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Population genetic structure and a possible role for selection in driving phenotypic divergence in a rainbowfish (*Melanotaeniidae*)

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Quantitative genetic divergence may be driven by drift or selection. The rainbowfish *Melanotaenia australis* exhibits phenotypic divergence among populations in Western Australia, although the mechanisms driving this divergence are unknown. We used microsatellites to assess neutral genetic divergence (F_{ST}), and found a hierarchical pattern of subdivision consistent with low divergence between upstream and downstream populations (within drainages), moderate divergence between drainages (within regions), and high divergence between regions. Using a common-garden approach, we measured quantitative genetic divergence in phenotypic traits (Q_{ST}). By comparing this to expectations from neutral processes (F_{ST}), we concluded that the effect of selection varies depending on the spatial scale considered. Within drainages, selection may be causing divergence between upstream and downstream phenotypes but, between regions, selection appears to homogenize phenotypes. This highlights the importance of spatial scale in studies of this kind, and suggests that, because variance in selection pressures can drive speciation, polymorphism in *M. australis* may represent speciation in action. *Biological Journal of the Linnean Society*, 2011, **102**, 144–160.

ADDITIONAL KEYWORDS: gene flow – genetic subdivision – local adaptation – *Melanotaenia splendida australis* – microsatellite – neutral variation – polymorphism.

INTRODUCTION

In natural populations, genetic changes accrue through neutral drift (Wright, 1929) and selection (Darwin, 1871; Fisher, 1958). Divergence at quantitative trait loci occurs through drift when the reproductive success of individuals is altered by random factors other than selection pressure. For example, historical population fragmentation, in the absence of long-term geographic barriers, is associated with drift-induced divergence among *Melanoplus* grasshoppers (Knowles & Richards, 2005). Under certain conditions, such as after bottlenecks or founder events, drift can even cause the loss of alleles favoured by selection, as a result of the greater chance of their elimination from small populations (Lande, 1989). Of course, selection itself also alters allele frequencies for loci subject to selection pressures. Tutt's (1896) differential bird predation hypothesis, which predicted that phenotypic change in *Biston* peppered moths was an adaptation to environmental change, is a classic example.

Although selection and drift are not mutually exclusive, the relative importance of each as an evolutionary force has been debated (Ohta, 1992). Species that exhibit polymorphism at quantitative trait loci can provide models with which to test whether local adaptation (as a result of selection) or drift is more likely to be the cause of among-population divergence. Inferences are often made from studies of variation at neutral marker loci, although this is generally only a poor guide to other types of genetic variation (Butlin & Tregenza, 1998). Indeed, if strong selection for particular traits is present, genetic variation in quantitative traits can occur despite extensive gene flow (Gockel *et al.*, 2001; Whibley *et al.*, 2006). It is also

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unwise to infer patterns of quantitative genetic divergence from phenotypic data alone because environmental factors can induce a plastic response in phenotype (Wimberger, 1992; Kinsey *et al*., 1994; Bouton, Witte & van Alphen, 2002). Common-garden experiments (CGEs), in which phenotypic variance arising from direct and indirect environmental effects is eliminated, are required to ensure that estimates of among-population phenotypic divergence are representative of variation at quantitative trait loci (Storfer, 1996).

If estimates of divergence at quantitative trait loci and neutral loci are available, the two can be compared (Merilä & Crnokrak, 2001; McKay & Latta, 2002). Neutral genetic divergence, for example F_{ST} (Wright, 1951) calculated from microsatellite data, can be used as a null hypothesis; it gives an estimate of the degree of divergence expected among populations in the absence of selection. Quantitative genetic divergence, calculated as Q_{ST} (Spitze, 1993) from CGE-derived phenotypic traits, is expected to equal F_{ST} when traits are controlled by purely additive genes and are neutral with respect to selection (Lande, 1992). Thus, when Q_{ST} is greater than or less than F_{ST}, it may be a result of diversifying or homogenizing selection, respectively, on the traits of interest (Merilä & Crnokrak, 2001). When Q_{ST} is equal to F_{ST}, the effects of drift and selection are indistinguishable; for a discussion of situations in which this does not apply, such as when variation in mutational inputs among loci is present, see Hendry (2002).

The western rainbowfish, *Melanotaenia australis* (Castelnau 1875), is an endemic freshwater fish distributed throughout the Pilbara and Kimberley regions of north-western Australia. Males are brightly coloured with elaborate fins, and male phenotype differs markedly among populations (see Appendix, Fig. A1) (Allen & Cross, 1982; Allen, Midgley & Allen, 2002). The evolutionary biology of this species is poorly understood. Moderate to high levels of neutral genetic subdivision in this species are present among populations within the Kimberley region (Phillips, Storey & Johnson, 2009), although the status of Pilbara populations has not been examined. The extent to which colour polymorphism within this species represents quantitative genetic variation, and the degree to which selection could be responsible for generating variation, are unknown.

The present study had two main objectives. The first was to describe neutral genetic differentiation among populations of the western rainbowfish in Western Australia using microsatellite markers, and to determine whether population structure across the bulk of the distribution of the species is congruent with that described using allozymes within the Kimberley region alone (Phillips, Storey & Johnson, 2009). The second was to determine whether the phenotypic divergence exhibited among these populations is likely to be driven by local adaptation, by comparing pairwise Q_{ST} and F_{ST} among populations.

MATERIAL AND METHODS

Fish collection and field methods

Rainbowfish were captured at 20 locations in the north of Western Australia (Fig. 1). These locations spanned the entire distribution of the species within Western Australia, and the bulk of the overall distribution of the species (Allen, Midgley & Allen, 2002). Collection of brood stock for our CGE was given priority during sampling; thus, the first 40–60 fish captured at each site were not used for data collection but were packed immediately for return to the laboratory.

At 13 of the 20 sites, there were sufficient fish remaining to use for phenotypic measurements in the field (for sample sizes, see Fig. 1). These fish were killed with a 10 × overdose of AQUI-S® (Young, 2009). Each fish was photographed and reflectance measures were taken at five points on the body (the tail, the coloured spot on the operculum, the beginning and end of the lateral line, and the lower flank) using a USB-4000 spectrometer (Ocean Optics). The reflectance measurement methodology is described in Young, Simmons & Evans (2010a).

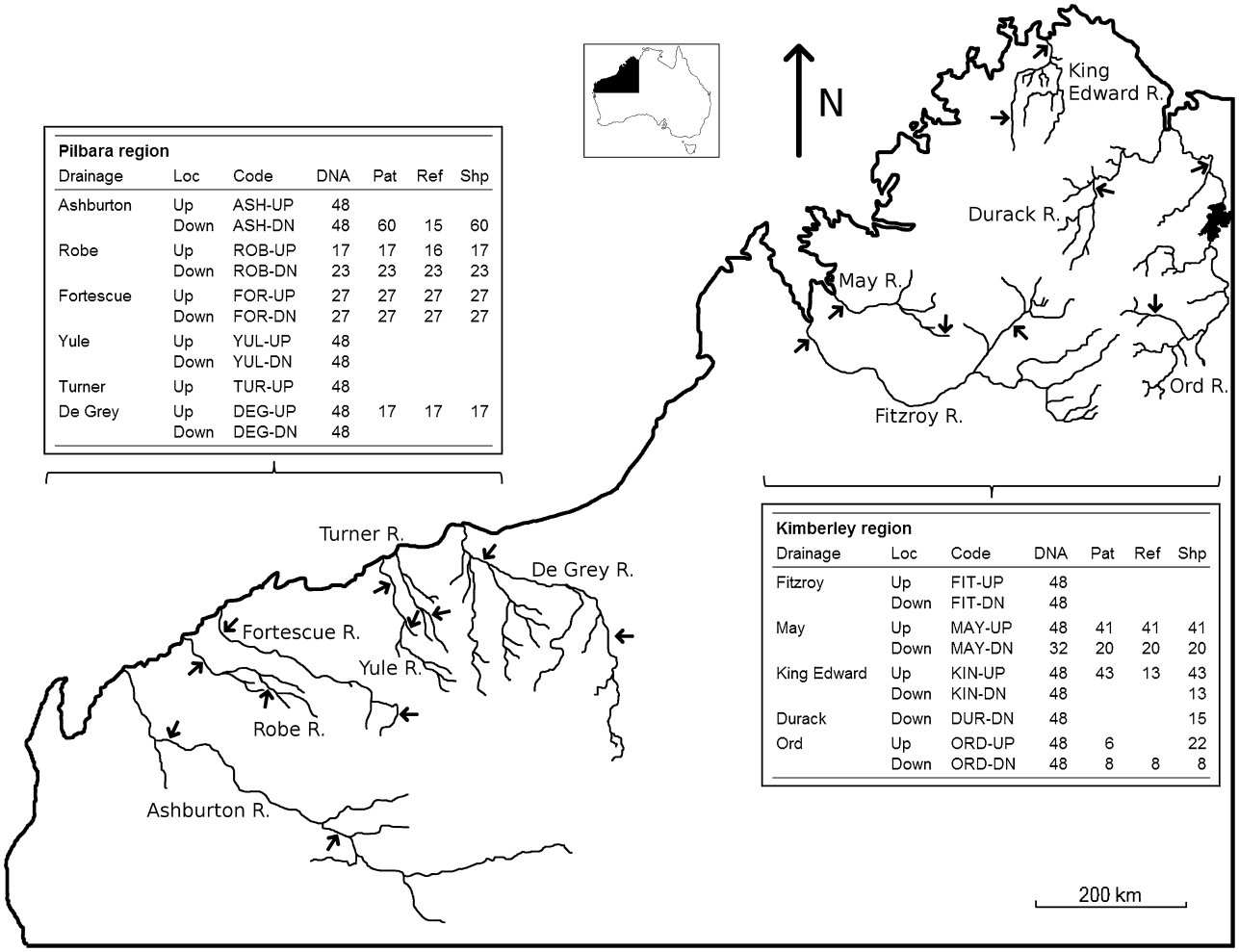
From each fish, a fin clip was taken and fixed in 95% EtOH. Finally, fish were preserved in Dietrich's fixative [by volume 58% H_2O, 30% EtOH (95%), 10% formalin (37% formaldehyde), 2% glacial acetic acid] and returned to the laboratory.

Laboratory methods: phenotypic measures

From the photographs taken in the field, we measured the total number of coloured lateral lines and the size of the coloured spot present on the operculum, on the left side of each individual. These are referred to as pattern variables.

The reflectance spectra, taken in the field, were decomposed (using data distillation methods and principal components analyses; Young, Simmons & Evans, 2010a; see also Endler, 1990; Cuthill *et al.*, 1999; LeBas & Marshall, 2000) into 11 orthogonal variables describing overall levels of reflectance, and specific colours reflected, of each rainbowfish. These are referred to as reflectance variables.

The preserved rainbowfish were photographed at × 7.60 magnification, and the photographs were used in a geometric morphometrics analysis (Zelditch *et al*., 2004). This generated 15 relative warps, each describing different aspects of the morphology of



Pilbara region

Drainage	Loc	Code	DNA	Pat	Ref	Shp
Ashburton	Up	ASH-UP	48			
	Down	ASH-DN	48	60	15	60
Robe	Up	ROB-UP	17	17	16	17
	Down	ROB-DN	23	23	23	23
Fortescue	Up	FOR-UP	27	27	27	27
	Down	FOR-DN	27	27	27	27
Yule	Up	YUL-UP	48			
	Down	YUL-DN	48			
Turner	Up	TUR-UP	48			
De Grey	Up	DEG-UP	48	17	17	17
	Down	DEG-DN	48			

Kimberley region

Drainage	Loc	Code	DNA	Pat	Ref	Shp
Fitzroy	Up	FIT-UP	48			
	Down	FIT-DN	48			
May	Up	MAY-UP	48	41	41	41
	Down	MAY-DN	32	20	20	20
King Edward	Up	KIN-UP	48	43	13	43
	Down	KIN-DN	48			13
Durack	Down	DUR-DN	48			15
Ord	Up	ORD-UP	48	6		22
	Down	ORD-DN	48	8	8	8

Figure 1. Map showing locations of rainbowfish collection sites in the Pilbara and Kimberley regions of Western Australia. The regions are separated by the Great Sandy Desert. The inset map displays the location of the study area relative to the Australian continent, and the main map shows the location of study sites in each drainage system (marked with arrows). An upstream and a downstream site were surveyed in each drainage (except in the Durack and Turner rivers, which had one site each). The insets show, for each site: the drainage, location (upstream or downstream), site code (used throughout this paper), and the number of fish collected for fin clips used in genetic analyses (DNA). Also provided are the sample sizes for each of the datasets used in the calculation of pairwise P_{ST}s: colour pattern data (Pat), reflectance data (Ref), and shape data (Shp).

individuals, and a measure of centroid size, which is a measure of body size that is uncorrelated with shape in the absence of allometry (Zelditch *et al*., 2004). These 16 scores are referred to as shape variables.

A discussion of the pattern, reflectance, and shape variables, and the extent of their variation among natural populations of rainbowfish, is the focus of another study (Young, Simmons & Evans, 2010b); thus, we do not present their specific characteristics here. Rather, we use these data to calculate pairwise estimates of among-population phenotypic divergence.

LABORATORY METHODS: CGE

The rainbowfish that were returned to the laboratory alive were used in a CGE. Once acclimatized to laboratory conditions, fin clips were taken from randomly chosen individuals within each population to add to those taken in the field, giving a total library of fin clips from wild-caught fish across 20 populations (Fig. 1). Twelve populations were chosen for inclusion in the CGE: ASH-UP, ASH-DN, FOR-UP, FOR-DN, DEG-UP, DEG-DN, FIT-UP, FIT-DN, KIN-UP, KIN-DN, ORD-UP, and ORD-DN. These populations were chosen because they span the majority of the

distribution of the species (Fig. 1). From each population, four randomly selected pairs were chosen and allowed to breed, and one son was raised to adulthood. This resulted in four first-generation male offspring for each of the 12 populations, and these offspring were used for phenotypic measurements. It was not possible to produce sets of full-sibling second generation offspring; thus, it should be noted that the phenotypes measured in our CGE may incorporate variation as a result of maternal or dominance effects.

The common-garden rearing environment consisted of indoor glass aquaria ($500 \times 800 \times 310$ mm), all sharing a common recirculating water supply filtered by a single large biological filter. Water was heated to 25 °C (±5 °C, seasonally variable) under a 12 : 12 h light/dark photoperiod; water supply into each aquarium was standardized and each aquarium was fed the same type and amount of food (dosed volumetrically; daily: Spectrum Premium Fish Food, New Life International, Inc.; weekly: Bio-Pure frozen bloodworms, Hikari, Inc.). Within each aquarium, there were two artificial spawning mops constructed of nylon wool (Allen, 1996).

It took approximately 1 year for rainbowfish to begin producing eggs on a regular basis, and for the CGE to begin. Within a 1-week period, we collected as many eggs as possible from each spawning mop in every aquarium, thus ensuring that resulting first-generation offspring were approximately the same age. Eggs were placed into plastic containers ($200 \times 120 \times 120$ mm), in turn placed within a large water bath. The water bath was part of the larger CGE recirculating system, and the plastic containers had 200-μm mesh inserts to allow water flow while retaining fry. Fry were fed *ad libitum* on a liquid containing *Paramecium* spp., Micron powdered food (Sera GmbH), vinegar eels (*Turbatrix aceti*), *Artemia* sp. nauplii (AusAqua, Pty Ltd), and powdered Spectrum fish food suspended in water. Each fry container was given an identical quantity of the mix, dosed volumetrically, twice daily.

As soon as the sexes could be identified within each sibling group, one male was chosen randomly to be retained in the CGE, and the other offspring were removed. This typically took place after 2 months of rearing. Up until this point, different sibling groups had different densities within containers, as a result of variation in the number of eggs collected and their hatching rates. We therefore cannot rule out an effect of variation in early-life stocking density on the phenotype of the resulting male used in our analyses, although we assume that any such effect would be minimal for two reasons. First, feeding was *ad libitum*, such that food availability did not differ according to stocking density. Second, water recirculated among all containers and the larger CGE system, so differences in water quality would not have occurred as a result of density.

Once individual males had reached approximately 30 mm in length, they were moved into the larger CGE system. Here, they were raised to 1 year of age (approximately 80 mm), after which they were euthanased with AQUI-S® and used for phenotypic measurements. The phenotypic measurements made on these first-generation CGE offspring were identical to those made on the wild-caught fish (data for two pattern variables, 11 reflectance variables, and 16 shape variables were recorded).

Collection and analysis of microsatellite data

DNA was extracted from the fin clips taken from each population (for sample sizes, see Fig. 1), using the EDNA HiSpEx Tissue Kit (Saturn Biotech). Polymerase chain reaction was used to genotype fish at twelve microsatellite loci (Ma01-Ma12). The loci, labelled primers, reaction conditions, and methods of fragment analysis are described elsewhere (Young, Simmons & Evans, 2009).

Preliminary microsatellite analyses were performed for each locus, within each population. Expected heterozygosities (calculated with Levene's correction; Levene, 1949), observed heterozygosities, and number of alleles present were determined using GENEPOP, version 4.0.10 (Raymond & Rousset, 1995). Deviations from Hardy–Weinberg equilibrium (HWE) were tested for using GENEPOP with an exact test (Markov chain parameters: 1000 dememorization steps, 100 batches, 1000 iterations per batch) *sensu* Guo & Thompson (1992). Loci were tested for linkage disequilibrium in FSTAT, version 2.9.3.2, using a log-likelihood ratio *G*-statistic (Goudet, 1995). For all significance tests involving multiple comparisons, the nominal α level of 0.05 was reduced using sequential Bonferroni adjustment (Rice, 1989). On the basis of these preliminary analyses, data from four loci were discarded (see Results); thus, data from eight loci across all populations were used in the remaining analyses.

Multilocus, pairwise F_{ST} values were calculated in FSTAT *sensu* Weir & Cockerham (1984), and were tested for significance using a log-likelihood *G*-statistic (Goudet, 1995). Multidimensional scaling in SPSS, version 17 (SPSS Inc.) and histograms were then used to visualize patterns of genetic subdivision as described by pairwise F_{ST} values. To determine whether deviations from HWE had any bearing on the statistical significance of the results obtained, significance tests of pairwise F_{ST}s were first performed assuming no HWE within populations, by permuting genotypes among samples, and were then

reperformed assuming HWE within populations, by permuting alleles among populations (Goudet, 1995). To determine whether the choice of differentiation measure had any bearing on our estimates of pairwise divergence, multilocus pairwise R_{ST} values were also calculated. R_{ST} was calculated *sensu* Slatkin (1995), after standardizing the data to express alleles in terms of standard deviations from the global mean rather than repeat unit number, using RST CALC (Goodman, 1997). The matrix of pairwise R_{ST} was then compared with the matrix of F_{ST} using a Mantel test (10 000 permutations) in POPTOOLS, version 3.1 (Hood, 2009).

To examine overall divergence between rainbowfish from the Pilbara and Kimberley regions, populations were grouped within each region and FSTAT was used to calculate allelic richness, observed heterozygosity, gene diversity, F_{IS}, and F_{ST} across groups within regions (with estimates for each parameter weighted by sample size). For each of these parameters, the difference between the estimates for each of the two regions were tested for statistical significance using the 'comparisons among groups of samples' option in FSTAT (two-sided tests, 10 000 permutations; Goudet, 1995). Individuals were then pooled within each region (i.e. each region was treated as a single super-population), and F_{ST} across the two regions was calculated for each locus, and across all loci, *sensu* Weir & Cockerham (1984). The estimate of F_{ST} across all loci was tested for significance, and an estimate of its standard error was obtained by jackknifing across all loci (Goudet, 1995). Allele frequencies, calculated by FSTAT for each locus in each population, were then examined for the presence of fixed allelic differences between the two regions.

Comparisons of microsatellite and phenotypic data

We tested for evidence of adaptive variation in rainbowfish by comparing pairwise between-population values of phenotypic differentiation to those expected under a null hypothesis of neutral drift. In the literature, this is typically a comparison of quantitative genetic divergence (calculated as Q_{ST}) and neutral genetic divergence (F_{ST}) on a trait-by-trait basis. Whitlock (2008) identifies several problems with this approach; thus, instead, for each of the pattern, reflectance, and shape datasets, we calculated pairwise Q_{ST} for each trait and then compared Q_{ST} averaged over sets of traits with pairwise F_{ST} averaged over loci. Our comparisons therefore serve as a gauge of the likely overall importance of local adaptation in pattern, reflectance, and shape variation for this species (Whitlock, 2008), rather than a definitive assessment of whether variation in specific traits is a result of selection or drift.

Q_{ST} was calculated using the formula:

$$Q_{ST} = \frac{\sigma^2_{GB}}{2\sigma^2_{GW} + \sigma^2_{GB}}$$

where σ^2_{GB} and σ^2_{GW} are the between- and within-population components of genetic variance, respectively (Whitlock, 2008). These were estimated using one-way analysis of variance of CGE-derived trait values for the respective pairs of populations. In addition, we calculated P_{ST} ('pseudo'-Q_{ST}, or 'phenotypic'-Q_{ST}; Leinonen *et al.*, 2006) from our field phenotypic data, and performed the same comparisons as for Q_{ST}. This allowed us to assess the amount of among-population phenotypic variance that could be accounted for by environmental effects. P_{ST} was calculated in the same way as Q_{ST}, although with substitution of field-measured trait values for CGE-derived trait values. For each of the pattern, reflectance, and shape datasets, pairwise Q_{ST} and P_{ST} were compared visually to pairwise F_{ST} by examining the scatter of data relative to the $y = x$ line that constitutes the null hypothesis. In each case, a binomial test was used to determine the likelihood that the observed distribution of Q_{ST} or P_{ST} (the number of observations above versus below the line) was the result of chance and, in some instances (see Results), we used Fisher's method of combining probabilities (Sokal & Rohlf, 1995) to consolidate the outcomes of binomial tests across datasets.

RESULTS

Population structure in the western rainbowfish

Loci Ma02 and Ma09 exhibited linkage within four populations (YUL-DN, YUL-UP, ORD-DN, and ORD-UP; $P < 0.001$ in each instance) and across all populations combined ($P < 0.001$). Four populations were not in HWE at locus Ma02, whereas all populations were in HWE for locus Ma09; therefore, Ma02 was excluded from our analyses (see Appendix, Table A1). Loci Ma04, Ma08 and Ma11 exhibited poor genotyping rates across all populations, and were also excluded. The loci retained for all subsequent analyses, across all populations, were Ma01, Ma03, Ma05, Ma06, Ma07, Ma09, Ma10, and Ma12. Some loci exhibited deviations from HWE in some populations, each time as a result of heterozygote deficiency, although the majority of locus-population combinations were in HWE (see Appendix, Table A1).

Multilocus, pairwise F_{ST} data, tested for significance without assuming HWE within populations, revealed consistent population structuring in a

hierarchical fashion (Table 1). When tests on F_{ST} values were performed again assuming HWE within populations, the statistical significance of individual F_{ST} values did not change. Pairwise, multilocus R_{ST}s showed the same structure as F_{ST}s, and there was a high degree of similarity between the R_{ST} matrix and the F_{ST} matrix (Mantel test, $r = 0.839$, $P < 0.001$). Therefore, we use F_{ST} to describe genetic differentiation.

Differentiation between upstream and downstream sites within any given drainage was low and, in some cases, not significantly different from zero (Table 1). Differentiation between sites within each of the two regions, by comparison, was moderate, and between-site F_{ST}s within the Pilbara region were generally higher than those within the Kimberley region (Table 1). Differentiation between sites across the two regions was high (Table 1). The multidimensional scaling representation of pairwise F_{ST} data was consistent with this pattern of genetic differentiation (see Appendix, Fig. A2).

Allelic richness, observed heterozygosity, and gene diversity were all higher in the Kimberley than in the Pilbara region (respectively: 4.10 versus 2.77, $P < 0.001$; 0.42 versus 0.30, $P < 0.001$; 0.46 versus 0.33, $P < 0.001$), when calculated across grouped populations within the two regions. F_{IS} did not differ between the Kimberley and Pilbara regions (0.09 versus 0.07, $P = 0.758$), and F_{ST} was lower in the Kimberley than in the Pilbara (0.230 versus 0.320, $P = 0.009$; see also Table 1), when calculated in the same way. When individuals were pooled within each of the two regions and F_{ST} was calculated for each locus, values of F_{ST} were all significantly different from zero ($P < 0.001$ in every instance) and were in the range 0.028–0.845 (Table 2). F_{ST} across all loci was significantly different from zero ($P < 0.001$) and indicated moderate genetic differentiation between the two regions when each was treated as a single super-population (Table 2). Although private alleles were seen in both regions at most loci, alleles were shared between regions at all loci (Table 2). There was therefore no evidence for fixed allelic differences between these regions at these loci.

Genetic versus phenotypic divergence

Comparisons of Q_{ST} (and P_{ST}) with F_{ST} were structured (Fig. 2) in accordance with the observed structuring of neutral genetic differentiation (low pairwise F_{ST} between sites within the same drainage, moderate pairwise F_{ST} between drainages within the same region, and high pairwise F_{ST} between sites within different regions). Q_{ST} and P_{ST} were significantly different from F_{ST} only in comparisons made between regions and between drainages within the same region (Table 3). Q_{ST} was lower than F_{ST} in the between region comparisons of the reflectance and shape data; in all other comparisons, Q_{ST} was not significantly different from F_{ST} (Table 3). P_{ST} exceeded F_{ST} in all but one of the between drainage and between region comparisons; in all other comparisons, P_{ST} was not significantly different from F_{ST} (Table 3). Q_{ST} was typically lower than P_{ST} when comparing values within the same dataset (Fig. 2); thus, comparison of Q_{ST} and P_{ST} with F_{ST} did not usually result in the same outcome (Table 3).

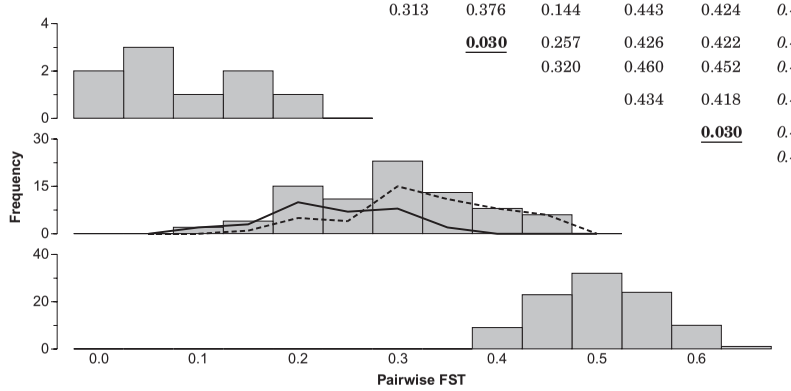
For comparisons between sites within the same drainage, Q_{ST} and P_{ST} differences from F_{ST} were never statistically significant after sequential Bonferroni correction (Table 3). Examination of the Q_{ST} and P_{ST} distributions for these within-drainage comparisons, however, showed that individual values for Q_{ST} and P_{ST} exceeded F_{ST} in almost every instance, and that F_{ST} was very low for these comparisons (Fig. 2). As a result, when the probability values of significance tests across all three trait datasets were combined, a statistically significant difference between Q_{ST} and F_{ST} was found between sites (from Table 3: $P_{pattern} = 0.219$, $P_{reflectance} = 0.031$, $P_{shape} = 0.031$; Fisher's method: $\chi^2 = 16.93$, d.f. = 6; $P_{combined} = 0.010$; see also Fig. 2). When all traits are considered collectively therefore Q_{ST} exceeds F_{ST} between sites within the same drainage (Fig. 2). The same approach also suggested a very strong, although not statistically significant, likelihood that P_{ST} exceeds F_{ST} between sites (from Table 3: $P_{pattern} = 0.125$, $P_{reflectance} = 0.250$, $P_{shape} = 0.063$; Fisher's method: $\chi^2 = 12.46$, d.f. = 6; $P_{combined} = 0.054$; see also Fig. 2).

DISCUSSION

Populations of *M. australis* in the Pilbara and Kimberley regions of Western Australia exhibit neutral genetic subdivision consistent with the hierarchical structure of those populations, according to current geography. In the present study, divergence was low within drainages, moderate between drainages within regions, and high between regions. In the east Kimberley region, *M. australis* exhibited a similar, hierarchical pattern of subdivision when populations were compared at smaller spatial scales, ranging from between pools within creeklines, to between drainages (Phillips, Storey & Johnson, 2009). Taken together, these studies provide a comprehensive assessment of the population genetics of *M. australis* at several spatial scales, and can be added to the body of empirical evidence (Shaw *et al*., 1994; Tibbets & Dowling, 1996; Baer, 1998; McGlashan & Hughes, 2002) supporting the stream hierarchy model of genetic subdivision in freshwater fishes (Meffe & Vrijenhoek, 1988).

Table 1. Pairwise F_{ST} values for 20 rainbowfish populations (Fig. 1)

		Pilbara region											Kimberley region								
		Ashburton		Robe		Fortescue		Yule		Tumer	De Grey		Fitzroy		May		King Edward		Durack	Ord	
		Down	Up	Down	Up	Down	Up	Down	Up	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Down	Up
Ashburton	Down		**0.004**	0.346	0.341	0.206	0.302	0.310	0.343	0.305	0.266	0.258	*0.424*	*0.512*	*0.509*	*0.471*	*0.471*	*0.499*	*0.556*	*0.451*	*0.398*
	Up			0.329	0.330	0.188	0.291	0.297	0.331	0.297	0.284	0.276	*0.428*	*0.515*	*0.508*	*0.474*	*0.476*	*0.499*	*0.556*	*0.458*	*0.403*
Robe	Down				**0.003**	0.181	0.357	0.308	0.371	0.357	0.434	0.423	*0.478*	*0.576*	*0.595*	*0.506*	*0.545*	*0.552*	*0.593*	*0.507*	*0.453*
	Up					0.190	0.367	0.315	0.380	0.357	0.412	0.398	*0.453*	*0.558*	*0.577*	*0.489*	*0.530*	*0.538*	*0.582*	*0.489*	*0.434*
Fortescue	Down						**0.127**	0.259	0.304	0.186	0.317	0.295	*0.395*	*0.495*	*0.527*	*0.460*	*0.492*	*0.504*	*0.556*	*0.464*	*0.406*
	Up							0.313	0.376	0.144	0.443	0.424	*0.443*	*0.538*	*0.605*	*0.520*	*0.556*	*0.570*	*0.632*	*0.528*	*0.471*
Yule	Down								**0.030**	0.257	0.426	0.422	*0.459*	*0.537*	*0.538*	*0.417*	*0.489*	*0.498*	*0.546*	*0.437*	*0.383*
	Up									0.320	0.460	0.452	*0.487*	*0.564*	*0.572*	*0.425*	*0.511*	*0.525*	*0.571*	*0.457*	*0.399*
Tumer	Up										0.434	0.418	*0.414*	*0.510*	*0.589*	*0.514*	*0.549*	*0.564*	*0.619*	*0.520*	*0.470*
De Grey	Down											**0.030**	*0.455*	*0.540*	*0.534*	*0.508*	*0.495*	*0.535*	*0.595*	*0.508*	*0.460*
	Up												*0.432*	*0.524*	*0.521*	*0.490*	*0.470*	*0.507*	*0.578*	*0.477*	*0.437*
Fitzroy	Down													**0.046**	0.229	0.184	0.243	0.299	0.306	0.219	0.241
	Up														0.231	0.194	0.250	0.303	0.325	0.227	0.323
May	Down															**0.204**	0.186	0.226	0.308	0.193	0.330
	Up																0.170	0.210	0.199	0.083	0.159
King Edward	Down																	**0.079**	0.149	0.180	0.291
	Up																		0.122	0.203	0.324
Durack	Down																			0.188	0.302
Ord	Down																				**0.169**



All values were significantly different from zero (sequential Bonferroni correction, nominal $P = 0.050$) except the four underlined. Values are organized according to region, drainage, and site (upstream or downstream). The inset histograms show frequency distributions of pairwise F_{ST} for between-region (lower histogram, italicized in Table), between-drainage (within region; middle histogram, normal typeset in Table), and between-site (within drainage; upper histogram, bold in Table) comparisons. The lines in the between-drainage histogram represent the sub-distributions of F_{ST} values for comparisons made within the Kimberley (solid line) and Pilbara (dashed line) regions as separate components of the total frequency for each bin.

Table 2. Comparison of microsatellite diversity between rainbowfish from the Pilbara and Kimberley regions (Fig. 1) at eight loci

Locus	Pilbara alleles (*n*)	Kimberley alleles (*n*)	Shared alleles (*n*)	Total (*N*)	F_{ST}
Ma01	0	0	3	3	0.028
Ma03	1	20	14	35	0.048
Ma05	1	2	1	4	0.845
Ma06	1	5	3	9	0.439
Ma07	4	3	2	9	0.810
Ma09	0	1	5	6	0.147
Ma10	4	5	17	26	0.044
Ma12	3	1	10	14	0.039
All	14	37	55	106	0.346 (±0.138)

For each locus, the total number of unique alleles found across both regions is given (*N*), and this total is broken down into the number of alleles shared between regions and the number of private alleles within regions (*n*). Between-region F_{ST} for each locus was calculated after pooling individuals within each region (i.e. treating each region as a single super-population), and the estimate of between-region F_{ST} across all loci is provided (±SE).

Table 3. Summary of results of binomial tests performed to determine whether the observed level of phenotypic divergence (P_{ST} or Q_{ST}) in rainbowfish is different to the level of neutral genetic divergence (F_{ST})

		Between site	Between drainage	Between region
Pattern	P_{ST}	NS[1]	$P_{ST} > F_{ST}$[2]	$P_{ST} > F_{ST}$[3]
	Q_{ST}	NS[4]	NS[5]	NS[6]
Reflectance	P_{ST}	NS[7]	$P_{ST} > F_{ST}$[8]	$P_{ST} > F_{ST}$[9]
	Q_{ST}	NS[10]	NS[11]	$Q_{ST} < F_{ST}$[12]
Shape	P_{ST}	NS[13]	$P_{ST} > F_{ST}$[14]	NS[15]
	Q_{ST}	NS[16]	NS[17]	$Q_{ST} < F_{ST}$[18]

The median of the distribution of P_{ST}/Q_{ST} versus that of F_{ST}, and the two-tailed probability of the binomial test, for each comparison: 1: 0.85 versus 0.15, $P = 0.125$; 2: 0.74 versus 0.30, $P < 0.001$; 3: 0.92 versus 0.50, $P < 0.001$; 4: 0.29 versus 0.06, $P = 0.219$; 5: 0.39 versus 0.28, $P = 1.000$; 6: 0.38 versus 0.48, $P = 0.099$; 7: 0.81 versus 0.13, $P = 0.250$; 8: 0.75 versus 0.30, $P < 0.001$; 9: 0.80 versus 0.51, $P < 0.001$; 10: 0.22 versus 0.06, $P = 0.031$; 11: 0.32 versus 0.28, $P = 0.035$; 12: 0.33 versus 0.48, $P < 0.001$; 13: 0.49 versus 0.13, $P = 0.063$; 14: 0.53 versus 0.23, $P < 0.001$; 15: 0.56 versus 0.51, $P = 0.020$; 16: 0.27 versus 0.06, $P = 0.031$; 17: 0.30 versus 0.28, $P = 0.011$; 18: 0.33 versus 0.48, $P < 0.001$.

Comparisons were made for pairwise P_{ST} and Q_{ST} (averaged across all pattern traits, reflectance traits, or shape traits) calculated between sites within drainages, between drainages within regions, and between regions. Each test determined whether there was a statistically significant difference between the proportion of cases where P_{ST} (or Q_{ST}) exceeded F_{ST} and the null expectation of 0.5 (after sequential Bonferroni correction of $\alpha = 0.050$; NS, not significant).

The significantly higher level of subdivision observed in *M. australis* within the Pilbara (within-region grouped population $F_{ST} = 0.320$ versus 0.230 in the Kimberley) may be a product of between-region differences in dispersal opportunities. In the Kimberley, watercourses are more permanent along their length year-round (Unmack, 2001) and are prone to consistent and extensive flooding during the monsoonal wet season (Bureau of Meteorology, Australian Government; www.bom.gov.au). Given that melanotaeniids are likely to have high dispersal abilities (Ivantsoff *et al*., 1988; Pusey, Kennard & Arthington, 2004), this may facilitate greater gene flow between drainage systems in this region. Phillips, Storey & Johnson (2009) suggest that wet-season dispersal, rather than dry-season isolation, may be the more important determinant of genetic structure in the Kimberley, in *M. australis* in particular and in freshwater fishes in general. By contrast, the influence of the wet season is diminished in the Pilbara region; rainfall is unreliable (Kay *et al*., 1999), and perennial surface water is scarce (Unmack, 2001). This may translate to fewer opportunities for dispersal, and hence higher genetic subdivision, in the Pilbara.

The estimate of neutral genetic divergence calculated between the Pilbara and Kimberley regions ($F_{ST} = 0.346$) fell within the range of values calculated for the between drainage estimates within these regions (pairwise F_{ST}s, range = 0.083–0.460). Historical gene flow between the two regions therefore is not likely to have been any less extensive than gene flow among some drainages within these regions. We found no evidence of fixed allelic differences between the two regions, and although private alleles were found at some loci, shared alleles were present at all loci. Collectively, these findings suggest that, although neutral loci in *M. australis* exhibit a degree of divergence between the Pilbara and Kimberley, individuals from these two regions are unlikely to represent different species. This is consistent with existing knowledge (McGuigan *et al*., 2000).

The distribution of *M. australis* is large compared with that of most rainbowfishes, which are typically highly endemic (Allen & Cross, 1982; Allen, Midgley & Allen, 2002). The Kimberley, in particular, supports high levels of endemism in its freshwater fish fauna (Allen & Leggett, 1990; Unmack, 2001). The marked

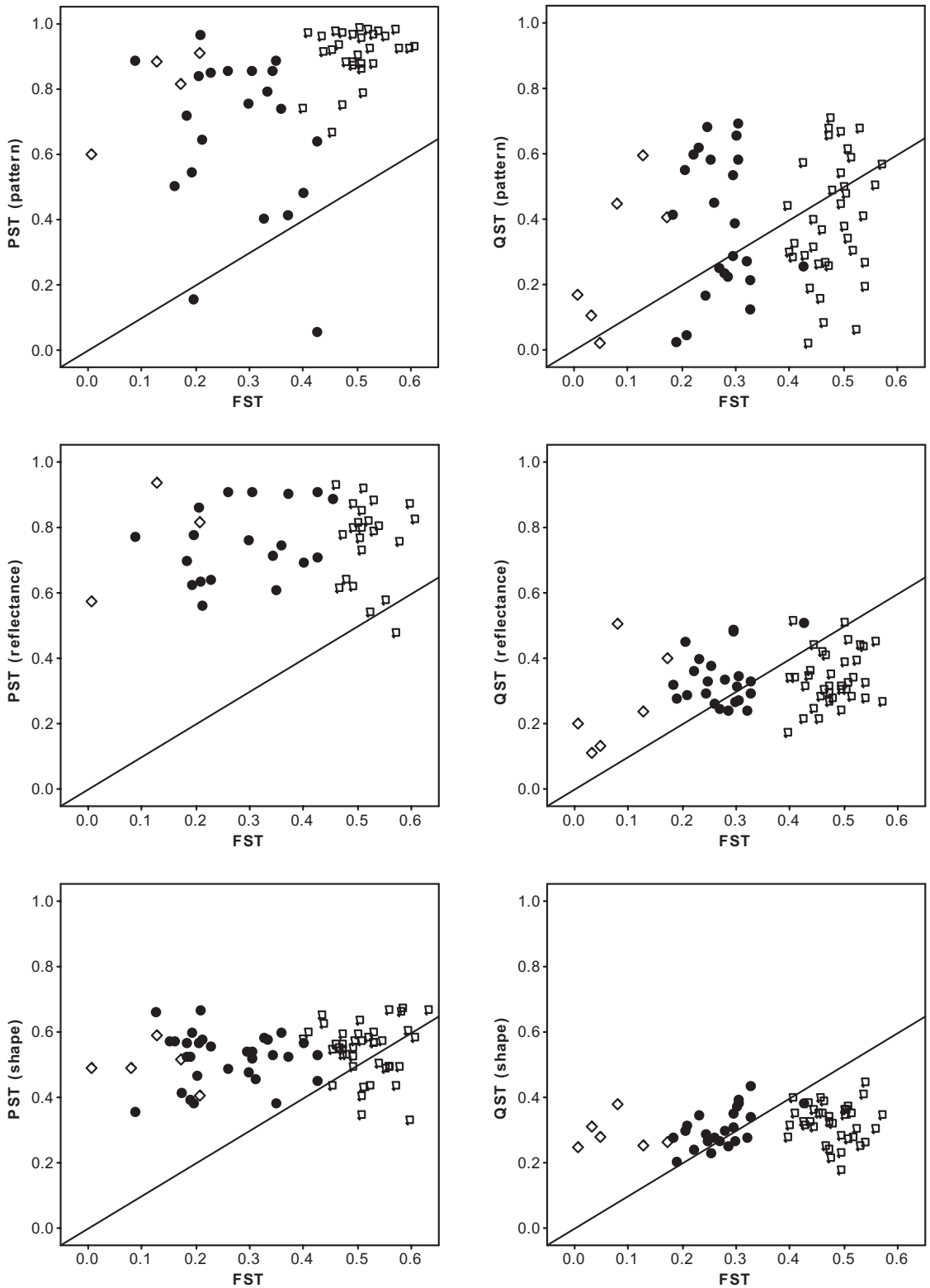


Figure 2. Comparisons of pairwise P_{ST} and Q_{ST} (averaged across traits) with pairwise F_{ST} (averaged across loci) for pattern, reflectance, and shape traits in rainbowfish. The line of $y = x$ is plotted to represent the null hypothesis of no difference. Data represent comparisons either between sites within the same drainage (diamonds), between sites within the same region (circles), or between sites within different regions (squares).

phenotypic divergence that *M. australis* exhibits among its populations (Allen & Cross, 1982; Allen, Midgley & Allen, 2002; Hieronimus, 2002) may therefore be a consequence of having a large distribution that encompasses a variety of habitat types and ecological conditions. Our comparisons of quantitative and neutral genetic divergence suggest that selection, the nature of which may differ from population to population across the wide range of *M. australis*, may have contributed to the evolution of phenotypic polymorphism in this species.

At small spatial scales (between sites), local adaptation is potentially driving overall phenotypic divergence between upstream and downstream habitats. In these between-site comparisons, Q_{ST} exceeded F_{ST} nearly every time and, when traits were considered collectively through combination of probabilities, this result was statistically significant. This is consistent with an overall effect of selection for local adaption within drainages in *M. australis*, although more data are clearly needed to test the robustness of this conclusion. At moderate spatial scales (between drainages), there were no differences between Q_{ST} and F_{ST}, and our data could therefore not distinguish between the effects of drift and selection for any traits.

At a large spatial scale (between regions), the finding that F_{ST} can exceed Q_{ST} appears to indicate homogenizing selection on reflectance and shape traits across the two regions (according to the conventional interpretation of QST-FST comparisons; Merilä & Crnokrak, 2001; McKay & Latta, 2002). We suggest, however, that the finding is an artefact of making comparisons between regions. If the effects of the aforementioned diversifying selection at small spatial scales are similar within each region, Q_{ST} between regions will remain small. Effectively, when Q_{ST} is considered from a broad-scale perspective, fine-scale resolution is lost and phenotypic divergence is 'averaged' across populations, erasing the evidence for selection processes. Phenotypic variation in *M. australis* is consistent with this hypothesis; phenotypic differences between up- and downstream sites are correlated with differences in predation regime and predation risk, although there are no such correlations between regions (Young, Simmons & Evans, 2010b).

Within each set of phenotypic trait data (pattern, reflectance, and shape), for between site, between drainage, and between region comparisons, P_{ST} exceeded Q_{ST}. This clearly indicates that a portion of the total phenotypic variance, removed by using the CGE, was a result of direct environmental effects. However, because we were only able to conduct a single generation of common-garden rearing in the present study, our Q_{ST} values may incorporate some carryover variation as a result of maternal effects. Currently, there is no evidence suggesting that maternal effects carry over to adult traits in *M. australis* but, given their prevalence in other systems, we cannot rule this out (Mousseau & Fox, 1998). Our conclusions are most likely robust to the influence of weak maternal effects because the outcome of Q_{ST} -F_{ST} comparisons would not be expected to change significantly. If maternal effects are strong, however, our current estimates of Q_{ST} may be inflated. Removal of maternal effects, if present, could in theory nullify the evidence for diversifying selection that our data offer. However, we consider this unlikely for several reasons. First, a considerable depression of Q_{ST} would have to occur because the estimates of pairwise F_{ST}s in these cases are so low. Second, in their recent meta-analysis of 55 studies incorporating Q_{ST}-F_{ST} comparisons, Leinonen *et al*., (2008) concluded that the effects of using either broad- or narrow-sense estimates of additive genetic variation are negligible when estimating Q_{ST}. Third, the wild-caught fish were acclimated to the laboratory for more than a year before offspring were sampled, which should assist in removing or reducing maternal effects. Finally, the traits examined (coloration and morphology) are probably less likely to be influenced by maternal effects than life history traits, where these effects are more often strong (Mousseau & Fox, 1998).

The majority of studies comparing Q_{ST} and F_{ST} typically identify diversifying selection as a driver of phenotypic divergence in specific traits of interest (Long & Singh, 1995; Rogers, Gagnon & Bernatchez, 2002; Storz, 2002; Palo *et al*., 2003; Gomez-Mestre & Tejedo, 2004). Such studies may be biased, however, toward demonstrating an effect of diversifying selection (Merilä & Crnokrak, 2001; Leinonen *et al*., 2008; Whitlock, 2008); specific traits are chosen for analysis because there is *a priori* knowledge that they differ in expression among populations and therefore estimates of Q_{ST} for those traits are predisposed to being higher than mean F_{ST}. A meta-analysis of all such studies conducted up until 2008 found that approximately 70% of Q_{ST}s exceeded their associated F_{ST}s (Leinonen *et al*., 2008). To address this, we calculated average Q_{ST} across all traits for which data were available, within trait sets and, in so doing, estimated only the overall importance of selection for those sets (as suggested by Whitlock, 2008). This is only a partial solution, however; if different traits are under different selective forces, it is difficult to use the results obtained by averaging to identify precise mechanisms of divergence.

The data obtained in the present study highlight an additional concern, which is that the outcome of Q_{ST} and F_{ST} comparisons may be strongly dependent upon the spatial scale at which a study is performed. If gene flow is stronger at smaller scales, as found here, there may be greater opportunity to detect

selection over short, rather than long, distances. As F_{ST} becomes high, it becomes hard, or even impossible, for Q_{ST} to exceed it (Hendry, 2002). Thus, the power of the F_{ST}-Q_{ST} comparison for detecting selection may be poor in situations with low gene flow, such as the between-region tests made in this study. Experimental designs using a clear hierarchical structure which incorporates multiple spatial scales, such as that presented in this paper, are uncommon in the literature but may represent a useful way forward.

Our results suggest some interesting hypotheses for future research. First, the finding that P_{ST} is generally greater than Q_{ST} implies that environmental effects are an important component of phenotypic variation among populations of this species. Phenotypic plasticity is widespread (Agrawal, 2001), and a better understanding of the extent and proximate causes of such plasticity is prerequisite for placing knowledge of heritable adaptive variation within a broader context. Second, the finding that Q_{ST} shows a similar range regardless of spatial scale could imply that phenotypes are converging on a series of possibilities within each drainage (as a result of selection), and that these possibilities are reasonably similar in the different drainages. If populations become more isolated, we may predict that diversifying selection across small scales will result in multiple local adaptation events. Alternatively, if gene flow does not decrease, speciation may be hindered and *M. australis* may continue to represent a single species across a very large range (in contrast to the high endemism exhibited by other rainbowfishes). Finally, the finding that F_{ST} is strongly associated with spatial scale implies, perhaps unsurprisingly, that gene flow is lower with greater physical separation and there is essentially zero gene flow between regions. More interestingly, F_{ST} values in an absolute sense are quite high even within drainages. This implies that gene flow is restricted among many populations, to the extent that adaptive divergence may potentially take place even in the absence of further isolation, provided that selection is sufficiently strong.

In conclusion, *M. australis* shows patterns of neutral genetic subdivision that are consistent with those seen in other freshwater fishes, and diversifying selection appears to be responsible for overall phenotypic divergence between populations at small spatial scales. The present study is one of few to simultaneously consider several spatial scales when testing for evidence of local adaptation, and our data suggest that the scale at which Q_{ST} and F_{ST} comparisons are made may have consequences for their correct interpretation. Knowledge of the overall prevalence of selection in a system is key to understanding the nature of selective pressures (such as predation and sexual selection) that may be acting, and which are likely to be common to many systems (Endler, 1978). Investigation of these selective pressures in *M. australis* is a promising avenue for future research, as a result of the hierarchical patterns of gene flow and local adaptation imposed upon this species by drainage geography.

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APPENDIX

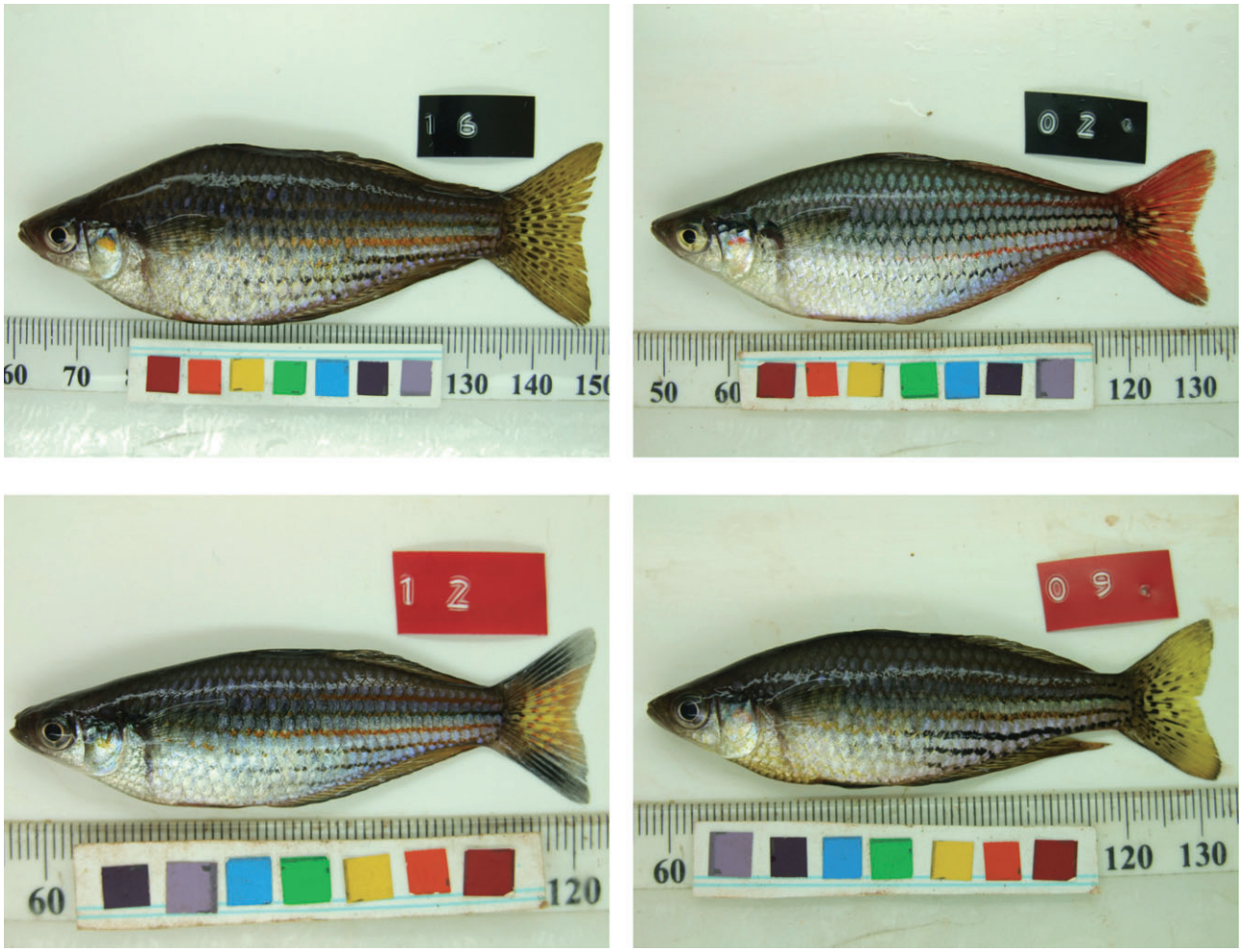

Figure A1. Photographs of *Melanotaenia australis* from four of the populations used in the present study (clockwise from top left: ASH-DN, KIN-UP, DEG-UP, ORD-UP; for key to population codes, see main text; Fig. 1).

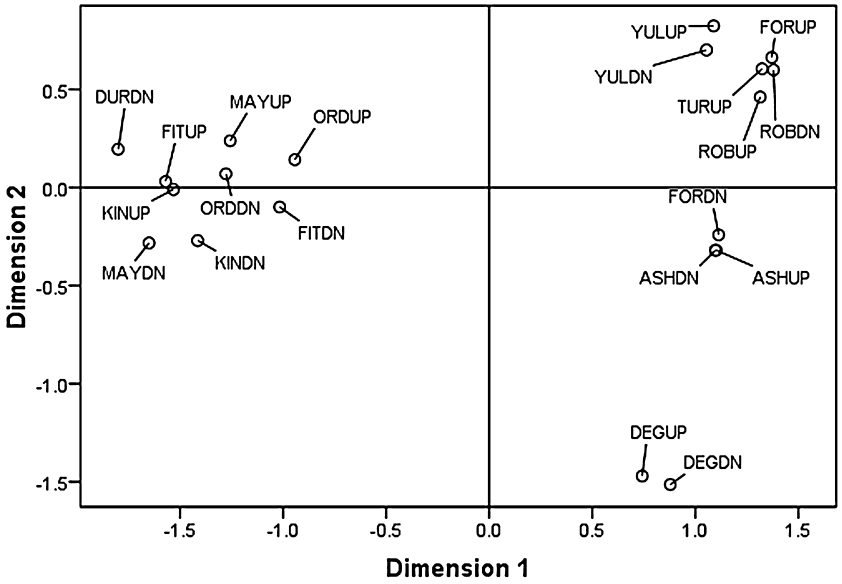

Figure A2. Multidimensional scaling output (stress = 0.102, $r^2 = 0.96$) of pairwise F_{ST} values derived from comparison of 20 rainbowfish populations (Fig. 1) genotyped at eight microsatellite loci. All populations on the right of the output (positive in dimension one) are from the Pilbara region, and all of those on the left (negative in dimension one) are from the Kimberley region. 'UP' and 'DN' suffixes on population labels denote upstream and downstream locations within drainages.

Table A1. Rainbowfish from 20 populations (for details, see Fig. 1) were screened at 12 microsatellite loci (Ma01–Ma12)

Population	*N*		Ma01	Ma02	Ma03	Ma04	Ma05	Ma06	Ma07	Ma08	Ma09	Ma10	Ma11	Ma12
ASH-DN	48	Typed (%)	97.9	68.8	64.6	89.6	97.9	87.5	85.4	89.6	89.6	95.8	83.3	70.8
		H_O/H_E (%)	10.6/10.2	84.8/86.3	**12.9/38.3**	7.0/9.1	0.0/0.0	40.5/45.8	19.5/26.3	0.0/0.0	25.6/29.1	84.8/85.4	92.5/94.0	64.7/63.6
		Alleles (*N*)	2	10	5	3	1	2	3	1	2	9	22	4
ASH-UP	48	Typed (%)	100.0	85.4	72.9	95.8	97.9	97.9	95.8	97.9	95.8	100.0	87.5	95.8
		H_O/H_E (%)	14.6/13.7	90.2/89.9	34.3/37.1	0.0/0.0	0.0/0.0	23.4/37.4	26.1/29.4	0.0/0.0	30.4/31.8	93.8/84.2	71.4/90.5	69.6/65.3
		Alleles (*N*)	2	13	5	1	1	2	3	1	2	11	17	5
ROB-DN	23	Typed (%)	100.0	100.0	100.0	4.3	100.0	100.0	100.0	100.0	100.0	100.0	8.7	100.0
		H_O/H_E (%)	0.0/0.0	91.3/92.1	13/12.5	0.0/0.0	0.0/0.0	52.2/46.4	0.0/0.0	0.0/0.0	65.2/50.7	52.2/60	0.0/66.7	39.1/33.6
		Alleles (*N*)	1	15	2	1	1	2	1	1	2	5	2	4
ROB-UP	17	Typed (%)	100.0	100.0	100.0	58.8	94.1	100.0	94.1	100.0	100.0	94.1	52.9	100.0
		H_O/H_E (%)	0.0/0.0	88.2/92.2	17.6/16.9	0.0/0.0	0.0/0.0	58.8/51.3	0.0/0.0	0.0/0.0	52.9/50.8	62.5/53.8	66.7/94.8	41.2/39.9
		Alleles (*N*)	1	12	3	1	1	2	1	1	2	4	11	3
FOR-DN	27	Typed (%)	100.0	100.0	100.0	18.5	100.0	100.0	100.0	100.0	100.0	100.0	59.3	100.0
		H_O/H_E (%)	0.0/0.0	92.6/92.5	66.7/56.5	0.0/0.0	0.0/0.0	18.5/23	22.2/25.7	0.0/0.0	18.5/23	74.1/84.4	**50.0/94.4**	63.0/62.7
		Alleles (*N*)	1	15	4	1	1	2	2	1	2	11	16	3
FOR-UP	27	Typed (%)	100.0	100.0	100.0	48.1	100.0	100.0	100.0	100.0	100.0	100.0	63.0	100.0
		H_O/H_E (%)	0.0/0.0	81.5/78.8	29.6/29.8	0.0/0.0	0.0/0.0	0.0/0.0	18.5/17.1	0.0/0.0	0.0/0.0	77.8/75.9	**52.9/83.1**	40.7/47.0
		Alleles (*N*)	1	11	5	1	1	1	2	1	1	9	9	4
YUL-DN	48	Typed (%)	97.9	91.7	100.0	58.3	97.9	97.9	97.9	97.9	100.0	83.3	41.7	100.0
		H_O/H_E (%)	4.3/4.2	20.5/30.2	**39.6/76.3**	0.0/0.0	2.1/2.1	8.5/8.3	27.7/24.1	0.0/0.0	56.3/60.2	35/53.5	95.0/94.0	75.0/79.5
		Alleles (*N*)	2	4	11	1	2	3	2	1	4	7	18	9
YUL-UP	48	Typed (%)	93.8	95.8	100.0	37.5	100.0	100.0	100.0	100.0	100.0	75.0	31.3	100.0
		H_O/H_E (%)	0.0/0.0	**26.1/59.0**	**22.9/54.3**	0.0/0.0	2.1/2.1	2.1/2.1	18.8/26.6	0.0/0.0	29.2/38.2	52.8/69.2	80.0/95.4	75.0/78.3
		Alleles (*N*)	1	3	9	1	2	2	2	1	4	8	17	7
TUR-UP	48	Typed (%)	97.9	95.8	97.9	97.9	95.8	93.8	91.7	91.7	97.9	97.9	97.9	97.9
		H_O/H_E (%)	6.4/6.2	69.6/73.3	55.3/63.5	0.0/4.2	0.0/0.0	0.0/0.0	36.4/32.9	0.0/0.0	0.0/0.0	76.6/66.3	59.6/77.7	44.7/49.4
		Alleles (*N*)	2	6	3	2	1	1	2	1	1	4	13	3
DEG-DN	48	Typed (%)	100.0	97.9	97.9	66.7	100.0	100.0	100.0	95.8	97.9	97.9	100.0	97.9
		H_O/H_E (%)	31.3/27.2	0.0/0.0	66/68.7	**6.3/74.4**	0.0/0.0	8.3/12.0	2.1/4.1	**26.1/46.9**	0.0/0.0	91.5/86.8	**83.3/93.1**	48.9/54.8
		Alleles (*N*)	3	1	6	6	1	3	3	3	1	18	22	4
DEG-UP	48	Typed (%)	97.9	64.6	97.9	93.8	100.0	100.0	22.9	41.7	97.9	97.9	97.9	97.9
		H_O/H_E (%)	8.5/8.3	0.0/0.0	63.8/72.1	64.4/69.1	0.0/0.0	8.3/8.1	9.1/43.7	0.0/18.5	0.0/0.0	95.7/90.2	89.4/94.6	66.0/59.1
		Alleles (*N*)	3	1	5	7	1	2	3	2	1	18	23	5
FIT-DN	48	Typed (%)	93.8	100.0	100.0	93.8	100.0	100.0	100.0	77.1	100.0	93.8	93.8	100.0
		H_O/H_E (%)	44.4/35.0	91.7/88.9	56.3/65.5	**33.3/71.3**	37.5/44.9	70.8/71.6	2.1/2.1	**64.9/67.4**	0.0/0.0	100/93.1	95.6/96.6	66.7/67.3
		Alleles (*N*)	2	12	4	9	2	6	2	4	1	18	33	4
FIT-UP	48	Typed (%)	100.0	100.0	100.0	100.0	100.0	100.0	97.9	52.1	100.0	100.0	100.0	100.0
		H_O/H_E (%)	47.9/36.8	95.8/88.2	68.8/70.2	41.7/45.5	16.7/15.7	47.9/45	0.0/0.0	84.0/59.8	0.0/0.0	81.3/89.1	93.8/94.4	52.1/57.5
		Alleles (*N*)	2	14	4	5	3	4	1	3	1	13	24	4

Table A1. *Continued*

Population	*N*		Ma01	Ma02	Ma03	Ma04	Ma05	Ma06	Ma07	Ma08	Ma09	Ma10	Ma11	Ma12
MAY-DN	32	Typed (%)	100.0	34.4	96.9	100.0	100.0	100.0	100.0	90.6	96.9	100.0	100.0	96.9
		H_O/H_E (%)	21.9/19.8	0.0/45.0	**48.4/66.4**	56.3/57.1	0.0/0.0	**21.9/47.7**	3.1/3.1	37.9/49.9	48.4/47.5	75.0/79.1	87.5/94.1	61.3/51.9
		Alleles (*N*)	2	3	10	5	1	5	2	2	3	10	24	4
MAY-UP	48	Typed (%)	97.9	81.3	91.7	97.9	97.9	97.9	93.8	45.8	97.9	100.0	95.8	97.9
		H_O/H_E (%)	8.5/8.2	0.0/0.0	**52.3/81.2**	38.3/48.6	23.4/27.1	51.1/58.3	0.0/0.0	59.1/58.7	61.7/51.5	85.4/91.6	87/90.8	89.4/75
		Alleles (*N*)	2	1	10	3	2	5	1	4	5	14	16	7
KIN-DN	48	Typed (%)	100.0	87.5	97.9	0.0	97.9	97.9	97.9	45.8	100.0	93.8	2.1	100.0
		H_O/H_E (%)	0.0/0.0	**23.8/55.9**	**59.6/85.2**	NA/NA	6.4/6.2	48.9/63.4	23.4/27.1	40.9/54.7	43.8/39.5	75.6/84.2	0.0/0.0	58.3/63.7
		Alleles (*N*)	1	4	14	NA	2	5	2	4	3	11	2	5
KIN-UP	48	Typed (%)	85.4	93.8	91.7	2.1	100.0	93.8	79.2	27.1	97.9	83.3	4.2	97.9
		H_O/H_E (%)	22.0/19.8	8.9/16.8	70.5/70.7	0.0/0.0	4.2/4.1	60.0/48.8	36.8/51.3	7.7/45.2	14.9/14.1	75.0/91.8	50.0/83.3	76.6/70.9
		Alleles (*N*)	2	3	14	1	2	5	3	4	3	18	3	4
DUR-DN	48	Typed (%)	100.0	97.9	97.9	100.0	75.0	31.3	43.8	0.0	97.9	100.0	100.0	97.9
		H_O/H_E (%)	37.5/30.8	0.0/0.0	80.9/83.8	0.0/4.1	0.0/0.0	46.7/52.2	0.0/0.0	NA/NA	0.0/0.0	85.4/89.3	91.7/93.5	61.7/63.6
		Alleles (*N*)	2	1	12	2	1	3	1	NA	1	16	19	6
ORD-DN	48	Typed (%)	97.9	33.3	100.0	95.8	97.9	97.9	54.2	4.2	100.0	97.9	97.9	100.0
		H_O/H_E (%)	6.4/6.3	**0.0/67.7**	83.3/94.6	78.3/86	0.0/4.2	**19.1/50.8**	**3.8/36.4**	0.0/66.7	85.4/75.4	87.2/90.8	97.9/91.6	64.6/63.9
		Alleles (*N*)	3	3	28	14	2	3	4	2	5	15	22	6
ORD-UP	48	Typed (%)	100.0	93.8	100.0	29.2	100.0	100.0	100.0	85.4	100.0	97.9	91.7	91.7
		H_O/H_E (%)	10.4/10	**31.1/72.8**	**52.1/91.5**	**0.0/69.8**	**18.8/45.6**	37.5/35.4	0.0/0.0	29.3/38.9	66.7/65	72.3/88.3	**47.7/92.8**	**56.8/74.9**
		Alleles (*N*)	2	7	18	5	2	4	1	3	4	15	21	5

Shown for each population are the number of fish screened, *N*; proportion of individuals genotyped for each locus, Typed (%); observed/expected heterozygosity for each locus, H_O/H_E (%); and number of alleles at each locus, Alleles (*N*). Bold entries indicate deviation from Hardy–Weinberg equilibrium.