several classes of molecular markers when studying gene flow in a given taxon (Arnaud-Haond et al., 2003; Hoarau et al., 2004), in addition to nonmolecular techniques (e.g., tagging, telemetry, otolith chemistry).

The black bream *Acanthopagrus butcheri* (Munro) is an estuarine sparid (Pisces: Sparidae) occurring throughout the southern half (27°40′–43°40′S) and almost entire longitudinal range of Australia (Allen et al., 2002). This is an important commercial and recreational fishery species (Kailola et al., 1993; Coutin, 2000), and its dependence on estuarine environments confers elevated conservation concern (Hodgkin, 1994). Fisheries in some estuaries have also experienced recent collapses (Blackwood River, Sarre and Potter, 2000; Gippsland Lakes, Coutin and Conron, 2006). Technologies for

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the captive breeding and rearing of *A. butcheri* have recently been developed with the aim of future production for human consumption (Doupé et al., 2005), and progeny have also been used to augment natural populations (Lenanton et al., 1999). Consequently, knowledge of stock structuring in this species is desirable for the future management and conservation of this resource (Carvalho and Hauser, 1995).

Acanthopagrus butcheri is a rare example of a fish that completes its entire lifecycle within estuaries (Potter and Hyndes, 1999). Movement between estuaries appears infrequent based on tagging studies, but has been documented for estuaries over 50 km apart (Butcher and Ling, 1962; Gorman, 1965; P.C. Coutin, unpublished). Consequently, populations in different estuaries are expected to be genetically divergent from one another (Bilton et al., 2002; Watts and Johnson, 2004), and represent distinct stocks for management purposes. However, in contrast to expectations based on ecology and tagging, studies of allozymes (nuclear genome) among southeast Australian populations of *A. butcheri* revealed low spatial structuring of variation; allele frequencies at three polymorphic loci were homogeneous throughout much of southeast Australia, with the exception of two peripheral populations (Figure 1; Farrington et al., 2000; Burridge et al., 2004). Conversely, variation in mitochondrial DNA (mtDNA) exhibited significant heterogeneity throughout the same range (Figure 1; Burridge et al., 2004), consistent with expectations.

From a management and conservation perspective, the previous genetic results for *A. butcheri* must be interpreted cautiously. Although the mtDNA structuring is strong and consistent with expectations, the predominantly maternal inheri-

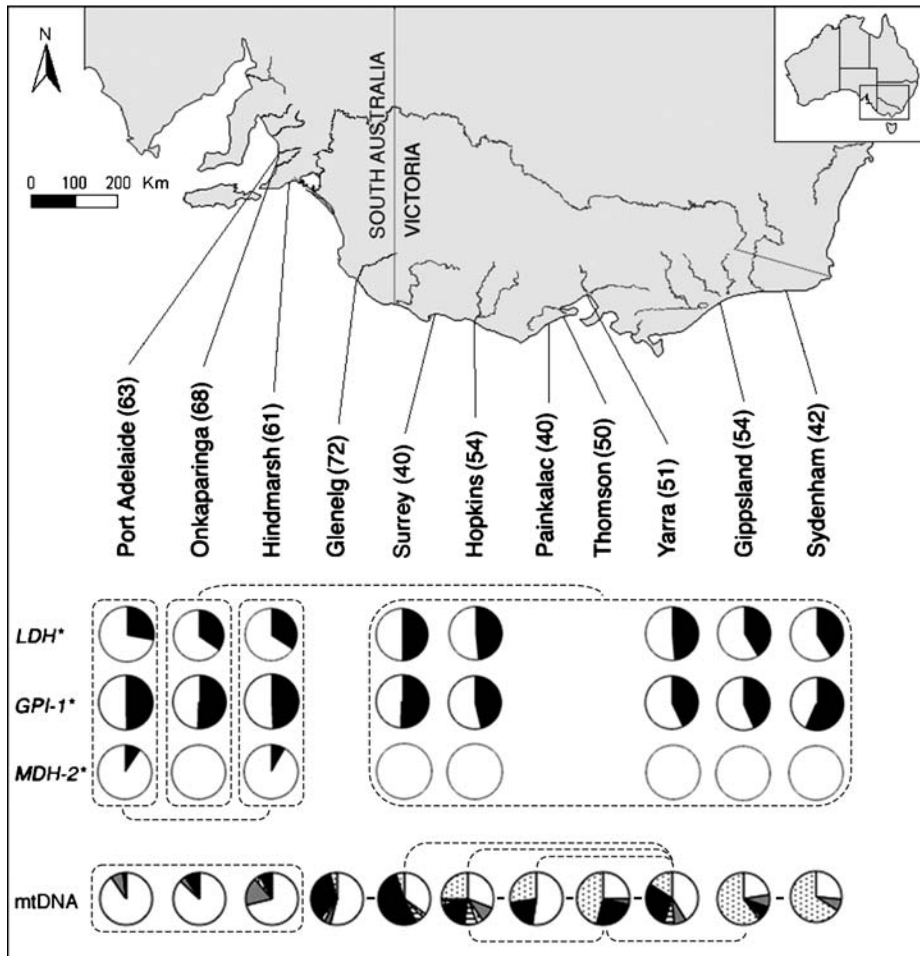

Fig. 1. *Acanthopagrus butcheri* sampling localities from estuaries in southeast Australia. Numbers in parentheses represent sample sizes of individuals scored for microsatellite variation during this study (and mtDNA variation of sites analyzed for the first time: Glenelg, Painkalac, and Thomson). Allozyme allele and mitochondrial haplotype frequencies are depicted below sites analyzed. (Allozyme data: Farrington et al., 2000; Burridge et al., 2004. Mitochondrial data: Burridge et al., 2004; this study). Populations that are homogeneous for allele or haplotype frequencies are either encircled or linked by dashed lines.

tance of this marker precludes any inference of male population structuring (Birky et al., 1989). Given the lack of allozyme structuring, it is possible that *A. butcheri* exhibits high male gene flow among estuaries, and hence the overexploitation of one estuary may have implications beyond that locality. The possibility of male-biased dispersal in fishes has only recently received attention, but has been documented in several species. Some rock-dwelling cichlids exhibit male-biased dispersal, as adjacent females have higher average relatedness than adjacent males (Knight et al., 1999). Male-biased dispersal has also been documented for salmonids, based on either tagging studies (Hutchings and Gerber, 2002) or microsatellite analyses and assignment tests (Bekkevold et al., 2004; Fraser et al., 2004). Male-biased dispersal has also been postulated for discrepancies between nuclear autosomal and mtDNA population structuring of other taxa (e.g., Rassmann et al., 1997; Lyrholm et al., 1999). Given that *A. butcheri* exhibits protogynous hermaphrodism in some estuaries (Rowland and Snape, 1994), male-biased dispersal could also result from size-biased dispersal. Consequently, rigorous assessment of *A. butcheri* population structuring requires a more thorough survey of autosomal variation to assess male gene flow. Previous surveys were constrained by the low polymorphism of allozymes; of more than 50 enzyme systems surveyed in *A. butcheri*, only *LDH**, *MDH*-2*, and *GPI*-1* exhibited scorable polymorphism (Chaplin et al., 1998; Farrington et al., 2000).

The aim of this study was to determine whether male gene flow in *A. butcheri* has been sufficient to homogenize nuclear genetic variation among southeast Australian estuaries. To address this question, we determined allele frequencies at five microsatellite loci among 11 populations (minimum sample of 40 individuals). Microsatellites are likely to represent a more accessible source of polymorphism than allozymes, and previous studies employing these markers have revealed population structuring not apparent from the analysis of allozymes (e.g., Bentzen et al., 1996; Shaw et al., 1999a,b; Wirth and Bernatchez, 2001), probably owing to the greater statistical power associated with higher allelic diversity (Goudet et al., 1996). Our null hypothesis is that male gene flow among estuaries is sufficient to homogenize microsatellite allele frequencies over large spatial scales in southeast Australia, equivalent to that observed for allozymes. We also surveyed estuaries intermediate to those previously distinguished by mtDNA, in an effort to address the finer spatial extent of population structuring (i.e., Glenelg, Painkalac, Thomson; Figure 1).

Materials and Methods

Study Design. At least 40 individuals from each of 11 estuaries were genotyped at five microsatellite loci. We utilized eight of the samples analyzed by Farrington et al. (2000) and Burridge et al. (2004), plus three samples collected at estuaries intermediate to those previously distinguished by mtDNA (Figure 1). The three new samples were analyzed for microsatellite and mtDNA variation, but not allozyme variation given the widespread homogeneity of allele frequencies already documented at these loci in southeast Australia (Farrington et al., 2000; Burridge et al., 2004) and tissue constraints (ethanol-preserved fin clips).

Microsatellite Analysis. Total DNA was isolated from tissue samples using a high-salt precipitation method (Crandall et al., 1999). Five microsatellite loci developed for species of sparid were analyzed (Table 1). Three loci were derived from *A. butcheri* (Yap et al., 2000). The remaining loci were derived from other sparids, *Pagrus auratus* (Adcock et al., 2000) and *A. schlegeli* (Jeong et al., unpublished), and were screened for polymorphism in *A. butcheri* for the first time herein. Loci were polymerase chain reaction (PCR) amplified with conditions comprising 1 × PCR buffer, 0.2 mM dNTPs, and 0.2 units of *Taq* DNA polymerase (Invitrogen), with $MgCl_2$ concentrations and primer sequences provided in Table 1. For all loci except pAb2A5, dye label incorporation followed Schuelke (2000); the forward primer was 5′ appended with an 18 bp M13 sequence (TGTAAAACGACGGCCAGT), and employed at 0.03 μM, while the reverse primer and a FAM- or HEX-labeled M13 primer were employed at 0.5 μM. This method is more economic than individually labeling a primer for each locus (Schuelke, 2000). Thermal cycling conditions for each locus except pAb2A5 were 3 min at 94°C, followed by 8 cycles of 94°C for 30 s, annealing temperature (Table 1) for 30 s, and 72°C for 1 min, followed by 33 cycles as before but with annealing at 53°C, and then a final extension of 72°C for 5 min. Locus pAb2A5 was amplified using a FAM-labeled forward primer and a reverse primer, both at 0.5 μM, and with thermal cycling conditions as above except that annealing was at 64°C throughout. PCR products were separated on a 6% denaturing polyacrylamide gel using an ABI 373 (Applied Biosystems) following the manufacturer's instructions. PCR product lengths were determined relative to the GS400 size standard (ABI).

Table 1. PCR Primers and Conditions for Five Microsatellite Loci Surveyed in *Acanthopagrus butcheri* from Southeast Australia

Locus	*Primer source or sequences*	*Dye label*	*Annealing*	*[$MgCl_2$]*
pAb1H1	Yap et al. (2000) [a,b]	FAM	64°C	2.0 mM
pAb2A5	Yap et al. (2000) [b]	FAM	64°C	2.0 mM
pAb2D11	Yap et al. (2000) [a,b]	HEX	64°C	2.0 mM
Pma1n	Adcock et al. (2000)[a]	HEX	57°C	1.5 mM
Asc21-218	CCTGACCAATAAGGGCTTTG[a,c] ATGCATTTACACTCGCACCA[b,c]	FAM	57°C	2.5 mM

[a]5′_end of forward primer appended with an 18 bp M13 sequence (TGTAAAACGACGGCCAGT), to facilitate the incorporation of a dye label during PCR (Schuelke, 2000).
[b]5′_end of reverse primer was modified to read GTTT, to avoid potential A-tailing problems (Brownstein et al., 1996).
[c]Derived from *A. schlegeli* sequence (Jeong et al., unpublished, GenBank accession number AB095014).

Mitochondrial DNA Analysis. MtDNA analysis was conducted on the three samples not previously analysed for this marker (Glenelg, Painkalac, Thomson). An approximately 1100-bp segment of mtDNA that included the noncoding control region, the $tRNA^{Phe}$ gene, and part of the 12S rRNA gene was chosen for PCR- restriction fragment length polymorphism (PCR-RFLP) analysis. The PCR conditions and primers PT and PU of Jean et al. (1995) were employed. Two restriction enzymes (four-base recognition) were employed that digest this fragment at sites known to be polymorphic, *NsiI* and *DpnII* (Burridge et al., 2004). Restriction enzyme digests of PCR products from each individual were carried out for 1 h at 37°C using 8 µl of amplified DNA, 1–3 units of enzyme, and the appropriate concentration of reaction buffer. The digested samples were electrophoresed through 2.0% agarose gels followed by ethidium bromide staining, and visualized under UV illumination.

Data Analysis. Concordances of microsatellite genotype frequencies with those expected under Hardy-Weinberg equilibrium, and the independence of genotypes among loci, were examined using exact tests implemented by Genepop 3.1c (Raymond and Rousset, 1995), followed by sequential Bonferroni correction of P values (Rice, 1989). Non-neutrality of mitochondrial DNA variation was assessed via the Ewens-Watterson-Slatkin test (Slatkin, 1996) implemented using Arlequin 2.001 (Schneider et al., 2000).

Microsatellite allele and mitochondrial haplotype frequency homogeneity among samples was assessed by exact tests using Genepop, with microsatellite results combined across loci using Fisher's method, following Ryman and Jorde (2001). Variance of allele and haplotype frequencies among samples (F_{ST}, Wright, 1978) was estimated by calculating θ (Weir and Cockerham, 1984) with FSTAT 2.9.3 (Goudet, 2001). Significance of observed θ values was estimated from 1000 permutations of alleles or

Table 2. Numbers of Alleles (a), Gene Diversity (H_e), and Population Structuring of Genetic Variation (θ, Weir and Cockerham 1984; G'_{ST}, Hedrick, 2005) among *Acanthopagrus butcheri* Samples in Southeast Australia

	Allozyme sites			*All sites*
Locus	a	H_e	G'_{ST}	θ
*LDH**	2	0.48	0.037	0.017
*MDH-2**	2	0.04	0.070	0.061[a]
*GPI-1**	3	0.50	0.003	−0.000
Mean/multilocus (allozymes)	2.3	0.34		0.012
Asc21_218	10	0.43	0.250	0.085[a]
pAb1H1	10	0.09	0.031	0.027[a]
pAb2D11	5	0.23	0.531	0.299[a]
pAb2A5	7	0.44	0.101	0.036[a]
Pma1n	14	0.53	0.065	0.023[a]
Mean/multilocus (microsatellites)	9.2	0.34		0.088[a]
Mitochondrial DNA	7	0.51		0.185[a]

Values were calculated based on samples in common across markers (eight samples, 'Allozyme sites') for comparative purposes, but estimates of population structuring were also calculated based on all samples (8 for allozymes, 11 for microsatellites and mtDNA). Allozyme data and results were derived from Farrington et al. (2000) and Burridge et al. (2004). Mitochondrial data and results were derived from Burridge et al. (2004) and this study. The significance of θ values was estimated from 1000 permutations of alleles or haplotypes among samples.
[a]$P < 0.05$.

haplotypes among samples. Values of G_{ST} (Nei, 1987) for microsatellites and allozymes were also calculated using FSTAT, and converted into Hedrick's (2005) standardized measure of genetic differentiation (G'_{ST}) to facilitate comparison between these nuclear markers.

The presence of a significant relationship between geographic isolation and the genetic divergence of samples was assessed using a Mantel test (10,000 randomizations), implemented by IBD 1.2 (Bohonak, 2002) and based on geographic distance and θ. Geographic distance was represented by the contemporary shoreline distance between estuaries, excluding the circumference of Port Phillip Bay for comparisons of localities east and west of the Yarra, given the comparatively narrow entrance to this bay (Figure 1).

Results

Microsatellite Variation. Each locus was polymorphic in all samples except locus pAb1H1 in Onkaparinga, Surrey, and Thomson, and locus pAb2D11 in Surrey (Appendix). Allelic diversity was substantially higher than that observed for allozymes across samples in common, although heterozygosities were similar (Table 2). Genotype frequencies did not differ from Hardy-Weinberg expectations following Bonferroni correction with the exception of locus Asc21–218 at Gippsland (P = 0.0029), and locus pAb2D11 at Hopkins (P = 0.0016). There was no evidence for genotypic disequilibrium among loci following Bonferroni correction ($P > 0.0235$).

Exact tests rejected microsatellite allele frequency homogeneity for the majority of sample comparisons (Table 3), with exceptions between several adjacent localities (Port Adelaide-Onkaparinga, Glenelg-Surrey, Hopkins-Painkalac, Painkalac-Thomson, Gippsland-Sydenham). Exclusion of locus Asc21–218 during comparisons against the Gippsland sample (deviating from Hardy-Weinberg expectations) did not alter the significance of allele frequency heterogeneity. The exclusion of pAb2D11 for comparisons against the Hopkins sample (deviating from Hardy-Weinberg expectations) did alter the significance of heterogeneity; Surrey, Yarra, and Gippsland were no longer significantly different from Hopkins. However, Hopkins was distinguished from all samples except Painkalac by analysis of microsatellite genotype—as opposed to allele—frequency homogeneity ($P < 0.05$ following Bonferroni correction), and this approach does not require Hardy-Weinberg equilibrium. mtDNA analysis also distinguished Hopkins from the majority of samples (Table 3). Structuring of microsatellite variation

Table 3. Pairwise Exact Test Probabilities of Microsatellite Allele and Mitochondrial DNA Haplotype Frequency Homogeneity Among Samples of *Acanthopagrus butcheri* from Southeast Australia

	Port Adelaide	*Onkaparinga*	*Hindmarsh*	*Glenelg*	*Surrey*	*Hopkins*	*Painkalac*	*Thomson*	*Yarra*	*Gippsland*	*Sydenham*
Port Adelaide		0.250	0.078	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]
Onkaparinga	0.308		0.037	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]
Hindmarsh	0.000[a]	0.000[a]		0.001[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]
Glenelg	0.000[a]	0.000[a]	0.000[a]		0.077	0.000[a]	0.003[a]	0.000[a]	0.027	0.000[a]	0.000[a]
Surrey	0.000[a]	0.000[a]	0.000[a]	0.026		0.001[a]	0.001[a]	0.000[a]	0.056	0.000[a]	0.000[a]
Hopkins	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]		0.037	0.051	0.364	0.000[a]	0.003[a]
Painkalac	0.000[a]	0.000[a]	0.000[a]	0.001[a]	0.000[a]	0.086		0.041	0.081	0.001[a]	0.000[a]
Thomson	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.002[a]	0.683		0.007	0.098	0.002[a]
Yarra	0.000[a]	0.000[a]	0.000[a]	0.001[a]	0.000[a]	0.001[a]	0.002[a]	0.004[a]		0.000[a]	0.000[a]
Gippsland	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]		0.424
Sydenham	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.000[a]	0.479	

Mitochondrial DNA values are given above the diagonal, and microsatellite values (probabilities combined across loci using Fisher's method) are below the diagonal. Populations are ordered from west to east.

[a]$P < 0.05$ following sequential Bonferroni correction for 55 simultaneous tests (and more than one standard error less than the critical P in the case of mitochondrial DNA).

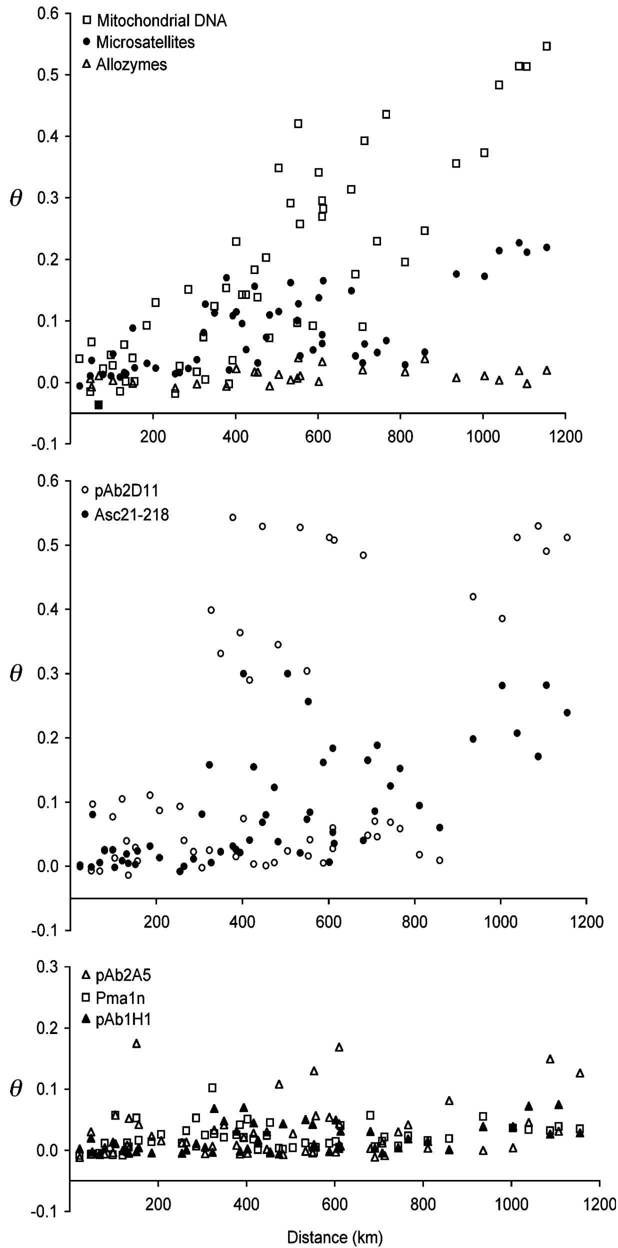

Fig. 2. Plots of θ against geographic distance (shoreline distance between sample sites) for molecular markers employed to assess population structuring in *Acanthopagrus butcheri* from southeast Australia. ***Top:*** θ derived from each class of molecular marker (allozyme, mitochondrial DNA, microsatellite) across all sites for which data were collected (Allozyme data: Farrington et al., 2000; Burridge et al., 2004. Mitochondrial data: Burridge et al., 2004; this study). ***Middle*** and ***lower:*** θ for individual microsatellite loci.

across all 11 populations (θ) was 0.088, and significantly greater than zero ($P < 0.001$).

Mitochondrial DNA Variation. Eight haplotypes were observed, all populations were polymorphic, and haplotype frequencies in each sample (Appendix) were consistent with selective neutrality according to the Ewens-Watterson-Slatkin test ($P > 0.059$). Genetic homogeneity was rejected for the majority of pairwise sample comparisons (Table 3). Nonrejection of homogeneity mostly involved comparisons of adjacent or second-most-adjacent samples, although the Yarra sample could not be distinguished from those collected across a wider range (Thomson to Glenelg, Table 3). Structuring of mtDNA variation across all 11 samples (θ) was 0.185, and was significantly greater than zero ($P < 0.001$) (previously 0.263 across eight populations, $P < 0.001$, Burridge et al., 2004).

Isolation by Distance. A significant positive relationship with geographic distance was observed for $\theta_{\text{microsatellites}}$ and θ_{mtDNA} (Figure 2; $r_{\text{microsatellites}} = 0.6920$, $P < 0.003$; $r_{\text{mtDNA}} = 0.5766$, $P < 0.005$). Inspection of the plot points revealed that the significant relationships were not due to exceptional divergence of peripheral populations, which could be reflective of vicariance, but rather a steady increase in genetic divergence with geographic distance.

Discussion

High Male Gene Flow?. The null hypothesis of this study was that gene flow among estuaries has been sufficient to homogenize microsatellite allele frequencies in *A. butcheri* over large spatial scales in southeast Australia. Given that strong spatial structuring of mtDNA variation has been observed for *A. butcheri* in this region (Burridge et al., 2004; this study), a lack of structuring in nuclear markers cannot be explained by female gene flow, but rather may be reflective of male movements. However, based on a survey of five microsatellite loci we reject our null hypothesis; microsatellites distinguished the majority of *A. butcheri* samples from one another. Only some of the populations that were adjacent to each other were genetically indistinguishable (Port Adelaide-Onkaparinga, Glenelg-Surrey, Hopkins-Painkalac, Painkalac-Thomson, Gippsland-Sydenham). Whenever mtDNA distinguished populations, microsatellites were in agreement, such that interpretation of population structuring can be extended to both sexes. In addition, microsatellites also distinguished several populations that could not be separated based on mtDNA haplotype frequencies. Most notedly, this represented the distinction of the Yarra population from a broad range of samples (between Glenelg and Gippsland), but also other sets of populations (i.e., Hindmarsh from sites further west; Hopkins vs. Painkalac; Thomson vs. Hopkins and Gippsland) (Table 3).

Isolation by Distance. The presence of significant positive relationships between genetic divergence and geographic isolation for both microsatellite and mtDNA variation in *A. butcheri* indicates low levels of contemporary gene flow within southeast Australia that is predominantly restricted to adjacent estuaries, consistent with the one-dimensional stepping stone model of Kimura and Weiss (1964). Isolation by distance relationships has been observed for estuarine fishes in other parts of Australia (Chenoweth and Hughes, 1997; Chenoweth et al., 1998; Jerry and Baverstock, 1998). There is also such a relationship in *A. butcheri* from Western Australia, based on reanalysis of the data presented by Chaplin et al. (1998) (not shown). It appears that isolation by distance relationships, and stepping-stone gene flow among demes may apply to the majority of Australian fishes that predominantly occupy estuaries.

Interestuary Movement. It has been previously hypothesized that periods of high river discharge facilitate gene flow between estuaries for *A. butcheri* (Burridge et al., 2004). There have been observations of individuals occurring out at sea, but still proximate to a river mouth, during periods of high discharge (Lenanton, 1977; Holt, 1978; Sherwood and Backhouse, 1982; Lenanton et al., 1999), and individuals may employ nearshore coalescing floodwater plumes to move between estuaries (e.g., Grimes and Kingsford, 1996), or undertake such movement in fully marine conditions which they can readily tolerate (Norriss et al., 2002). There is no evidence for interestuary gene flow mediated by egg or larval stages in *A. butcheri*, as none have been recorded out at sea. Spawning in estuaries open to the ocean also occurs during periods of low river discharge and is preceded by an upstream migration, followed by larval and juvenile development in the upper and mid reaches of estuaries (Sherwood and Backhouse, 1982; Newton, 1996; Haddy and Pankhurst, 1998).

Lack of Allozyme Structuring. The comparative homogeneity of allele frequencies at protein loci across southeast Australian populations could reflect balancing selection, given that these molecules are functionally significant and estuaries exhibit temporal and spatial heterogeneity in temperatures, salinities, and dissolved oxygen concentrations (Hodgkin, 1994). While several statistical tests exist for the presence of selection at allozymes, their application to *A. butcheri* is constrained by the low number of polymorphic loci and alleles per locus, and estimated $F_{ST} < 0$ (see Lewontin and Krakauer, 1973; Baer, 1999; Arnaud-Haond et al., 2003; Guinand et al., 2004). Other studies have inferred selection by contrasting allozyme population structuring against molecular markers thought immune to direct selection, such as microsatellites (e.g., Karl and Avise, 1992; Pogson et al., 1995; Estoup et al., 1998; Lemaire et al., 2000; Buonaccorsi et al., 2001; De Innocentiis et al., 2001; Dufresne et al., 2002; Arnaud-Haond et al., 2003). However, an appreciation is required for the broad variance in neutral structuring expected among loci (Lewontin and Krakauer, 1973; McDonald et al., 1996; Bierne et al., 2003), and the fact that the low homozygosity and higher mutation rates typical of microsatellites can constrain estimates of F_{ST} to small values, particularly when migration rates are low (Goudet et al., 1996; Hedrick, 1999; Balloux et al., 2000; Buonaccorsi et al., 2001).

Here we standardized measures of genetic differentiation at microsatellites and allozymes relative to theoretical maximums derived from expected heterozygosities (G'_{ST}; Hedrick, 2005). However, the structuring observed across allozymes was not significantly lower than that for microsatellites for samples in common to both marker types (Table 2; $U_{0.05(1),3,5} = 12$, $P > 0.10$). Therefore, we cannot reject selective neutrality of the allozyme variation; the difference in structuring between allozymes and microsatellites may simply reflect the broad variance in structuring expected among loci under neutral conditions and an inadequate sampling of protein loci. Alternatively, a recent colonization history could explain the lack of allozyme structuring.

Estuaries are generally ephemeral over geological and evolutionary timescales (Schubel and Hirschberg, 1978; Hodgkin, 1994; Kench, 1999; Bilton et al., 2002), and the coastline in the vicinity of Bass Strait (separating Tasmania from southeast Australia) has been particularly disrupted during glacial/interglacial cycles, most recently during the Holocene marine transgression (Kench, 1999). Consequently, if contemporary estuaries were colonized only recently, they may not have yet attained migration-drift equilibrium (Slatkin, 1987; Hutchinson and Templeton, 1999 Castric and Bernatchez, 2003). While the presence of a isolation by distance relationship for microsatellites suggests the attainment of migration-drift equilibrium for nuclear loci (Slatkin, 1987; Hutchinson and Templeton, 1999), an inspection of plots for individual loci reveal that Asc21-218 and pAb2D11 are mostly responsible for this pattern, whereas pAb1H1, pAb2A5, and Pma1n exhibit comparatively uniform and low θ regardless of spatial scale (Figure 2). Therefore, colonization of southeast Australian estuaries may have been sufficiently recent that some, but not all nuclear loci have attained migration-drift equilibrium and reveal population structuring, regardless of whether they are microsatellites or allozymes. The fact that

each of the same three allozymes revealed population structuring of variation in *A. butcheri* among Western Australian estuaries (*LDH** θ = 0.096, *MDH-2** θ = 0.268, *GPI-1** θ = 0.054, all $P < 0.001$ based on a reanalysis of data from Chaplin et al. 1998) could reflect differences in colonization times among southeast and Western Australian estuaries.

Conservation Implications. The microsatellite data support mtDNA and tagging study inferences of low contemporary gene flow, which is predominantly restricted to adjacent estuaries. Consequently, separate management of distinct populations (i.e., stocks) is desirable for the avoidance of regional overexploitation given that replenishment of depleted stocks by immigrants is likely to be slow (Carvalho and Hauser, 1995). In addition, given the varied environmental conditions likely to be encountered among estuaries, the possibility of local adaptation by *A. butcheri* populations cannot be discounted (Bilton et al., 2002). While differences in growth rates among some populations (Sarre and Potter, 2000) have been ascribed to environmental rather than genetic variation (Partridge et al., 2004), this does not in itself preclude local adaptation for other traits, nor indicate that translocation of individuals among distinct populations is without nongenetic risks (e.g., disease transmission).

While genetic data have not distinguished several of the estuaries we sampled, it should be noted that the absence of genetic divergence among samples does not necessarily indicate that such populations are panmictic or receive migrants at such a rate to rapidly recover from overexploitation (Carvalho and Hauser, 1995; Waples, 1998). It is worth noting that our interpretation of nuclear autosomal population structuring would be very different (lower) had we not sampled loci pAb2D11 or Asc21–218, and additional loci could reveal even finer spatial structuring of variation. This study illustrates the importance of surveying many loci for studies of population structuring, be they allozymes or microsatellites (Arnaud-Haond et al., 2003; Hoarau et al., 2004).

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Appendix

Frequencies of microsatellite alleles and mitochondrial haplotypes at 11 populations of *Acanthopagrus butcheri* from southeast Australia

Locus/allele	*Port Adelaide*	*Onkaparinga*	*Hindmarsh*	*Glenelg*	*Surrey*	*Hopkins*	*Painkalac*	*Thomson*	*Yarra*	*Gippsland*	*Sydenham*
Asc21–218	(124)	(106)	(120)	(142)	(74)	(108)	(80)	(100)	(100)	(108)	(82)
222	–	–	–	–	–	–	–	–	0.010	0.019	–
234	–	–	–	–	–	0.009	–	–	–	–	–
240	0.024	0.019	–	0.197	0.216	0.102	0.188	0.140	0.090	0.093	0.183
244	0.016	0.009	–	–	–	0.009	0.013	0.050	–	–	–
246	0.016	–	0.017	0.014	0.095	0.065	0.050	0.070	0.100	0.176	0.195
248	0.032	0.019	–	0.092	0.108	0.019	–	0.010	0.010	0.019	0.085
250	0.903	0.943	0.933	0.627	0.446	0.731	0.650	0.560	0.730	0.537	0.463
252	–	–	0.017	0.007	–	0.009	–	0.020	0.010	0.065	0.049
254	0.008	0.009	0.033	0.063	0.135	0.046	0.087	0.110	0.050	0.083	0.012
256	–	–	–	–	–	0.009	0.013	0.040	–	0.009	0.012
pAb1H1	(110)	(106)	(116)	(140)	(56)	(104)	(80)	(96)	(100)	(108)	(84)
151	–	–	–	–	–	–	–	–	–	–	0.012
153	–	–	–	–	–	–	0.013	–	–	–	0.024
155	–	–	–	0.007	–	–	–	–	–	0.009	0.012
157	–	–	–	–	–	–	–	–	–	–	0.012
159	0.009	–	–	0.007	–	–	–	–	–	–	–
161	0.964	1.000	0.974	0.964	1.000	0.981	0.988	1.000	0.970	0.870	0.869
163	–	–	–	0.007	–	0.010	–	–	–	0.019	0.024
165	0.027	–	0.009	0.014	–	–	–	–	–	0.046	0.012
167	–	–	–	–	–	0.010	–	–	0.020	0.037	0.024

Continues.

Appendix: Continued

Locus/allele	*Port Adelaide*	*Onkaparinga*	*Hindmarsh*	*Glenelg*	*Surrey*	*Hopkins*	*Painkalac*	*Thomson*	*Yarra*	*Gippsland*	*Sydenham*
169	–	–	0.017	–	–	–	–	–	0.010	0.019	0.012
pAb2D11	(126)	(132)	(120)	(144)	(80)	(108)	(80)	(100)	(102)	(108)	(84)
122	–	–	–	0.021	–	–	–	–	–	–	–
126	0.968	0.955	0.900	0.938	1.000	0.843	0.837	0.870	0.990	0.306	0.369
128	–	–	–	0.028	–	0.046	0.063	0.100	0.010	0.130	0.119
130	0.032	0.045	0.100	0.014	–	0.111	0.100	0.030	–	0.537	0.500
132	–	–	–	–	–	–	–	–	–	0.019	0.012
134	–	–	–	–	–	–	–	–	–	0.009	–
pAb2A5	(126)	(132)	(120)	(144)	(78)	(108)	(78)	(100)	(102)	(108)	(84)
108	–	–	–	–	–	–	–	–	0.010	–	–
110	–	–	–	–	–	0.046	–	–	–	0.056	0.071
114	–	–	–	–	–	0.009	–	–	–	0.019	–
116	0.460	0.598	0.758	0.701	0.731	0.741	0.603	0.620	0.667	0.713	0.690
118	–	–	0.025	–	–	–	–	–	–	–	–
120	0.540	0.402	0.217	0.299	0.269	0.204	0.397	0.380	0.314	0.213	0.238
122	–	–	–	–	–	–	–	–	0.010	–	–
Pma1n	(124)	(128)	(114)	(142)	(70)	(108)	(80)	(92)	(98)	(108)	(84)
151	–	–	–	–	0.029	–	–	–	–	–	–
153	0.008	–	–	–	–	–	–	–	–	–	0.012
157	0.742	0.742	0.518	0.796	0.714	0.685	0.637	0.587	0.612	0.639	0.595
159	–	–	–	0.028	0.029	0.028	0.013	0.033	0.031	0.056	0.024
161	0.105	0.070	0.193	0.035	0.114	0.074	0.087	0.087	0.061	0.046	0.083
163	–	–	0.035	0.007	0.014	0.037	0.038	0.033	0.020	0.120	0.119
165	–	0.016	–	–	–	–	–	–	–	0.009	0.024
167	–	–	–	0.007	–	0.046	0.013	0.011	0.041	0.037	0.012
169	–	–	–	–	–	0.019	–	–	–	0.009	0.036
171	–	–	0.009	–	–	0.009	–	–	0.010	0.028	0.036
173	0.145	0.164	0.237	0.127	0.071	0.093	0.213	0.228	0.224	0.056	0.060
175	–	0.008	0.009	–	–	0.000	–	–	–	–	–
177	–	–	–	–	–	0.009	–	0.011	–	–	–
179	–	–	–	–	0.029	–	–	0.011	–	–	–
mtDNA	(52)	(54)	(52)	(72)	(40)	(52)	(40)	(48)	(51)	(53)	(41)
AA	0.904	0.870	0.712	0.542	0.350	0.308	0.525	0.250	0.412	0.226	0.268
AB	0.077	0.037	0.173	0.042	–	0.096	–	0.042	0.078	0.076	0.073
AC	–	–	–	–	–	0.038	–	–	–	–	–
BA	–	–	–	–	0.050	0.077	–	–	0.059	–	–
BB	0.019	0.093	0.077	0.347	0.525	0.192	0.200	0.250	0.294	0.075	–
BD	–	–	0.038	0.028	0.025	–	–	–	–	–	–
CA	–	–	–	–	–	0.039	–	–	–	0.019	–
CB	–	–	–	0.042	0.050	0.250	0.275	0.458	0.157	0.604	0.659

Populations are ordered from west to east. Total numbers of alleles surveyed are given in parentheses, and microsatellite allele names represent their size in base pairs. Mitochondrial data were derived from Burridge et al. (2004) except for Glenelg, Painkalac, and Thomson.

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