Chapter 6: Greater genetic diversity on the edges of species' ranges: Secondary contact among differentiated lineages?

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Abstract

Using mtDNA (control region) and nuclear (ISSR) markers, I investigated the population genetic structure of three congeneric species pairs of pomacentrid reef fishes (Pomacentridae) in the context of species' borders theory. Each species pair consisted of one species sampled at two central locations within its geographic range, and one species sampled at the same locations, but for which these locations constituted one location toward the centre of its range and another close to its edge. Theory predicts that populations located on the periphery of a species' range should be smaller and more fragmented and hence, display greater genetic structure among populations and lower genetic diversities within populations, compared to more centrally located populations. Estimates of genetic structure did not differ among central and peripheral species as expected. Similarly in contrast to predictions from theory, genetic diversities were greater in species whose sampling included a population toward the edge of its geographic range compared to species sampled at two locations toward the centre of their range. In two of the three species pairs, the distribution of genetic variation indicated secondary contact among differentiated lineages in the species sampled towards its periphery, but not in its congener that was not sampled towards a range edge. Elevated mutation rates mediated by environmental stress on the species' margin may have contributed further genetic variability in these species.

Introduction

What limits the geographic ranges of species has long interested biologists (Darwin 1859, Mayr 1963), yet many issues in this field remain unresolved (Holt and Keitt 2005; Holt et al. 2005). Ultimately, the distributional range of a species will be determined by vital demographic rates and their variation across geographic ranges and through time, with borders forming where population growth rates approach zero beyond some point (Holt et al. 2005). Zero growth rates may result from physical barriers to dispersal, or because of discontinuities in suitable physical and biological environments (Gaston 2003; Holt et al. 2005). Species borders, however, commonly occur in the absence of such barriers, suggesting that range edges form in response to other demographic processes that result in a decline in fitness from the centre to the periphery or through changing metapopulation dynamics towards the species' margin (Lennon et al. 1997; Holt and Keitt 2000).

Generally, biological and environmental conditions are assumed to be optimal in the centre of a species' distribution and to decline towards its periphery (Hoffmann and Parsons 1991). As a result, population density should be highest in the centre of the species range and decline towards range edges (Brown 1984; Vucetich and Waite 2003; Guo et al. 2005 and references therein). Marginal populations should, therefore, become smaller and more fragmented (Vucetich and Waite 2003). They should also experience lower levels of migration among populations and hence display stronger genetic structure (Holt 1987; Lennon et al. 1997). Greater genetic structure has been reported towards species margins in some species (e.g., Gapare and Aitken 2005; Ayre and Hughes 2004) but not in others (Grant and Antonovics 1978). Consequently, the role of reduced gene flow towards the edge of the range in determining species borders is at present unclear.

The demographic processes operating on at a species border should be evident in the effective population sizes and genetic diversities of such populations. At migration – drift equilibrium, genetic diversity can be expressed as the effective number of alleles:

$$H = \frac{4N_e\mu}{1 + 4N_e\mu} \qquad \text{(Eq. 1)}$$

where N_e is the effective population size and μ is the mutation rate per site per generation. $N_e\mu$ is multiplied by 4 to account for the biparental origin of a diploid marker. Assuming mutation rates are equal among populations, genetic diversity

becomes a function of the effective population size and generation time. The effective population size is almost always smaller than the actual size of the population (N) (Hartl and Clark 1997). Differences between N and N_e may arise when N fluctuates (Hartl and Clark 1997). Under such circumstances and because it is the harmonic mean of N, N_e tends to be the most affected by the smallest value of N. N_e will be low in populations that have undergone a severe reduction in size, or that have been founded by a small number of individuals. Marginal population should, therefore, display lower effective population sizes and lower genetic diversities compared to more centrally located populations (Holt 1987). Empirical investigations of variation in genetic diversities across a species' range have reported lower genetic diversities in peripheral populations in some species (e.g., Jain et al. 1981; Kat 1982; Schnabel and Hamrich 1990; Palumbi et al. 1997; Bowen et al. 1997; Durka 1999; Pedersen and Loeschcke 2001; McCauley and Ballard 2002; Hoffman and Blouin 2004; Lecomte et al. 2004) but not in others (Tigerstedt 1973; van Rossum et al. 1997; Betancourt et al. 1991; Planes and Fauvelot 2002; Garner et al. 2004; Gapare et al. 2005). Consequently, empirical support for this relationship remains equivocal and warrants further study.

Estimates of genetic diversity may also be affected by the mutation rate (Eq. 1). Mutation rates may vary among markers (Nei and Graur 1984), but are commonly assumed to be constant within markers among populations and closely related species (Avise 2000). Indeed, constant mutation rate is an explicit assumption in most commonly used population genetic analyses (e.g., AMOVA, Weir and Cockerham 1984). Emerging evidence, however, suggests that mutation rates of neutral genetic markers may be increased by environmentally induced stress (Parsons 1987; Hoffmann and Parsons 1991). This may occur through a variety of processes including a stress-induced error prone DNA repair mechanism (Walker 1984; MacPhee 1984). Elevated mutation rates following sub-lethal stress has only been demonstrated under controlled laboratory conditions (Lindgren 1972; Kerkis 1975; Belyaev and Borodin 1982). Consequently, it is not clear how widespread this mechanism is and how it may affect the population genetic structure of wild populations.

Coral reef fishes provide an excellent model for examining the evolution of species borders because they are speciose and their ranges are relatively well known. Their diversity enables the design of comparative studies that allow issues of species borders evolution to be addressed. Biological and ecological factors which may affect patterns of genetic variation such as ecological specificity (Nevo 1978; Smith and Fujio

1982), demographic and reproductive characteristics (e.g., Selander and Kaufman 1973; Mitton and Lewis 1989) can be controlled through the selection of species that display similar ecological and biological attributes. Despite this, coral reef fishes have not previously been used as a model system to test species border theory.

Here I examine the evolution of species borders in coral reef fishes on the Great Barrier Reef (GBR) using a comparative design. Using a mtDNA sequence marker (Control region) and nuclear genetic fingerprints (ISSR) I examine the population genetic structure of species sampled towards their range margin and compare this to congeneric species sampled in the centre of their ranges. I test the hypotheses that 1) species experience higher genetic structure towards the species' margin and 2) demographic processes such as smaller and more fragmented populations result in decreased genetic diversity in peripheral populations.

Materials and methods

Study species and locations

Three congeneric species pairs were selected for use in this study. Each species pair was selected from a different pomacentrid genus and was collected from a combination of three locations separated by 800-1200 km. There are no known dispersal barriers separating any of these locations. From each species pairs, one species had a distribution that allowed it to be collected from a location toward the centre of that species' geographic range and a location close to a geographic range limit. These species are hereafter referred to as the peripheral species. The congeneric species of each of the peripheral species had a geographic range that extended well beyond the sampling locations allowing it to be collected from two central locations. These species are hereafter referred to as the central species. Species pairs were also selected to control for other biological and ecological attributes that could otherwise confound the population genetic structure of these species. Species were selected that had similar dispersal potentials (Chapter 5), habitat use, diets, reproductive modes and generation time (Table 1). Distributional information was obtained from guidebooks (e.g., Randall et al. 1997; Kuiter 1993), Fishbase (www.fishbase.org) and the Australian Museum fish distribution database. Approximately 25 – 30 individuals per species were collected from each of the two locations used for each species (Table 1).

Table 1: Sampling locations, biological and ecological attributes of the six species. Sampling locations: One Tree Island (OTI) 23°30S; 152°05E; Orpheus Island (OI) 18°38S; 146°28E; Lizard Island (LI) 14°40S; 145°28E. Position in the species range (C = centre, P = peripheral) and sample sizes for (mtDNA/ nucDNA) data sets, latitudinal spread (Lat. spread) (W = widespread, R = restricted), pelagic larval duration (PLD) (min – max), diet (P = planktivore, H = herbivore), reproductive mode (Rep.) (B = benthic spawning), generation time (years) and proportional local abundance (Prop. abund) of widespread species (W) vs. restricted (R) species. = indicates approx. equal abundance of widespread and restricted species within a genus and W > R indicate a greater local abundance of the widespread species.

	Sampling locations			Geography, biology and ecology					
Species	One Tree Island	Orpheus Island	Lizard Island	Lat. spread [1]	PLD	Diet [1]	Rep. [1]	Gen. time [1]	Prop. abun
Amphiprion melanopus	C (22/22)		C (20/24)	40 (W)	11 (8 – 14)[2]	P	B	1.4 – 4.4	W > R [5]
Amphiprion akindynos	C (24/24)		P (20/23)	22 (R)	11 (9 – 13)[2]	P	B	1.25	
Pomacentrus moluccensis	C (21/ 24)		C (25/24)	55 (W)	15 (14 – 21)[2]	P	B	1.25	W > R [6]
Pomacentrus wardi	C (20/23)		P (22/20)	22 (R)	26.1 (19 – 28)[2]	H / P	B		
Chromis atripectoralis	C (20/ 20)	C (22/ 24)		62 (W)	21.2 (18 – 22)[3]	P	B	1.25	W = R [7]
Chromis nitida	C (17/ 23)	P (17/ 17)		23 (R)	16 (10 – 24)[4]	P	B	1.4 – 4.4	

Source: [1] www.fishbase.org; [2] Chapter 4; [3] Bay unpubl. data; [4] Doherty et al. 1995, Thorrold and Milicich (1990); [5] Srinivasan unpubl. data; [6] Fulton unpubl. data; [7] Eagle unpubl. data

Fish were collected by hand-held spears, fence nets, clove oil and hand-held dip-nets. Fishes were transported live, or on ice, to the nearest shore where a sample (fin clip) was preserved in 100% EtOH for later analysis.

Molecular techniques

MtDNA: DNA was extracted, 335 to 398 base pairs of the mitochondrial hyper variable control region I were amplified, sequenced in the forward and reverse directions and aligned in 39 to 46 individuals from all species following methods outlined in Chapter 5. Representative sequences have been deposited in GenBank under accession numbers DQ250449 – DQ250526, DQ212240 – DQ212281, DQ212323 – DQ212410.

ISSR: Genetic fingerprints were obtained from 17 – 24 individuals per location using Inter Simple Sequence Repeats (ISSR) using 5 universal primers following the methods outlined in Chapter 5. Presence and absence of bands between 50 and 850 base pairs in length were scored using MegaBACE Fragment Profiler 1.2 (Amersham Biosciences), then converted into binary data matrices and concatenated. Raw binary data matrices are available from the authors upon request.

Statistical procedures

Sequence data: The best fitting substitution model and associated rate heterogeneity were estimated separately for each species using PAUP* 4.0b10 (Swofford 1998) and Modeltest 3.5 (Posada and Crandall 1998) and these, where possible, were implemented in all subsequent analyses (Table 2). Genetic diversity estimates for haplotype and nucleotide diversity (Nei, 1987, Tajima 1983) and their associated standard deviations were calculated using Arlequin 2.000 (Schneider et al. 2000). Standard deviations were converted to 95% confidence intervals (95% CI = $\pm$1.96*(SD/$\sqrt{}$ (n))). Estimates of genetic structure were calculated as pairwise Φ_{ST} values following the methods implemented in Arlequin and significance levels were corrected for multiple comparisons following the Dunn-Sidak method (Sokal and Rohlf, 1995). Transition – transversion ratios indicated that saturation may have occurred in some species (Table 3). Therefore, all analyses were repeated using transversions only. The demographic history of species was analysed using mismatch analysis using Arlequin and 1000 bootstrap replicates.

Table 2: Number of base pairs, transition – transversion ratios (ts-tv) substitution models, gamma distribution shape parameter (γ), invariable sites and their likelihoods (determined by Modeltest) for the six species included in this study

					Among-site rate variation		
Species	No base pairs	Ts-Tv ratio	Model selected	-ln Likelihood score	Invariable sites	γ	Model implemented
Amphiprion melanopus	335	6.3	HKY	576.73	0	0	TN(93)
Amphiprion akindynos	354	9.8	HKY	956.9848	0	0.1410	TN(93) +G
Pomacentrus moluccensis	349	5.9	HKY	652.70	0	0	TN(93)
Pomacentrus wardi	359	8.1	HKY	1211.3286	0	0.3002	TN(93) +G
Chromis atripectoralis	349	7.7	HKY	924.56	0	0	TN(93)
Chromis nitida	347	6.2	HKY	1058.0591	0.6167	0.7053	TN(93) +G

The distribution of genetic variation within species was compared among species using haplotype networks and the frequency distribution of pairwise differences among individuals pooled from the two sampling locations.

ISSR data: Due to the large number of fragments amplified by the 5 primers, only bands with a minimum frequency of 0.25 within any particular species were analysed. No differences were detected among analyses of 0.05, 0.1 and 0.25 minimum frequency data sets conducted for a subset of species. Therefore, this data reduction did not appear to have affected the results significantly. Mendelian segregation of fragments with a single dominant (amplified) and recessive (absent) allele at each banding position was assumed. Because dominant data do not allow within-individual heterozygosities to be estimated, Hardy-Weinberg equilibrium was assumed in the analysis of these data. Given the large population sizes and the potential for considerable pelagic larval dispersal, this assumption was deemed reasonable in this study. Analogues of codominant genetic diversity measures including within-population diversity H_e (here H_j), total heterozygosity H_t and mean heterozygosity across populations H_s (here H_w) were calculated following the methods of Lynch and Milligan (1994) using AFLP-Surv 1 (Vekemans et al. 2002). Genetic structure (Φ_{PT}) was estimated using Genalex 5 (Peakall and Smouse 2001) and significance levels were corrected for multiple comparisons (Sokal and Rohlf 1995). Genetic diversity estimates were interpreted as statistically different when confidence intervals did not overlap.

Results

Estimates of genetic differentiation based on the mtDNA were generally low (Φ_{ST} -0.005 – 0.01) and statistically insignificant in all species when based on transitions and transversions or on transversions alone (Table 3). In contrast, estimates of genetic differentiation were higher when based on the ISSR data (Φ_{RT} 0.018 – 0.188) and indicated significant genetic structuring between northern and southern GBR locations in all species except *P. moluccensis* (Table 3). Patterns of gene flow were significantly lower in the peripheral species compared to central species when based on transversions of the Control region data ($Z_{3,3} = 1.964$, P = 0.0495) but not when including transitions ($Z_{3,3} = 0.655$, P = 0.654) or when based on ISSR data ($Z_{3,3} = 0.577$, P = 0.564).

Table 3: Estimates of genetic differentiation in the six species. Mitochondrial fixation indices (Φ_{ST}) based on transitions and transversion (ts-tv) and transversions alone (tv) and fixation index based on ISSR data (Φ_{PT}). Significance: * P < 0.01, ns = not significant (insignificance following sequential Bonferroni correction in bold).

Species	Φ_{ST} (ts – tv)	p	Φ_{ST} (tv)	p	Φ_{PT}	p
Amphiprion melanopus	-0.00487	0.47 ns	-0.0019	0.38 ns	0.188	0.001*
Amphiprion akindynos	-0.02405	0.65 ns	-0.0186	0.51 ns	0.119	0.001*
Pomacentrus moluccensis	0.00553	0.25 ns	0.0049	0.04 **ns**[1]	0.018	0.022 *
Pomacentrus wardi	-0.02021	0.75 ns	-0.0249	0.93 ns	0.034	0.002*
Chromis atripectoralis	-0.01551	0.70 ns	0.0131	0.17 ns	0.159	0.001*
Chromis nitida	0.01328	0.26 ns	-0.0366	0.89 ns	0.114	0.001*

[1] Bonferroni corrected significance level $\alpha = 0.008$

Mitochondrial (haplotype and nucleotide) and ISSR diversities (H_w) varied among species and locations (Fig. 1 – 3). Haplotype diversities were generally high (but lower in *P. moluccensis*) and significantly lower in the peripheral population in a single species (*A. akindynos*) when transitions and transversions were included (Fig. 1a). Haplotype diversities were low when considering only transversions and were similar between populations of all species except two. In *A. melanopus* and *P. wardi* haplotype diversities were greater in the northern population coinciding with the northern range margin in *P. wardi* (Fig. 1b). Haplotype diversities were consistently greater in both populations of the peripheral species compared to haplotype diversities of both populations of central species (Fig. 1b). Nucleotide diversities were generally high and did not vary between locations in any of the species except *A. akindynos* where nucleotide diversities greater in the peripheral population (Fig. 2a). When based on transversions, nucleotide diversities did not differ between populations of any of the species, but were generally higher in the peripheral species compared to their central congeners (Fig. 2b). Expected heterozygosities (H_j) were significantly lower in the northern location of *A. akindynos* and *A. melanopus* but similar between locations of the other four species (Fig. 3).

Significant differences were observed in overall genetic diversity (haplotype, nucleotide and mean heterozygosity) among species (Fig. 4 - 6). Haplotype and nucleotide diversities were significantly higher in the peripheral species compared to their central congeners when based on transitions and transversions (Fig. 4a, 5a) and this pattern was particularly evident when based on transversions only (Fig. 4b, 5b). Likewise, levels of heterozygosity (H_w) were significantly greater in the peripheral species compared to the central congeners in two genera (Fig. 6). Intraspecific variation in H_j of two species was large (*A. akindynos* and *A. melanopus* Fig. 3) and hence, the estimate of H_w was variable in these species. Nucleotide diversities were significantly greater in peripheral compared to central species (ts - tv: $Z_{3,3} = -1.963$ $p = 0.0495$; tv: $Z_{3,3} = -1.96$, $P = 0.0495$) but haplotype and expected heterozygosities were not (ts - tv: $Z_{3,3} = -0.65$, $P = 0.51$; tv: $Z_{3,3} = 1.527$, $P = 0.126$; H_w: $Z_{3,3} = 1.547$, $P = 0.248$).

The haplotype networks and mismatch distributions revealed large and consistent differences between peripheral (Fig. 7a – c) and central species (Fig. 7d – e). The haplotype networks of peripheral species were complex; central haplotypes were less frequent and distal haplotypes were separated by many mutations.

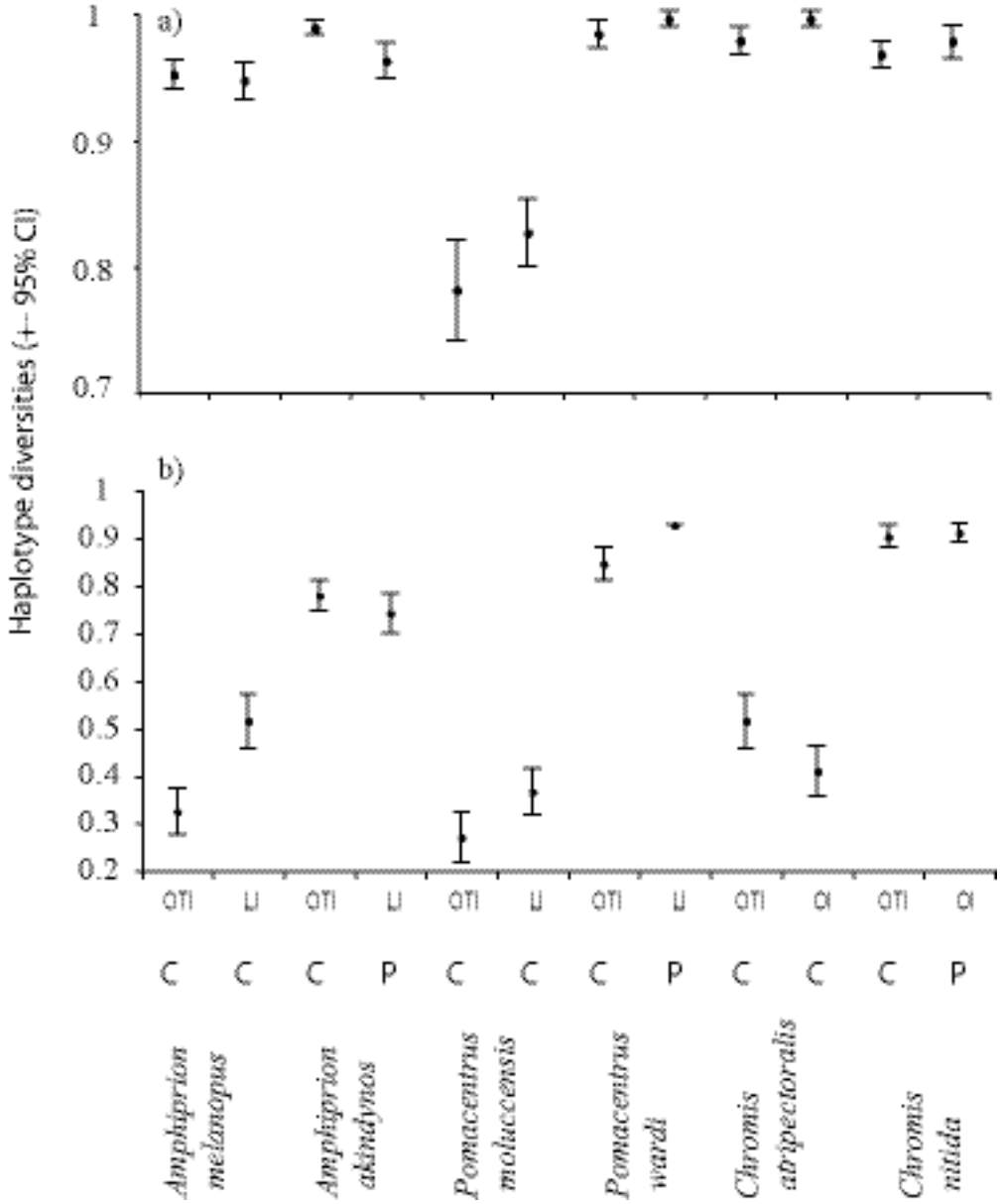

Fig. 1: Haplotype diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions alone in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

Mismatch distributions were characterised by larger means (13.7±SE 0.09) and were bimodal in two of the three species (*A. akindynos* and *P. wardi* Fig. 7 a, b). The mismatch distribution in *C. nitida* was unimodal with a large mean and variance (13.57 ±SD 6.96). In contrast the haplotype networks of the central species were characterised by one, or a few, central haplotypes of higher frequency with distal haplotypes being separated by one or a few mutations. Mismatch distributions were unimodal with small

means (4.6 ±SE 2.19) (although a slightly higher mean of 8.99 was obtained for *C. atripectoralis*). This pattern was also evident in comparisons using only transversions, although mismatch means and their variation were lower (unpublished data).

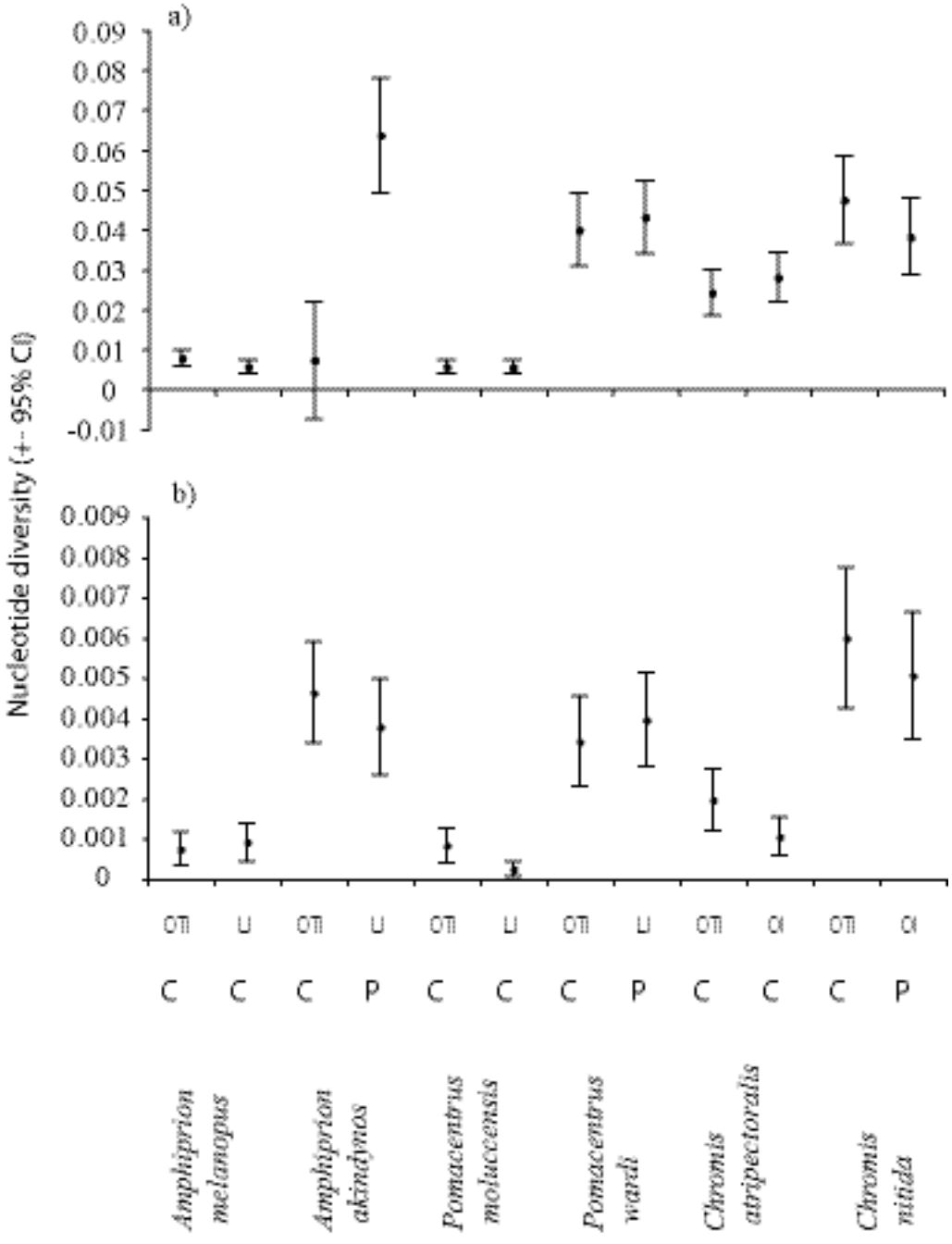

Fig. 2: Nucleotide diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

These differences in genetic diversities could not be explained by differences in demographic histories as all species appeared to have a signal of demographic expansion (the null hypothesis of sudden expansion was retained in all species, Table 4) and experienced similar expansion times, regardless of positions in the species' range of the populations sampled (Table 4).

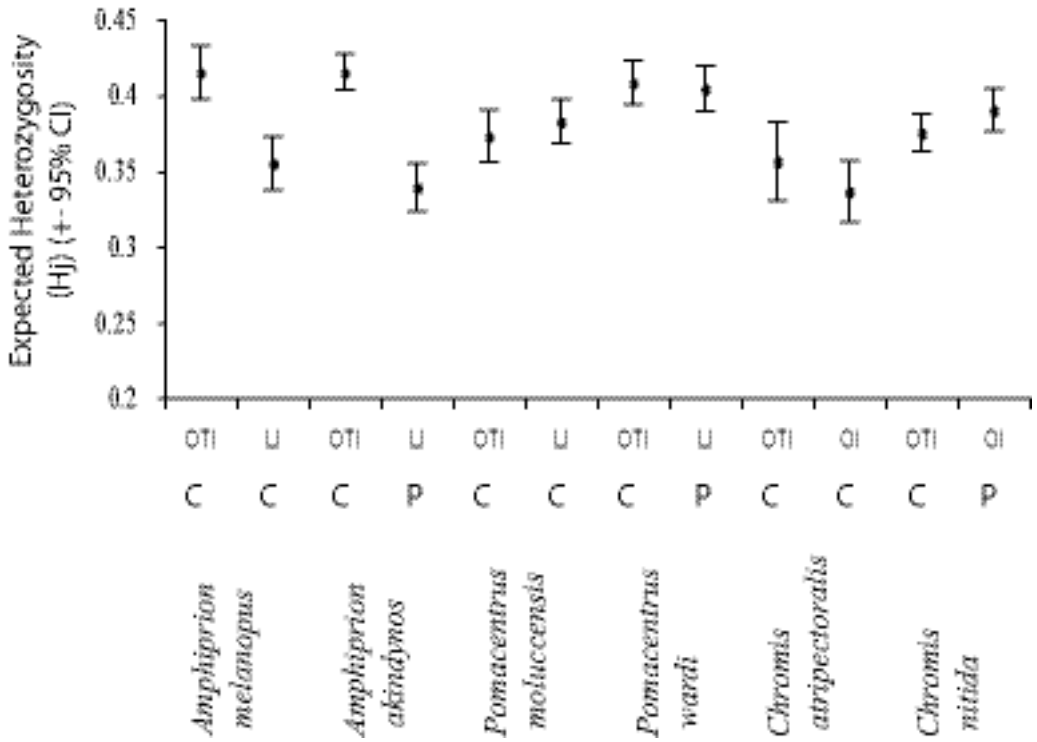

Fig. 3: Expected heterozygosities (± 95% confidence intervals) in central (C) and peripheral (P) populations of the six species. LI = Lizard Island, OTI = One Tree Island.

Discussion

Gene flow and genetic diversities on the species margin

Although the population genetic effects of species' borders determined by demographic processes are well established in theory, empirical support remains equivocal. In general, this study found no evidence to support the predictions from species' borders theory of greater genetic structure and lower genetic diversities towards the species margin. All species displayed high levels of gene flow although the ISSRs indicated that some genetic isolation was present (Table 3). Reduced gene flow towards the edge of the range was only evident from analyses of transversions (Table 3).

Table 4: Demographic history analysis of all species including mismatch mean, summed square deviations (SSD) and Bonferroni corrected p (ns = not significant, bold indicates insignificance following sequential Bonferroni correction), expansion parameter (τ) and its 95% confidence interval.

Species	Mismatch mean	SSD	p	τ	Lower bound of 95% CI	Upper bound of 95% CI
Amphiprion melanopus	2.416	0.007	0.091 ns	2.59	1.147	3.369
Amphiprion akindynos	13.853	0.022	0.035 **ns**[1]	5.023	1.312	26.895
Pomacentrus moluccensis	2.416	0.007	0.091 ns	2.896	0.727	5.652
Pomacentrus wardi	13.804	0.011	0.329 ns	3.589	1.481	16.545
Chromis nitida	13.569	0.002	0.954 ns	13.774	9.059	23.071
Chromis atripectoralis	8.988	0.001	0.966 ns	9.986	6.096	12.753

[1] Bonferroni corrected significance level $\alpha = 0.008$

This result may indicate that historical gene flow was lower than present day patterns, although the reduction in data may have influenced this result. Consequently, all species, regardless of position in the species range, were characterised by relatively low levels of genetic structure consistent with moderate gene flow between sampling locations.

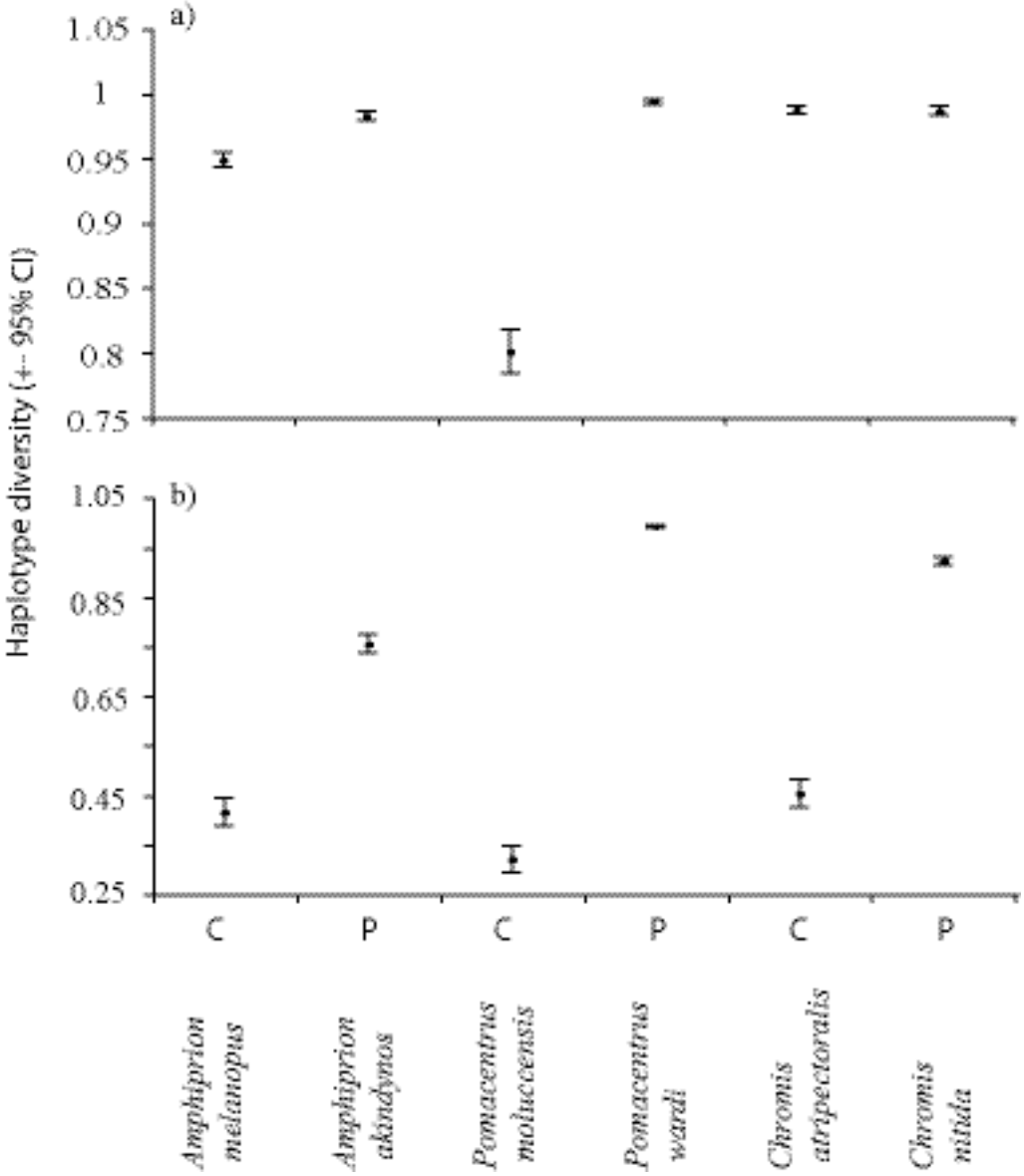

Fig. 4: Haplotype diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions in the three central (C) and the three peripheral (P) species.

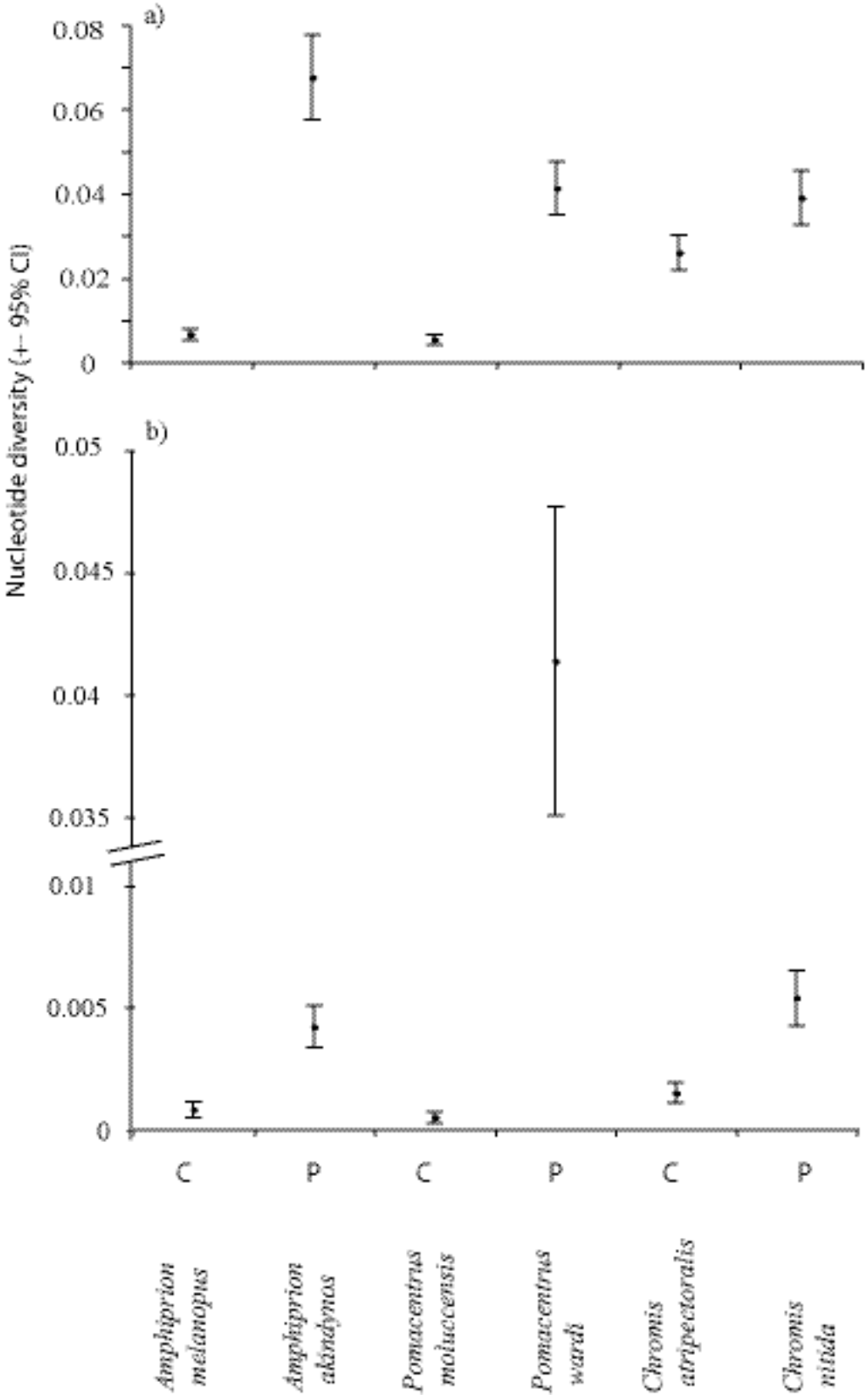

Fig. 5: Nucleotide diversities (± 95% confidence intervals) based on a) transitions and transversions and b) transversions in the three central (C) and the three peripheral (P) species.

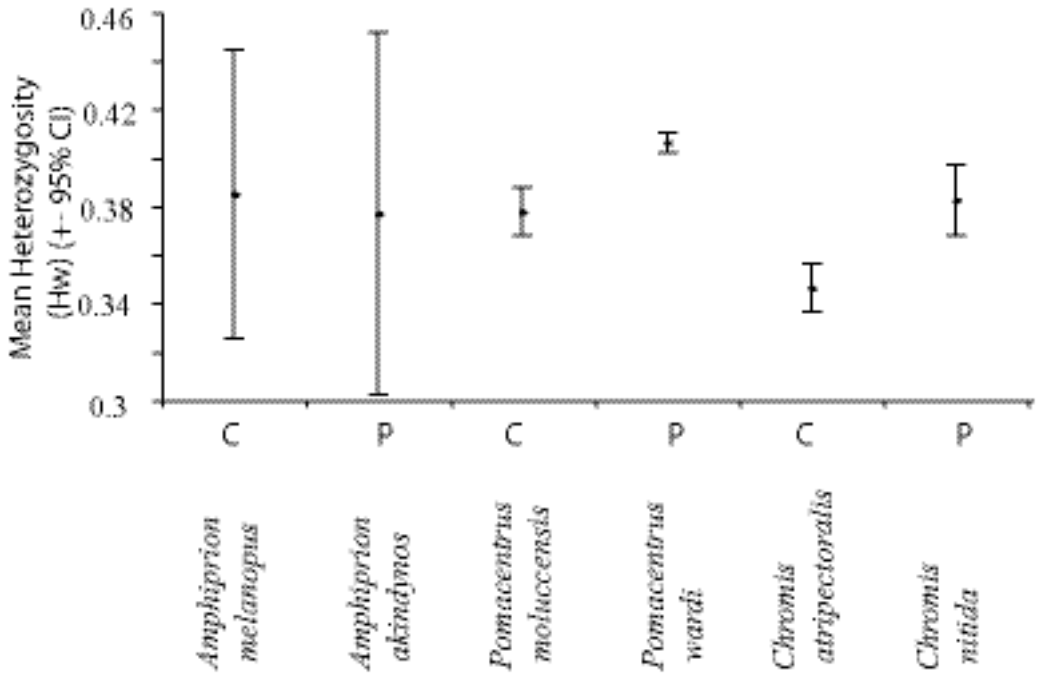

Fig. 6: Mean heterozygosity (± 95% confidence intervals) in the three central (C) and the three peripheral (P) species.

Genetic diversities varied among species and locations, but were not consistently lower in the populations sampled close to their geographic limits. For example, *A. akindynos*, a peripheral species, had lower ts-tv haplotype (Fig. 1a) and expected heterozygosity (Fig. 3) in the peripheral population, but nucleotide diversities were higher at this location (Fig. 2). Similarly, *A. melanopus*, a central species, had lower haplotype diversity (Fig. 1b) and lower expected heterozygosity in the northern population (Fig. 3), centrally located in its range. Therefore, the lower genetic diversities in the *Amphiprion* spp. appear to be associated with the northern location rather than the peripheral position in *A. akindynos*. While it is possible that I did not detect a genetic signature of declining populations at the species margin because I did not sample close enough to the border (Lennon et al. 1997), high genetic diversities can be maintained in peripheral populations, even if these are effectively sinks, by high levels of gene flow (Vucetich and Waite 2003). The estimates of genetic structure of the species included in this study all indicated relatively high levels of gene flow between central and peripheral populations. Consequently, the high genetic diversities maintained on the species margins recorded by this study may be maintained by high gene flow from more centrally located populations.

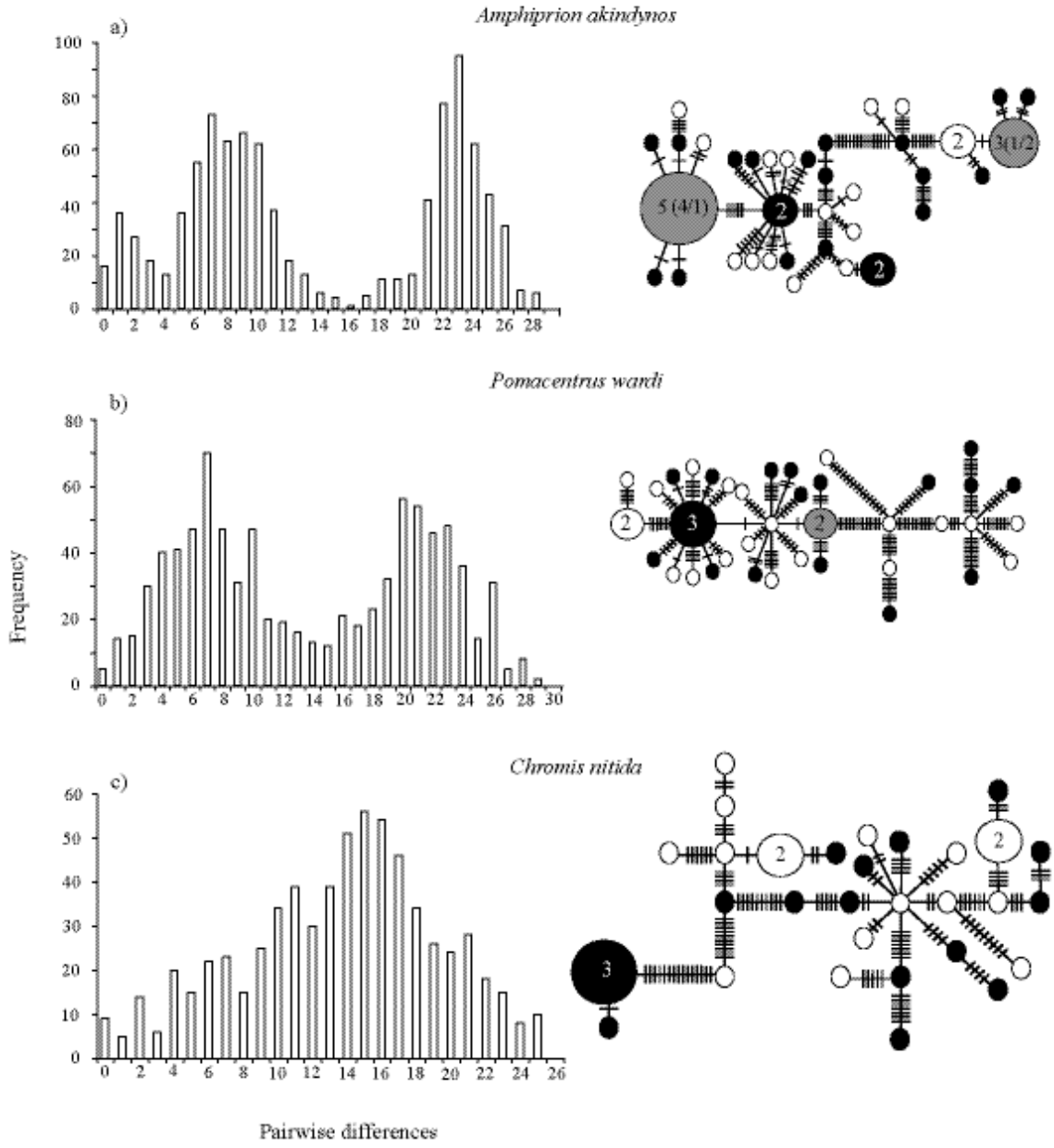

Fig. 7: Mismatch distributions and haplotype networks of the peripheral species (a – c) and the central species (d – f). Haplotype networks: Haplotypes indicated by circles where their size and the embedded number indicates its frequency with the frequency of shared haplotypes indicated in brackets (LI/OI, OTI), the location indicated by colour (white = LI/ OI, black = OTI, grey = shared) and the number of mutations separating haplotypes represented by bars.

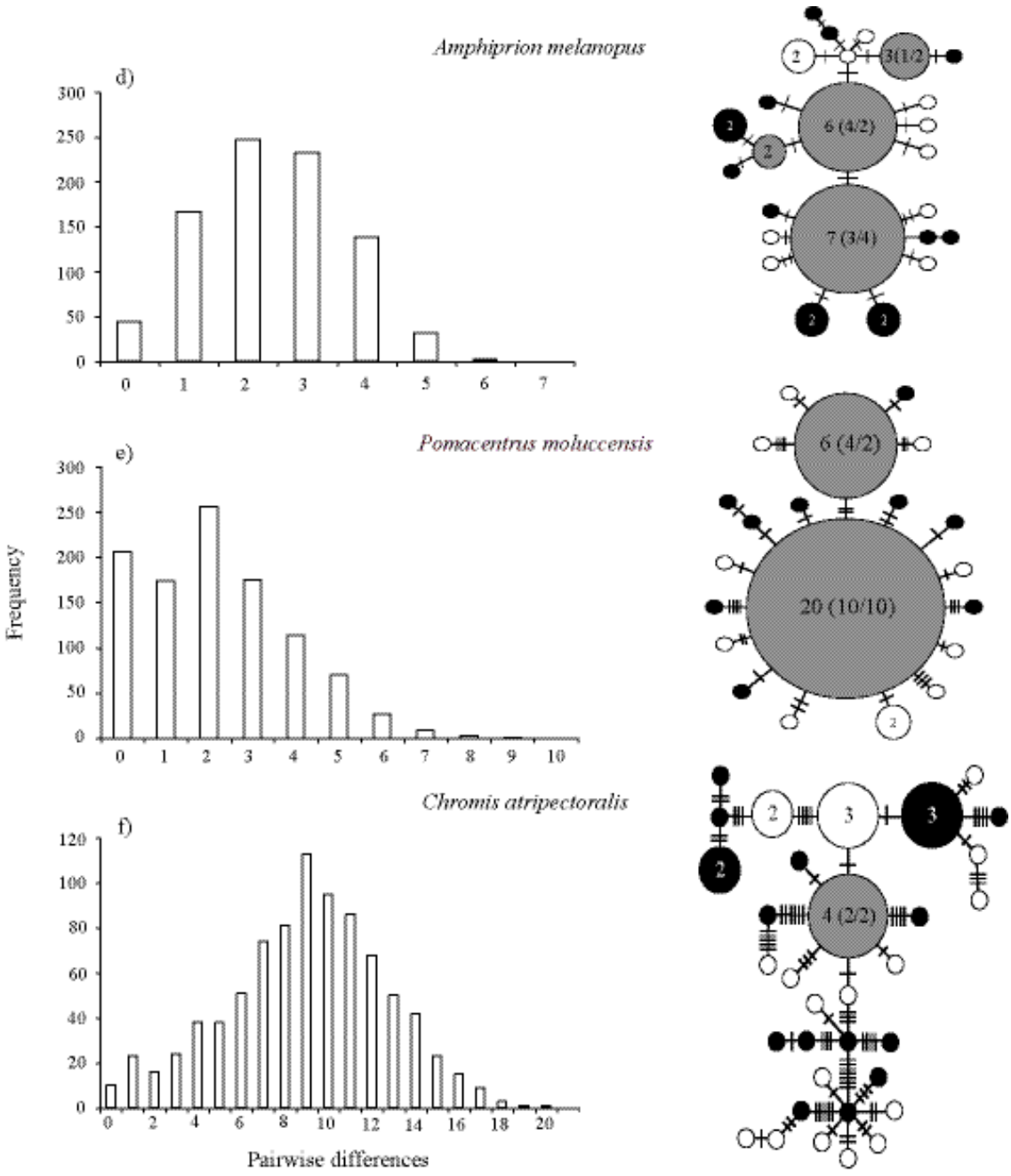

Fig. 7 Continued

Genetic diversities in peripheral and central species

Genetic diversities were consistently greater in the peripheral species compared to central species (Fig. 4b, 5, 6), despite very similar levels of gene flow in all species. Levels of genetic diversity in the central species were similar to those reported for widespread coral reef fishes that have experienced long stable evolutionary histories (e.g., Fauvelot et al. 2003). In contrast, the genetic diversities of the peripheral species

were higher than most other values reported for reef fishes (e.g., Grant and Bowen 1998; Planes 2002; Fauvelot et al. 2003). The concordance of this pattern among species from three genera suggests that a general mechanism may be underlying this pattern. A number of possible explanations can be erected to explain the higher genetic diversities in the peripheral species and I consider these in turn below.

Large population sizes on the species' periphery

The higher genetic diversity in peripheral species could arise if they have higher local abundances (Soulé 1976) and if true would suggest that Lawton's universal rule (of a positive relationship between local abundance and geographic distribution) does not apply to coral reef fishes. Here, the central species were either similarly or more abundant than the peripheral species at all sampling locations (Table 1). Local abundance patterns do not therefore appear to provide an adequate explanation for the observed differences in genetic diversities.

Peripheral species are older or have inhabited the GBR for longer

If mutations accumulate at a constant rate then the higher genetic diversity in peripheral species may be expected if the taxa are older, or if they have occupied the GBR for longer (Soulé 1972). The phylogenetic relationships of the majority of species used here (except *P. wardi*) were examined by Quenouille et al. (2004). Branch lengths of peripheral species were not significantly longer than branch lengths of central species ($F_{2, 1} = 0.17$, $P = 0.13$) indicating that these taxa are not older. Likewise, peripheral species did not appear to have occupied the GBR for longer than the central species; all species displayed a signal of sudden expansion and expansion times did not differ among species (Table 4). These results indicate that the populations of the species included in this study may not be at migration – drift equilibrium, however, the potential degree of disequilibrium did not appear to differ among species. The current population genetic structure of these species may have been affected by a genetic bottleneck potentially associated with the initial colonisation of the GBR following the last glacial maximum. If so, this did not appear to have had a greater effect on central species compared to peripheral ones. It therefore appears that neither taxon age, nor duration of local occupancy provide adequate explanations for the observed patterns.

Peripheral species have greater genetic diversity because of their evolutionary histories

High haplotype and nucleotide diversities may arise when populations that have diverged during historical isolation come into secondary contact, or if species have experienced a long and stable evolutionary history (Grant and Bowen 1998). Secondary contact among differentiated lineages should be evident as bimodal or multimodal distributions of pairwise differences whereas long stable evolutionary histories should produce broad unimodal mismatch distributions (Avise 2000). I observed strong bimodal mismatch distributions in two of the three peripheral species (i.e., *A. akindynos* and *P. wardi*) and a broad unimodal mismatch distribution in *C. nitida* (Fig. 7a - c) contrasting with the narrow unimodal distributions obtained for all three central species (Fig. 7d – f). This pattern indicates that the high genetic diversities in at least two of the peripheral species could be the result of secondary contact. It is possible that the peripheral species persisted and diverged in isolated off-shore refugia during the last glacial maximum (Davies 1989) and that these lineages came into contact when the GBR was formed approximately 6000 - 9000 years ago (Hopley and Thom 1983; Larcombe 2001). Consequently, the observed pattern in genetic diversities could have been produced if the GBR was colonised by several genetically differentiated lineages of the peripheral species, but only one of the central species. While this mechanism is plausible, it is not particularly parsimonious. It is unclear why such isolation would only apply to some of the species, given that all commonly co-occur on many reefs of the GBR. It is also plausible that this pattern could have been generated in sympatry if gene flow were historically lower in peripheral species compared to their central counterparts. I detected lower gene flow in the spatially restricted species based on analyses of transversions (Table 3). These rates, however, were still too high to allow for this level of divergence. Conversely, if historically the dispersal potential of the peripheral and central species were similar, patterns of genetic diversity may have been generated if they did not occupy the same Pleistocene refugia to the east of the GBR, or if the central species colonised the GBR from northern refugia. Genetic diversities commonly decline with increasing distance from Pleistocene refugia (reviewed by Gaston 2003; Briggs 2004). Consequently the differences in the genetic diversities between central and peripheral species may by explained by their contemporary proximity to such refugia. This explanation bears superficial resemblance to the centrifugal speciation hypothesis initially proposed by Brown (1957) and advocated by Briggs (2000). In this model species disperse out of the centre of diversity and

populations at the periphery of the ranges become isolated and speciate in allopatry during repeated cycles of range expansion and contraction (Brown 1957; Briggs 2000). The peripheral species are not able to colonise the centre of diversity potentially due to interactions with already established species (Briggs 1974). Such unidirectional dispersal filters have been proposed for the east and west of the Indo-pacific centre of diversity (Briggs 1974), but have never previously been implicated in explaining the species distributions to the south of the centre of diversity. Some of the tenets of the centrifugal speciation hypothesis, such as peripheral species being plesiomorphic and extinction prone relics (Brown 1957; Briggs 1974) are not met by the current study. Here the peripheral species were not older and did not appear extinction prone given their very high effective population sizes. However, the predictions regarding the direction of dispersal and the presence of barriers erected by the centrifugal speciation hypothesis could explain the pattern of genetic diversity found by this study, although this explanation is not very parsimonious.

Species have higher mutation rates on the periphery of the range

The differences in genetic diversities between the peripheral species and the central species could be generated if mutation rates differ between the two groups. Mutation rates can be elevated by sub-lethal temperature stress (*Drosophila melanogaster,* Lindgren 1972) or by other stressful conditions that disrupt intracellular homeostasis (*Mus musculus*, Kerkis 1975; Belyaev and Borodin 1982). Environmental conditions are generally assumed to be effectively more extreme and stressful on populations at the species margin compared to populations closer to range centres (Hoffmann and Parsons 1991; Parsons 1991). If so, such a process may have led to a higher mutation rate at this location. The moderate levels of gene flow (Table 3) could have then distributed these mutations across the species' range. It is, however, unclear how elevated rates of mutation on the species margin could have produced the bimodal mismatch distributions observed in two of the peripheral species without genetic isolation among lineages (Fig 7 a – b). Consequently, elevated mutation rates on the species margin may play an important role in producing the very high genetic diversities in the peripheral species. This hypothesis alone, however, does not provide a satisfactory explanation for patterns of genetic diversities observed here.

The species' borders are the result of physical barriers or physiological stress

It is possible that the species' borders examined are not the product of declining demographic processes in peripheral populations but rather a result of a physical barrier to dispersal. If so, barriers would have to occur in at least two different locations and be species specific or unidirectional (i.e., permeable from the north but not the south) (Briggs 1974) given the distributional patterns of the species studied here. At present there is insufficient evidence of the population genetic structure of marine organisms at this spatial scale to evaluate this hypothesis. Further sampling incorporating a population genetic examination of central species from locations north of the GBR may elucidate the potential presence and role of such putative barriers.

It is possible that the species borders examined here are determined by physiological stress at the species margin, a lack of genetic variation in stress tolerance related traits and/or a failure of natural selection to produce local adaptive optima because of gene flow from central locations (Hoffmann and Parsons 1991). The examination of neutral genetic variation here does not permit an examination of these hypotheses, but the high levels of gene flow recorded indicate a potential important role of migration into marginal populations.

Conclusion

Contrary to expectations, gene flow did not differ among central and marginal species and genetic diversities were not less in peripheral populations compared to central populations or species sampled in the centre of their ranges. Indeed, genetic diversities were much higher in the spatially restricted border species compared to their more widespread counterparts indicating that they have much larger effective population sizes. Based on the distribution of genetic diversity in the peripheral and central species, it appears most likely that historical isolation and subsequent secondary contact has produced the patterns of genetic diversities detected here. Higher mutation rates mediated by environmental stress on the species margin may have further enhanced genetic diversities in the peripheral species. Here, I can only speculate on the processes generating the very high genetic diversities in the species sampled at the species margin. The genetic patterns uncovered by this investigation, however, may form the foundation for further investigations examining the genetic consequences of species' borders in coral reef fishes.