

# Latitude-wide genetic patterns reveal historical effects and contrasting patterns of turnover and nestedness at the range peripheries of a tropical marine fish

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Few studies have examined core–periphery genetic patterns in tropical marine taxa. The core–periphery hypothesis (CPH) predicts that core populations will have higher genetic diversity and lower genetic differentiation than peripheral populations as a consequence of greater population sizes and population connectivity in the core. However, the applicability of the CPH to many tropical marine taxa may be confounded by their complex population histories and/or high (asymmetric) population connectivity. In this study we investigated genetic patterns (based on mtDNA) across the latitudinal range of the neon damselfish *Pomacentrus coelestis* (36°N, Japan – 37°S, east Australia). We suggest a novel hypothetical framework for core–periphery genetic patterns and extend typical analyses to include genealogical analyses, partitioned  $\beta$ -diversity measures (total  $\beta_{\text{SOR}}$ , turnover  $\beta_{\text{SIM}}$ , and nestedness-resultant  $\beta_{\text{SNE}}$ ), and analyses of nestedness. We found that the existence of two divergent lineages of the neon damselfish led levels of genetic diversity to deviate from CPH expectations. When focusing on the widespread lineage (Pacific clade) nucleotide diversity was higher in the core, supporting the CPH. However, genetic patterns differed toward the northern and southern peripheries of the Pacific clade. The turnover of haplotypes (pairwise- $\beta_{\text{SIM}}$ ) increased over distance in the north, indicative of historical colonization with little contemporary migration. In contrast, although turnover was still dominant in the south ( $\beta_{\text{SIM}}$ ), there was no relationship to distance (pairwise- $\beta_{\text{SIM}}$ ), suggesting the influence of more contemporary processes. Moreover, the haplotype compositions of populations in the south were nested according to latitude, indicating immigration from lower latitudes toward the southern periphery. By extending the typical characterizations of core–periphery genetic patterns we were able to identify the effects of lineage sympatry on measures of genetic diversity and contrasting demographic histories toward the latitudinal peripheries of the neon damselfish's range.

Range-wide genetic patterns provide a window into a species' history, the contemporary demography of populations, and can ultimately help inform conservation priorities. Despite the importance of range-wide genetic information, predictive hypotheses of genetic patterns across a species' entire range are rare. The core–periphery hypothesis (CPH; da Cunha et al. 1950, Brussard 1984, Eckert et al. 2008) can be considered a null hypothesis for range-wide genetic patterns, although the model is very simple. The CPH considers two factors that influence the balance of migration, mutation, and drift across a species range to predict range-wide genetic patterns. First, it assumes that the close geographic arrangement of populations in the core of a species range will lead populations to exchange migrants frequently, reducing compositional differences among core populations. Second, the

CPH predicts that greater standing genetic variation will be maintained in the core of a species range (Lammi et al. 1999) based on the assumption that population sizes will be larger in the core than in the periphery (i.e. the abundant-center hypothesis: Antonovics 1976, Hengeveld and Haack 1982). Thus, the CPH predicts two genetic patterns across a species range: core populations will have higher genetic diversity than peripheral populations; and peripheral populations will be more genetically differentiated from each other relative to comparisons among core populations (Fig. 1a I).

Although several studies have described range-wide genetic patterns in agreement with the CPH expectations (reviewed by Eckert et al. 2008), the historical and contemporary demographic processes underlying those genetic patterns likely vary. A number of demographic processes

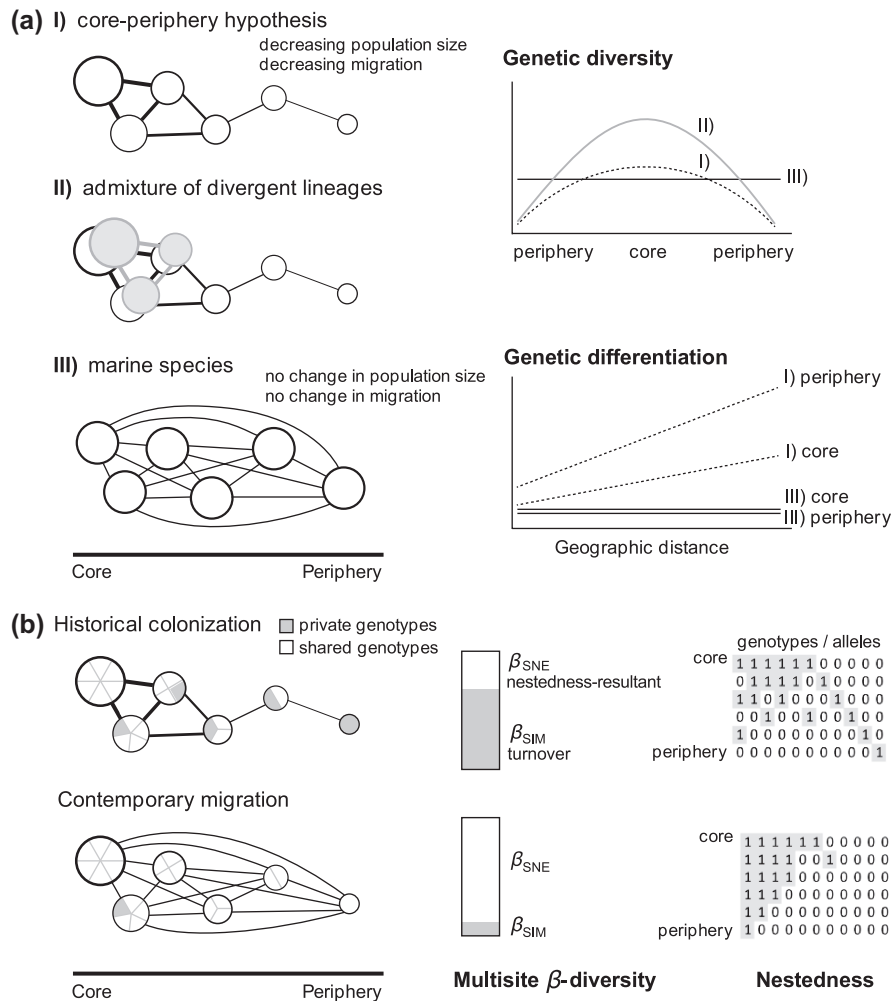


Figure 1. Hypothetical scenarios underlying range-wide genetic patterns of Indo-Pacific marine species. (a) Expected patterns across the range for genetic diversity and genetic differentiation over geographic distance based on: I) the core-periphery hypothesis; II) sympatry of divergent lineages in the range core; and III) high levels of migration and large population sizes that may be typical of a marine species. Circles represent relative population sizes and weighted lines represent migration rates. (b) The contribution of the turnover ( $\beta_{SIM}$ ) and the nestedness-resultant ( $\beta_{SNE}$ ) components to total multisite  $\beta_{SOR}$ -diversity (total bar height) and expected patterns of nestedness in the range periphery under a scenario of historical colonization and contemporary isolation, versus asymmetric contemporary migration into the range periphery. Circle partitions denote genotypic composition (private or shared) and relative diversity (number of partitions).

affect patterns of genetic diversity and differentiation and are not considered in the CPH. For example, genetic diversity can be elevated by admixture, such as when divergent lineages become sympatric following periods of demographic isolation (Petit et al. 2003). Furthermore, genetic diversity is generally higher in older populations and decreases predictably according to colonization timing (Hewitt 1996, Hardie and Hutchings 2010). Lastly, populations toward the range periphery are less likely to be in migration–drift equilibria and therefore may have highly variable patterns of genetic diversity and differentiation. Classical tests of the CPH relying on patterns in genetic diversity and differentiation cannot capture the contribution of such underlying population histories.

Methods that extend the usual characterizations of core–periphery genetic patterns may help disclose the demography underlying range-wide genetic patterns. For example, genealogical analyses prior to range-wide analysis can identify divergent lineages that are now sympatric. Furthermore,

concepts and methods used in community ecology to describe analogous spatial patterns of species diversity can be applied to genetic data (Diniz-Filho and Bini 2011, Liggins et al. 2013). In particular, two methods could be useful for characterizing population histories toward a species range periphery: the partitioning of  $\beta$ -diversity (Baselga 2010, 2012) and analyses of nestedness (Darlington 1957, Daubenmire 1975).

Traditionally,  $\beta$ -diversity has been used as a measure of the variability in species composition among sites or communities (Whittaker 1960), but it can also measure the variability in the genetic composition among populations (Diniz-Filho et al. 2012). Sørensen's dissimilarity,  $\beta_{SOR}$  (Sørensen 1948) can be partitioned into components due to the replacement of genotypes among populations (the turnover component,  $\beta_{SIM}$ ; Simpson's dissimilarity, Simpson 1943) and a component describing differences in genotype richness caused by nestedness among populations (the nestedness-resultant component,  $\beta_{SNE}$ , Baselga 2010, 2012,

Legendre 2014). Thus, the partitioning of  $\beta$ -diversity can be used to reveal the contribution of private (the turnover component,  $\beta_{\text{SIM}}$ ) versus shared genotypes (the nestedness-resultant component,  $\beta_{\text{SNE}}$ ) to diversity changes and the differentiation among populations. Such partitioning of  $\beta$ -diversity complements  $F$ -statistics and genetic diversity measures that are typically used in spatial genetic analyses. For example, when pairwise  $F_{\text{ST}}$  is equivalent among populations, and/or genetic diversity is consistently high across populations, there are still several scenarios for how these genetic patterns could be conferred by private versus shared genetic diversity (contributing to  $\beta_{\text{SIM}}$  and  $\beta_{\text{SNE}}$ , respectively). Moreover, the relative contribution of private versus shared genotypes is meaningful in a population genetic context. We would predict that a greater contribution of private genotypes ( $\beta_{\text{SIM}}$ ) to  $\beta$ -diversity ( $\beta_{\text{SOR}}$ ) among populations would be indicative of mutation and drift (Slatkin 1985), whereas high rates of genotype sharing ( $\beta_{\text{SNE}}$ ) would be indicative of migration (Fig. 1b).

The concept of nestedness, or the way in which communities form subsets of other communities (Wright and Reeves 1992, Almeida-Neto et al. 2008), can also be used to describe the nature of genetic diversity changes among populations. Where there is a spatial gradient of interest, such as a core–periphery transect, the nested structure of genotype compositions along that gradient can be statistically compared to null expectations (Ulrich et al. 2009). In a population genetic context, populations that share the same genotypes are likely to have migration between them. Furthermore, populations that have only a reduced subset of the shared genotypes (i.e. nested populations) are likely to represent demographic ‘sinks’ (Fig. 1b). Thus, our understanding of the demographic conditions underlying the distribution of genetic diversity across species ranges could be enhanced by using these methods adopted from community ecology.

There have been comparatively few range-wide investigations of population genetic patterns and thus few tests of the CPH in either tropical or marine systems (reviewed by Eckert et al. 2008, but see Palma-Silva et al. 2009, Miller et al. 2010, Liggins et al. 2014). Tropical systems may be especially useful for testing the CPH as there is the opportunity to address patterns in two latitudinally peripheral regions, presumably limited by similar environmental conditions. Marine systems, however, introduce several challenges for the limited parameters of the CPH. Many marine species have high levels of dispersal (Hellberg 2009) and large population sizes throughout their ranges (Sagarin and Gaines 2002) potentially compromising the expectations of the CPH for reduced migration and smaller population sizes toward the range periphery (discussed in Liggins et al. 2014). Furthermore, the dispersal of many taxa (particularly those with pelagic larvae) is highly subject to ocean currents that can disconnect proximal populations and connect geographically distant populations (Riginos and Liggins 2013). Thus, range position may be less relevant in determining range-wide genetic patterns of some marine species as compared to more sedentary terrestrial taxa (Fig. 1a III).

Nonetheless, the population histories of tropical marine organisms might lead to genetic patterns that inadvertently support the expectations of the CPH. The range

core of many tropical marine species sits around the Indo-Australian-Archipelago where the Indian and Pacific Oceans meet (IAA, Briggs and Bowen 2012). This region, and specifically the Coral Triangle, is posited to be the ‘Centre of Origin’ for several tropical marine species, and the ‘Centre of Overlap’ and ‘Centre of Accumulation’ for others (Bowen et al. 2013). Cryptic lineages are common in marine populations (Knowlton 1993, Rocha and Bowen 2008) and cases of lineage sympatry have been described within the IAA (reviewed by Carpenter et al. 2010). Furthermore, many reef-associated fishes that originated in the Coral Triangle, have since colonized and expanded their ranges into the Pacific and Indian Oceans (Cowman and Bellwood 2012). Thus, there is cause to expect that genetic diversity will be elevated in the range core of many tropical reef-fish species due to the sympatry of divergent lineages, and that genetic diversity will decline toward their range peripheries according to colonization timing. Such a pattern in genetic diversity would support the expectations of the CPH, but would result from the history of the populations, rather than contemporary range position (Fig. 1a II).

In this study we investigate the latitude-wide genetic patterns of a common coral reef fish, the neon damselfish species complex (*Pomacentrus coelestis*). A previous study of the neon damselfish reported the existence of two genetically divergent, yet morphologically cryptic lineages (Liu et al. 2012). The authors describe a Micronesian clade (re-described as *Pomacentrus micronesicus* in Liu et al. 2013) that is found at low latitudes, but co-occurs in parts of the Coral Triangle with another widespread clade (*P. coelestis*; herein referred to as the Pacific clade sensu Liu et al. 2012; Fig. 2). In this study we first address the impacts of population history on a test of the CPH, using the extreme case whereby two divergent lineages co-occur in the core of a species range, but not the periphery. We expect that genetic diversity will be elevated in the core of the species range due to the sympatry of divergent lineages, providing a pattern in support of the CPH, but resulting from an entirely different process (e.g. lineage sympatry, not core abundance and high connectivity, Fig. 1a II). We then focus on the widespread Pacific clade of the neon damselfish. Across the latitudinal range of the Pacific clade, we expect either: genetic patterns that support the CPH (Fig. 1a I); or no change in genetic diversity or differentiation as might be expected for a relatively high dispersal marine species (pelagic larval duration ~19.5 d, Thresher et al. 1989; Fig. 1a III).

Finding patterns of genetic diversity and genetic differentiation in support of, or contrary to, the CPH alone may be inadequate to understand the historical and contemporary processes acting toward the range periphery of the Pacific clade. There are two demographic scenarios likely, either: historical colonization toward the range periphery; or contemporary migration into the range periphery driven by western boundary currents (the Kuroshio current in the north and the East Australian Current in the south; Fig. 1b). In both of these scenarios we might anticipate patterns that concur with those described by the CPH – a decrease in genetic diversity toward the range periphery, and an increase in genetic differentiation (although patterns could deviate unpredictably in the case of non-equilibrium populations). To distinguish between our alternate hypotheses we extend

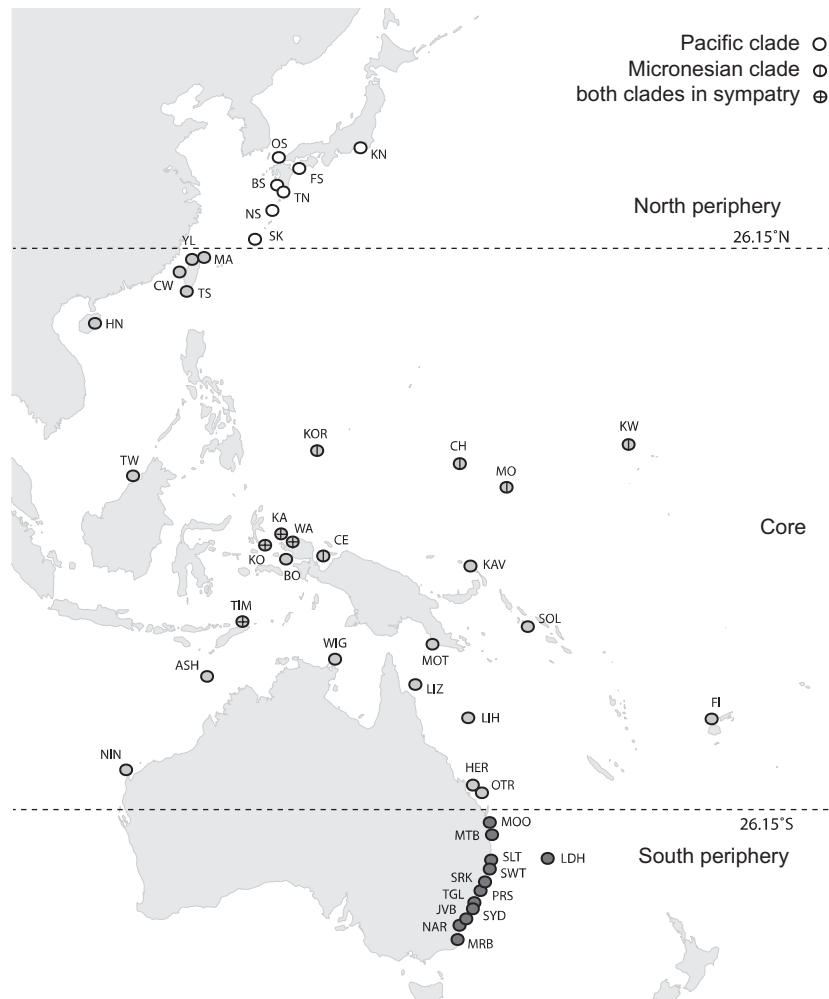


Figure 2. Map of the study area including the latitudinal core-periphery range limits of the neon damselfish. Populations from which genetic diversity measures ( $\pi$ ,  $Hd$ ) were attained are shown by the points. Attributes of populations are designated as follows: range position – white = north periphery, light gray = core, dark gray = south periphery; clade affinities – no bars = Pacific clade, vertical bar = Micronesian clade, crossed = Micronesian and Pacific clades in sympatry.

the usual characterizations of genetic patterns across a species range to include partitioned  $\beta$ -diversity and nestedness analyses. Under an historical colonization scenario, we expect that turnover ( $\beta_{SIM}$ ) will be high in the peripheral regions indicating contemporary demographic isolation. Conversely, under a scenario of contemporary migration into the range periphery, we expect that the nestedness-resultant component ( $\beta_{SNE}$ ) will be high (indicating shared genotypes) and the genotypic composition of populations will be nested toward the range periphery (Fig. 1b).

## Methods

### Data acquisition

Neon damselfish were collected from 21 locations throughout the species range using pole spears and hand nets while on SCUBA or snorkel. The mitochondrial control region (CR) and cytochrome *c* oxidase subunit I (COI) were sequenced for these fish and were combined with sequences available on GenBank from previous studies (Liu et al. 2008,

2010, 2012, Mirams et al. 2011; laboratory methods and data integration is detailed in the Supplementary material Appendix 1). Where the sequence data from GenBank had insufficient relating information, data were excluded from some analyses and in some cases statistics were taken from the original publication (detailed in Supplementary material Appendix 1 and 2).

### Identification of clades and core-periphery range limits

We categorically designated populations as being in the ‘core’ or ‘periphery’ of the species range according to the method of Channell and Lomolino (2000) where the core and periphery are of equal area and the area defining the core-boundary sits equidistant from the species range edge in each dimension. We used a rectangle to represent the range extremes rather than any other polygon, as our focus is on the latitudinal extremes (range limits were taken from Allen 1991, and extended to include populations sampled herein). The latitudinal core-boundaries



designated three regions: north periphery (NP), core, and south periphery (SP).

Maximum likelihood gene trees were constructed in MEGA ver. 6 (Tamura et al. 2013; bootstrap values estimated by 1000 replicates) using both the CR and COI datasets (described in Supplementary material Appendix 1) to identify whether individuals belonged to the Micronesian or Pacific clades and any locations of clade sympatry (hereafter also treated as, and referred to as ‘populations’). To view the genetic structure and genealogical relationships among regions (NP, core, SP) a median-joining network was constructed based on the CR sequences for individuals of the Pacific clade using Network 4.6.1.0 and Network Publisher 2.0 (fluxus-engineering.com, Bandelt et al. 1999).

### Core–periphery patterns of genetic diversity

According to the CPH, genetic diversity should be higher in the core of the neon damselfish’s range, and we expected this trend to be exacerbated by the sympatry of divergent lineages. To test these hypotheses we calculated measures of nucleotide diversity  $\pi$  (Nei 1978) and haplotype diversity  $Hd$ , for our sampled populations and populations studied in Mirams et al. (2011) in the same manner as Liu et al. (2008, 2010, 2012; using Arlequin 3.5, Excoffier and Lischer 2010), so that values could be compared (for CR sequences only). In total, our dataset for the genetic diversity analyses included 46 populations from across the range of the neon damselfish.

We used two methods to investigate the influence of range position on genetic diversity. First, to examine patterns in genetic diversity across the latitudinal range we used second-order polynomial regression. Hence, a significant, negative curvature coefficient would indicate a peak in genetic diversity in the core of the species range, and a significant linear coefficient would reveal any north–south trends in genetic diversity (base package, R ver. 2.15.3, R Core Team). Second, we used ANCOVA to examine whether the relationship between distance from the core and genetic diversity for the northern and southern hemispheres differed. In ANCOVA analyses, genetic diversity was the response, hemisphere was a fixed factor with two levels (north and south), and absolute latitude was the covariate. All analyses were conducted using both the combined Micronesian and Pacific clade dataset and the Pacific clade only dataset to understand the influence of divergent lineages and sympatry. Model residuals were visualized in all cases to check that the data met the assumptions of normality and homogeneity of variance.

To further understand any influence of clade affinity and clade sympatry on levels of genetic diversity we used ANOVA. Clade was included in the model as a fixed factor with three levels: Micronesian (Mic), Pacific (Pac), and sympatric (Sym). Tukey’s test for honestly significant differences (HSD) was used to uncover pairwise differences where we found a significant main effect of clade.

### Core–periphery patterns of genetic differentiation

Isolation by distance (IBD) analyses were used to understand how patterns of genetic differentiation changed across the

range of the Pacific clade. An IBD pattern (Wright 1943) can indicate the geographic scale of approximate migration–drift equilibrium (Hutchison and Templeton 1999). According to the CPH expectations, we expected to find a greater intercept value and a greater positive relationship between genetic differentiation and geographic distance in the range periphery (Fig. 1a I). We generated pairwise  $\Phi_{ST}$  values for our populations and those studied in Mirams et al. (2011) in the same manner as Liu et al. (2008, 2010; in Arlequin using uncorrected pairwise differences, 1000 permutations, for CR only). We compared the IBD relationship between  $\Phi_{ST}$  and Euclidean geographic distances for the NP, SP and two sub-regions of the core (i.e. populations within 1580 km of each other, comparable to the smaller pairwise geographic distances of the peripheral regions, Supplementary material Appendix 2). Reduced major axis regression was used to derive intercepts and measures of fit ( $R^2$ ); Mantel tests were used to assess correlation ( $r_M$ ) and significance (p-values based on 10 000 permutations).

The differentiation within and among populations for each region was inferred using analyses of molecular variance (AMOVA; using Arlequin 3.5). This method of variance partitioning can include information regarding either the frequency of haplotypes within populations (global  $F_{ST}$ ), or the frequency of haplotypes and the pairwise distance among haplotypes (global  $\Phi_{ST}$ ). AMOVAs were conducted separately for each region using all Pacific clade populations possible (NP = 7 populations, core = 12 populations, and SP = 12 populations; detailed in Supplementary material Appendix 2). Based on the CPH, we expected that the variation among populations would be greatest in the peripheral regions.

Measures of multisite  $\beta$ -diversity (Sørensen’s  $\beta_{SOR}$ ) and its turnover ( $\beta_{SIM}$ ) and nestedness-resultant ( $\beta_{SNE}$ ) components were also calculated for these regions. In contrast to AMOVA, the multisite  $\beta$ -diversity measures are based on haplotype presence and absence only. A matrix of haplotype presence–absence for each region was constructed and multisite  $\beta_{SOR}$  and its  $\beta_{SIM}$  and  $\beta_{SNE}$  components were calculated for the NP, core, and SP using the beta.multi function of the package ‘betapart ver. 1.2’ in R (Baselga and Orme 2012; see Supplementary material Appendix 3 for the haplotype matrix). Regions were resampled for the minimum number of populations within a region using the beta.sample function so that the means and standard errors (SE) could be estimated and measures of multisite  $\beta$ -diversity could be compared among regions. According to the CPH, we expected to find higher values of  $\beta_{SOR}$  in the range periphery of the Pacific clade as compared to the core (corresponding to peripheral populations being more genetically differentiated). In the range periphery, we expected that higher values of the turnover component  $\beta_{SIM}$  would be indicative of historical colonization and contemporary isolation. Alternatively, finding larger values of  $\beta_{SNE}$  in the range periphery would indicate a greater influence of contemporary migration (Fig. 1b).

### Genetic patterns toward the northern and southern periphery

Pairwise  $\beta$ -diversity measures were calculated using the beta.pair function of the package ‘betapart’ in R. These distance

matrices (pairwise- $\beta_{\text{sor}}$ , pairwise- $\beta_{\text{sne}}$  and pairwise- $\beta_{\text{sim}}$ ) were each individually analysed in an IBD framework for the maximum transect possible in the north (2387 km) and a similar transect in the south (2269 km) surpassing the previous core–periphery distinctions (Supplementary material Appendix 2). For comparison, we also conducted IBD analyses over the same transects using standard genetic differentiation measures:  $F_{\text{ST}}$ ,  $\Phi_{\text{ST}}$  (in Arlequin), and  $D_{\text{est}}$  (Jost 2008, in Genodive ver. 2.0b23, Meirmans and van Tienderen 2004). Under a scenario of historical colonization toward the range periphery we expected to find: a positive relationship between pairwise- $\beta_{\text{sor}}$  (as well as  $F_{\text{ST}}$ ,  $\Phi_{\text{ST}}$  and  $D_{\text{est}}$ ) and geographic distance; the same or a stronger positive relationship between pairwise- $\beta_{\text{sim}}$  and geographic distance; and a nil or negative relationship between pairwise- $\beta_{\text{sne}}$  and geographic distance (i.e. richness differences caused by nestedness between populations decrease over distance or have no relationship to distance). In contrast, under a scenario of contemporary migration, we did not expect to find a relationship significantly different from 0 between geographic distance and any of the standard genetic differentiation measures (or  $F_{\text{ST}}$ ,  $\Phi_{\text{ST}}$  and  $D_{\text{est}}$ , Slatkin 1993) or pairwise- $\beta_{\text{sim}}$ . Moreover, where contemporary migration is asymmetric, we expected to find a positive relationship between pairwise- $\beta_{\text{sne}}$  and geographic distance (i.e. richness differences caused by nestedness between populations increase over distance) and therefore an overall positive relationship between pairwise- $\beta_{\text{sor}}$  and geographic distance (pairwise- $\beta_{\text{sor}}$  = pairwise- $\beta_{\text{sim}}$  + pairwise- $\beta_{\text{sne}}$ ).

A targeted analysis of nestedness (based on overlap and decreasing fill, Almeida-Neto et al. 2008) using the nestednif function of the package ‘vegan ver. 2.0-7’ (Oksanen et al. 2007) addressed the nested structure of population haplotype composition in relation to latitude for the north and the south of the Pacific clade’s range (transects as described above). The rows (representing populations) of the haplotype presence–absence matrices were organized according to latitude and columns (representing haplotype identity) were organized using a custom, standardized procedure to optimize their arrangement for ‘decreasing fill’. This enabled us to attain the maximum value for  $\text{NODF}_{\text{rows}}$  (nestedness according to latitude) in both the northern and southern transects, ranging from 0 (non-nested) to 100 (perfectly nested). The organization of rows was subsequently randomized 1000 times, each time followed by an optimization of their arrangement for ‘decreasing fill’, to test the significance of the nested relationship of haplotype composition with latitude. Under a scenario of historical colonization and contemporary isolation we did not expect to find nestedness according to latitude, however for a scenario of asymmetric contemporary migration into the periphery we expected that haplotypes would be nested predictably according to latitude (Fig. 1b).

## Results

### Identification of clades and core–periphery range limits

The polygon representing the core of the species range was bounded by 100.5°W, 160.5°E, 26.15°N, 26.15°S

designating 27 populations into the core, 7 populations into the NP, and 12 populations into the SP (Fig. 2; further population information is provided in Supplementary material Appendix 2). For both the COI and CR dataset, our maximum likelihood gene trees portrayed two distinct and divergent lineages across the sampled range of the neon damselfish species complex, with high bootstrap support (Fig. 3a and b, respectively). Our de novo dataset contained individuals from both the Micronesian clade and the Pacific clade identified in Liu et al. (2012). The complete dataset included 5 populations consisting of the Micronesian clade only, 4 locations comprising both clades, and 37 populations including the Pacific clade only (Fig. 2). The median-joining haplotype network recovered 240 unique haplotypes within the Pacific clade. There were several shared haplotypes among the core, NP and SP of the species range. Although there were many equally parsimonious network configurations (Polzin and Daneshmand 2003), most network differences related to the position of single haplotypes separated by only 1–2 bp (one random network shown in Fig. 3c).

### Core–periphery patterns of genetic diversity

ANOVA revealed that populations comprising only one clade had significantly lower  $\pi$  than the locations with sympatric clades (Table 1; HSD: Mic–Sym, diff = –0.029,  $p < 0.001$ ; Pac–Sym = –0.021,  $p < 0.001$ ). The Micronesian clade also had significantly lower  $\pi$  than the Pacific clade (diff = –0.008,  $p = 0.008$ ). Populations of the Micronesian clade had significantly lower  $Hd$  than locations of sympatry (diff = –0.222,  $p = 0.029$ ) and Pacific clade populations (diff = –0.341,  $p < 0.001$ ), however populations of the Pacific clade did not have significantly different levels of  $Hd$  than locations with sympatric clades (diff = 0.119,  $p = 0.178$ ).

As expected under the CPH expectations, the second-order polynomial regression revealed a peak in  $\pi$  at low latitudes (Fig. 4). The peak was highest in the overall dataset where locations of sympatry generally had high values (except WA, Fig. 4), however this was not significant, probably owing to very low  $\pi$  of Micronesian clade populations also located at low latitudes (Table 1; note that all  $t$ -values and the significance of relationships were evaluated using orthogonal second-order polynomial regression analyses, the presented coefficients are on the raw data scale). The ANCOVA also indicated that in the dataset comprising both clades  $\pi$  was higher at low latitudes ( $F_{1,43} = 3.229$ ,  $p = 0.079$ ). For the Pacific clade, the polynomial regression term for curvature was significant ( $t_{2,35} = -2.561$ ,  $p = 0.014$ , Table 1) and the ANCOVA analyses also indicated  $\pi$  had a negative relationship with absolute latitude as expected under the CPH ( $F_{1,35} = 4.806$ ,  $p = 0.035$ ).

In contrast to the CPH expectations for patterns in genetic diversity, the curve derived from the polynomial regression of  $Hd$  reached its lowest point at low latitudes for the overall dataset ( $t_{2,43} = 1.868$ ,  $p = 0.069$ , Table 1). The dip of the curve for the overall dataset appeared to be due to low  $Hd$  for populations consisting of the Micronesian clade only, and low  $Hd$  for a few locations of sympatry (KO and WA, Fig. 4). The ANCOVA also revealed that  $Hd$  increased with latitude ( $F_{1,43} = 5.345$ ,  $p = 0.026$ , Table 1). When the dataset was

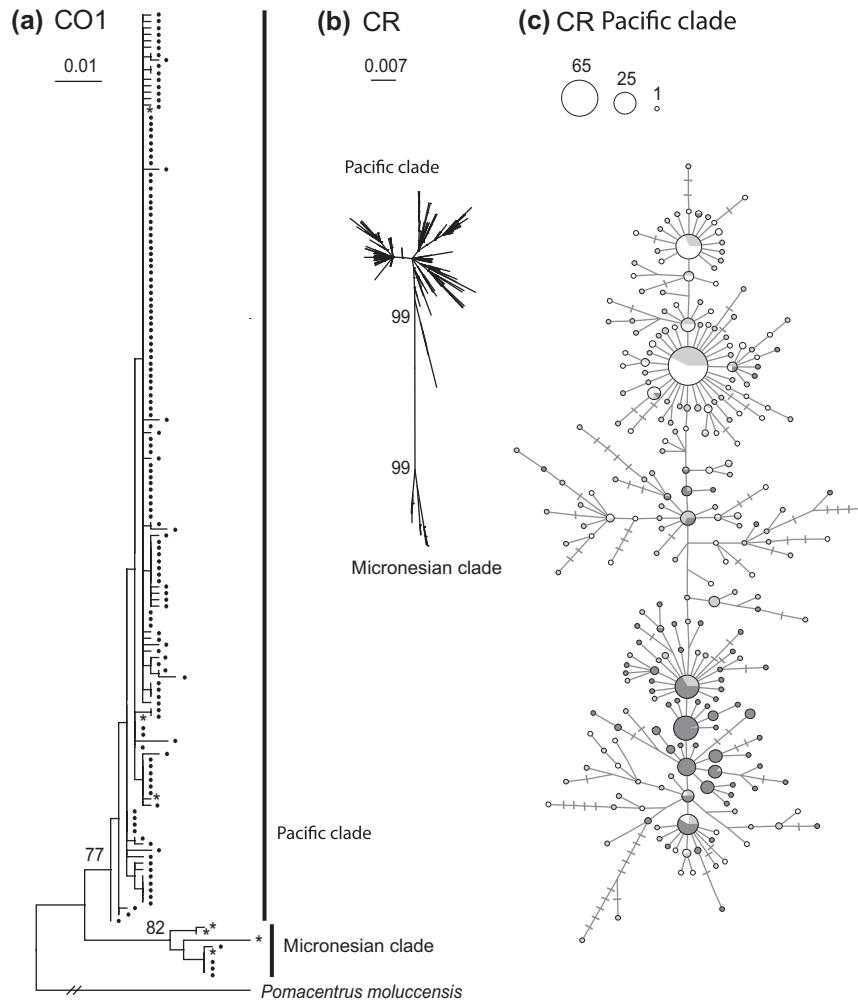


Figure 3. (a) Maximum likelihood gene tree based on cytochrome *c* oxidase subunit I (COI) sequences showing the relationship between the Micronesian and Pacific clades of the neon damselfish. Black points denote our de novo sequences; asterisks denote reference sequences from Liu et al.'s (2012) original description of the clades. (b) Unrooted maximum likelihood gene tree based on all control region (CR) sequences used to designate studied individuals into clades. Bootstrap support (%) based on 1000 replicates is indicated. (c) Maximum parsimony median-joining network showing unique CR haplotypes and their frequencies (see size key, top) across the range of the Pacific clade. Haplotypes are shaded according to range position: white = north periphery, light gray = core, dark gray = south periphery. Edges between haplotypes represent one mutational step, with additional steps indicated by cross bars.

reduced to include individuals from the Pacific clade only, a slight peak in *Hd* at low latitudes could be observed but this was not significant in the polynomial regression analysis or the ANCOVA (Fig. 4, Table 1). The ANCOVAs and polynomial regressions detected no significant differences in the genetic diversity trends in the northern hemisphere versus the southern hemisphere, and no overall trends from north–south in genetic diversity values, for either dataset (Table 1).

### Core–periphery patterns of genetic differentiation

According to the expectation of the CPH, we expected to find a stronger IBD pattern and a higher intercept in the peripheral regions of the Pacific clade's range than in the core (Fig. 1a I). Although the pattern of  $\Phi_{ST}$  differentiation in the NP had a positive relationship to distance that was stronger than the core regions, this was not significant

( $r_M = 0.356$ ,  $R^2 = 0.127$ ,  $p = 0.182$ ). There was no detectable IBD relationship in either of the core sub-regions (near the NP:  $r_M = -0.119$ ,  $R^2 = 0.012$ ,  $p = 0.509$ ; near the SP:  $r_M = -0.075$ ,  $R^2 = 0.006$ ,  $p = 0.665$ ) or in the SP ( $r_M = -0.050$ ,  $R^2 = 0.002$ ,  $p = 0.520$ ). The value of the intercept was higher in the SP than the neighboring core sub-region (0.153 and 0.111, respectively), but this was not mirrored in the NP (−0.068 and 0.381, respectively).

AMOVAs based on both frequency of haplotypes and the pairwise distance among haplotypes revealed that the greatest population differentiation was found in the core of the Pacific clade's range (global  $\Phi_{ST} = 0.226$ ,  $p < 0.001$ ), contrary to what was expected according to the CPH. The NP also had significant population differentiation (global  $\Phi_{ST} = 0.044$ ,  $p < 0.001$ ), whereas the genetic variation within the SP could not be attributed to among population differences (global  $\Phi_{ST} = -0.002$ ,  $p = 0.510$ ). In contrast, based on haplotype frequencies only, the SP had the greatest level of population differentiation (global  $F_{ST} = 0.072$ ,

Table 1. Table of coefficients for ANOVA, ANCOVA, and second-order polynomial regression (Regression). Left: relationships between the clade affinity of populations (Pacific = Pac; Micronesian = Mic; Pacific and Micronesian = Sym) and population-level genetic diversity ( $\pi$ ,  $Hd$ ), and range position (latitude, hemisphere) and population-level genetic diversity, for the pooled Micronesian and Pacific clades. Right: relationships between range position and population-level genetic diversity for the Pacific clade (Pac) only. Degrees of freedom for  $F$ - and  $t$ -ratios are indicated by subscripts. Significance of results, and significance following the exclusion of populations with small sample sizes ( $n < 10$ ), are indicated as demonstrated by inset key (bottom left). All  $t$ -values and the significance of relationships were evaluated using orthogonal second-order polynomial regression analyses; however, coefficients are presented on the data scale for interpretation.

Analysis	Factors	Micronesian + Pacific clades				Pacific clade			
		$\pi$ $F$ or $t$	$p$	$Hd$ $F$ or $t$	$p$	$\pi$ $F$ or $t$	$p$	$Hd$ $F$ or $t$	$p$
ANOVA	clade	34.130 <sub>2,43</sub>	*** <sup>10</sup>	17.160 <sub>2,43</sub>	*** <sup>10</sup>	—		—	
ANCOVA	latitude <sub>0–37°</sub>	3.229 <sub>1,43</sub>	.	5.345 <sub>1,43</sub>	* <sup>10</sup>	4.806 <sub>1,35</sub>	*	0.811 <sub>1,35</sub>	
	hemisphere × latitude <sub>0–37°</sub>	0.089 <sub>1,42</sub>		0.015 <sub>1,42</sub>		0.155 <sub>1,34</sub>		0.010 <sub>1,34</sub>	
	hemisphere	0.165 <sub>1,43</sub>		0.015 <sub>1,43</sub>		0.235 <sub>1,35</sub>		0.037 <sub>1,35</sub>	
Regression	latitude <sub>37–0–37°</sub> curvature	–1.661 <sub>2,43</sub>		1.868 <sub>2,43</sub>	. <sup>10</sup>	–2.561 <sub>2,35</sub>	* <sup>10</sup>	–0.811 <sub>2,35</sub>	
	latitude <sub>37–0–37°</sub> linear	–0.218 <sub>2,43</sub>		–0.307 <sub>2,43</sub>		–0.055 <sub>2,35</sub>		0.357 <sub>2,35</sub>	

<sup>10</sup> significant excluding populations with  $n < 10$

\*\*\* $< 0.0001$

\*\* $< 0.001$

\* $< 0.05$

. $< 0.1$

$p < 0.001$ ) and the core (global  $F_{ST} = 0.067$ ,  $p < 0.001$ ) and NP (global  $F_{ST} = 0.063$ ,  $p < 0.001$ ) had very similar levels of overall population differentiation.

Differences in overall haplotype composition among populations ( $\beta_{SOR}$ ) were highest in the core of the species range (mean  $\beta_{SOR} = 0.953$ ,  $SE = 0.003$ ), followed by the SP

(mean  $\beta_{SOR} = 0.897$ ,  $SE = 0.003$ ) and NP ( $\beta_{SOR} = 0.860$ ; Fig. 5). Partitioning of  $\beta_{SOR}$  revealed that the composition differences among populations in the core region of the species range were mostly due to turnover (mean  $\beta_{SIM} = 0.939$ ,  $SE = 0.004$ ), as were those for the SP (mean  $\beta_{SIM} = 0.884$ ,  $SE = 0.011$ ) and NP ( $\beta_{SIM} = 0.841$ ; Fig. 5). The greatest

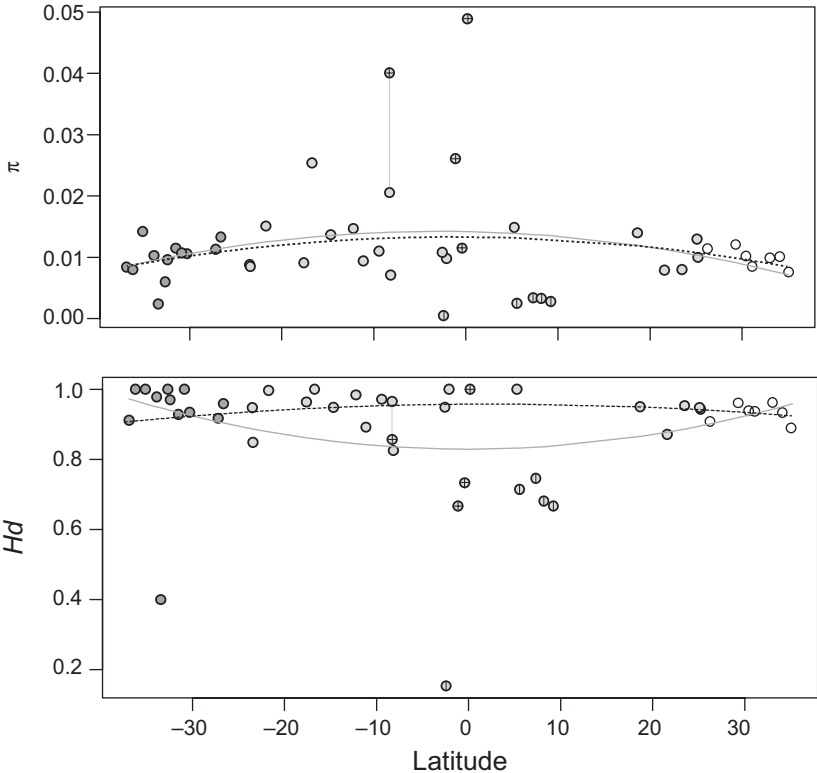


Figure 4. Relationship between latitude and genetic diversity measures ( $\pi$ ,  $Hd$ ) for populations of both the Micronesian and Pacific clades (gray line) and the Pacific clade only (dashed line). Lines indicate predicted values from the second order polynomial regression. Clade affinities and range position of populations are designated as in Fig. 2. Thin vertical gray line connects two representations of Timor-Leste, one comprising the Pacific clade only, the other including the Pacific clade and Micronesian clade.



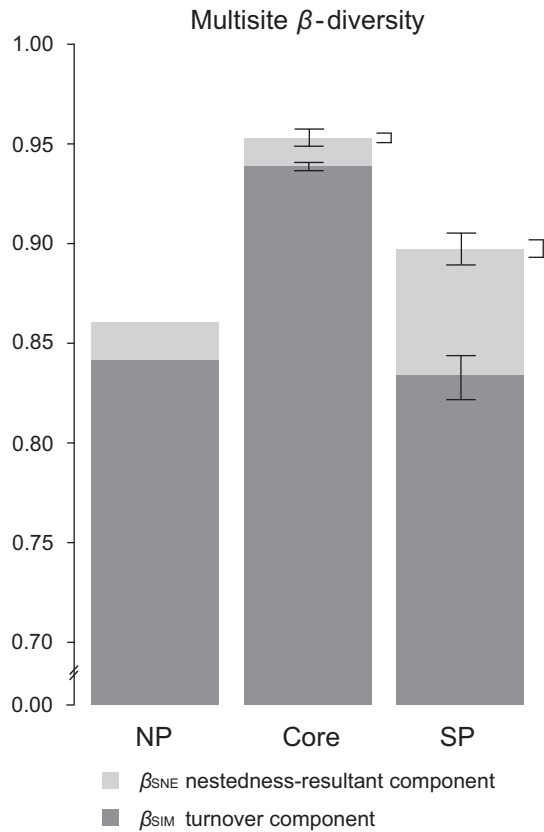


Figure 5. The turnover ( $\beta_{SIM}$ , dark gray) and nestedness-resultant ( $\beta_{SNE}$ , light gray) components of total multisite  $\beta_{SOR}$ -diversity (total bar height) for the north periphery (NP), core, and south periphery (SP) of the Pacific clade. Mean values and standard error bars for the core and SP were estimated via resampling of populations within each region so that values may be compared with the smaller number of populations sampled in the NP ( $\beta_{SOR}$  standard error bars are offset right).

contribution via the nestedness-resultant component ( $\beta_{SNE}$ ) was in the SP (mean  $\beta_{SNE} = 0.064$ ,  $SE = 0.008$ ) followed by the NP ( $\beta_{SNE} = 0.019$ ) and core (mean  $\beta_{SNE} = 0.014$ ,  $SE = 0.002$ ; Fig. 5).

### Genetic patterns toward the northern and southern periphery

Along a transect in the northern part of the range pairwise- $\beta_{sor}$  had a positive and significant relationship with geographic distance ( $R^2 = 0.376$ ,  $r_M = 0.613$ ,  $p_{perm} = 0.011$ ) as did pairwise- $\beta_{sim}$  ( $R^2 = 0.375$ ,  $r_M = 0.612$ ,  $p_{perm} = 0.013$ ) and all of the genetic differentiation measures ( $\Phi_{ST}$   $R^2 = 0.582$ ,  $r_M = 0.763$ ,  $p = 0.001$ ;  $F_{ST}$   $R^2 = 0.698$ ,  $r_M = 0.835$ ,  $p_{perm} = 0.001$ ;  $D_{est}$   $R^2 = 0.272$ ,  $r_M = 0.521$ ,  $p_{perm} < 0.001$ ), whereas the richness component decreased with distance (pairwise- $\beta_{sne}$   $R^2 = 0.160$ ,  $r_M = -0.400$ ,  $p_{perm} = 0.946$ , Fig. 6). Overall, this suggests that the genetic patterns in the northern part of the Pacific clade's range support a scenario of historical colonization. In the southern part of the clade's range none of the genetic differentiation measures ( $\Phi_{ST}$   $R^2 = 0.002$ ,  $r_M = 0.045$ ,  $p_{perm} = 0.334$ ;  $F_{ST}$   $R^2 = 0.009$ ,  $r_M = -0.093$ ,  $p_{perm} = 0.624$ ;  $D_{est}$   $R^2 = 0.025$ ,  $r_M = -0.157$ ,

$p_{perm} = 0.907$ ) or pairwise- $\beta_{sim}$  ( $R^2 < 0.001$ ,  $r_M = 0.013$ ,  $p_{perm} = 0.541$ , Fig. 6) had a significant relationship with geographic distance, supporting a scenario of contemporary migration. However, pairwise- $\beta_{sne}$  had only a slightly positive trend over geographic distance ( $R^2 < 0.001$ ,  $r_M = -0.007$ ,  $p_{perm} = 0.448$ ) and pairwise- $\beta_{sor}$  had a slightly negative trend ( $R^2 < 0.001$ ,  $r_M = 0.018$ ,  $p_{perm} = 0.494$ ) providing only tentative support for asymmetric contemporary migration.

The nestedness of the haplotype compositions of populations organized according to latitude was higher along the southern transect of the clade's range than the northern transect ( $NODF_{rows} = 28.687$  and  $10.556$ , respectively). Our randomization test confirmed that the nestedness statistic of the southern transect was significantly higher than would be expected from the chance arrangement of our study populations ( $p_{perm} = 0.020$ ), whereas the statistic for the northern transect was no different to what may be expected by chance ( $p_{perm} = 0.336$ ). These results from the southern transect (and those above for the SP) fitted with our suggested scenario of asymmetric contemporary migration into the periphery (Fig. 1b).

### Discussion

Our novel approach to characterizing range-wide genetic patterns in a tropical marine species offers a more nuanced understanding of the demographic context and conservation value of peripheral populations than conventional analyses used in spatial population genetics. For the neon damselfish (*Pomacentrus coelestis* species complex), we found that population history, in terms of divergent lineages that were in sympatry, confounded our test of the CPH. Although locations with both clades had higher levels of genetic diversity, lower values for  $\pi$  and  $Hd$  in the Micronesian clade, and the presence of this clade only in the core of the species range, detracted from there being significantly higher levels of genetic diversity in the core.

Across the range of a single clade (Pacific clade) we found mixed support for the CPH using conventional genetic measures. There was an increase of  $\pi$  in the core compared with the periphery, but no pattern in  $Hd$  (Fig. 4) or evidence of greater genetic differentiation over distance in the peripheral regions ( $\Phi_{ST}$ -based IBD). Overall population differentiation based on haplotype frequencies indicated that populations within the peripheral regions of the Pacific clade were more differentiated than those of the core (global  $F_{ST}$ ) in accordance with the CPH. However, population differentiation based on haplotype frequencies and pair-wise distances among haplotypes indicated population differentiation was greatest in the core of the Pacific clade's range (global  $\Phi_{ST}$ ).

The use of partitioned  $\beta$ -diversity and nestedness measures enabled us to better characterize the spatial arrangement of genetic diversity across the Pacific clade's latitudinal range. We found a strong relationship between geographic distance and pairwise- $\beta_{sor}$ , its turnover component (pairwise- $\beta_{sim}$ ), and several genetic differentiation measures (Fig. 6) toward the northern periphery, consistent with a historical colonization scenario. In contrast, we found no significant relationships between the pairwise differentiation measures and geographic distance toward the southern

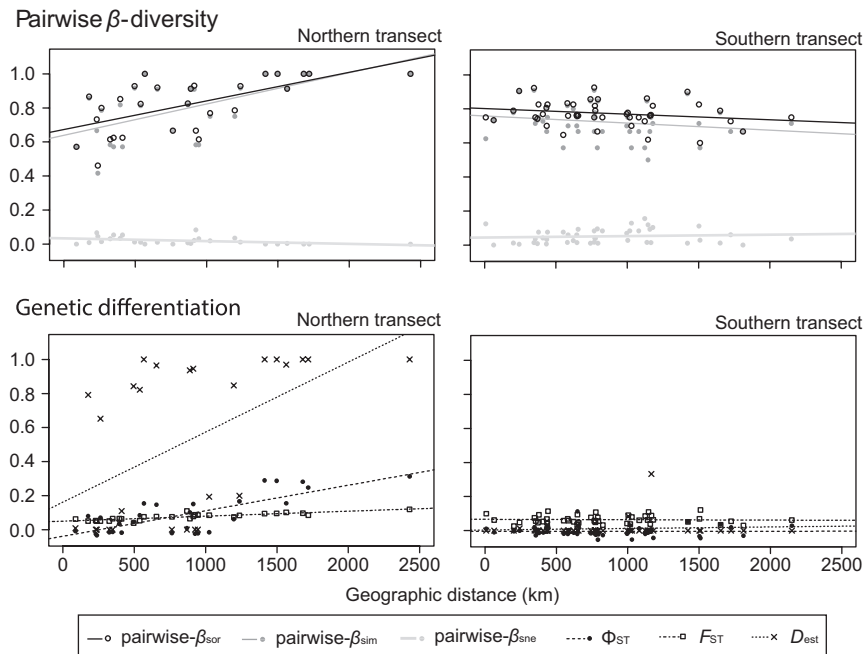


Figure 6. Isolation-by-distance relationships across the northern and southern parts of the Pacific clade's range. Top panel: black circles and black line represent pairwise- $\beta_{\text{SOR}}$ , dark gray filled circles and dark gray line represent pairwise- $\beta_{\text{SIM}}$ , and light gray filled circles and light gray line represent pairwise- $\beta_{\text{SNE}}$ ; bottom panel: black filled circles and dashed line represent  $\Phi_{\text{ST}}$ ; black squares and dot-dash line represent  $F_{\text{ST}}$ ; and crosses and dotted line represent  $D_{\text{EST}}$ .

periphery, suggesting an influence of contemporary migration. Although turnover ( $\beta_{\text{SIM}}$ ) was still the greatest contributor to the overall haplotypic compositional difference among populations ( $\beta_{\text{SOR}}$ ), the nestedness-resultant component ( $\beta_{\text{SNE}}$ , Fig. 5) was greater in the south than in the north. Furthermore, the haplotypic composition of populations was nested with latitude, consistent with asymmetric contemporary migration into the southern range periphery (Fig. 1b).

### Latitude-wide genetic patterns in the neon damselfish

Historical impacts and population history can strongly influence contemporary patterns of genetic diversity for any taxon. Yet, few studies have considered population history in their tests of the CPH (Eckert et al. 2008, but see Pfeifer et al. 2009, Pinheiro et al. 2011). In marine systems, sympatry of divergent lineages or clades has been found to increase genetic diversity in several North Atlantic species (Maggs et al. 2008) and Indo-Pacific reef fishes (Bay and Caley 2011, Gaither et al. 2011). In our study, the population history of the neon damselfish in the Coral Triangle led some 'populations' to be perceived as having significantly higher genetic diversity than others due to sympatric divergent clades. Surprisingly though, the inherently different levels of genetic diversity within clades had the greatest effect on range-wide genetic patterns. Genetic diversity ( $\pi$ ,  $Hd$ ) within the Micronesian clade was significantly lower than in the Pacific clade, obscuring the signature of higher  $\pi$  found in the core of the Pacific clade's range. Based on our results we caution against genetic diversity surveys and

testing range-wide hypotheses using datasets of unknown genealogical history.

Several factors other than those associated with range position have a role in shaping the genetic patterns in the core of the neon damselfish's range. Although the Micronesian clade occurs in the range core, its pure populations are peripheral to the Indo-Australasian Archipelago (IAA), and largely isolated to the Micronesian Islands. The low genetic diversity found in this clade is likely due to this geographic context. Liu et al. (2012) suggest that the Micronesian Islands were colonized in a stepping-stone manner from the Coral Triangle that would have entailed serial genetic bottlenecks. Moreover, small, isolated populations tend to have low genetic diversity (Allendorf and Luikart 2007); this is an assumption that underlies the predicted genetic patterns for the range periphery in the CPH, but is evidently not restricted to the range periphery for the neon damselfish species complex.

Although some of our findings for the Pacific clade were in accordance with the CPH expectations ( $\pi$  peaked in the core and population differentiation was slightly greater in the periphery according to global  $F_{\text{ST}}$ ), overall, we did not find compelling support for the hypothesis across the clade's range. We found no latitudinal pattern in  $Hd$  and populations in the core were more differentiated than predicted by the CPH, and for a high dispersal marine organism (based on global  $\Phi_{\text{ST}}$  and high values of  $\beta_{\text{SOR}}$  and particularly the turnover component,  $\beta_{\text{SIM}}$ ). Such high genetic differentiation is not unusual among populations in the IAA that may have been separated during periods of the Pleistocene when sea levels were lowered (Voris 2000). Thus, there are several geographic factors that affect genetic patterns across a seascape other than range position.

## Contrasting patterns in the northern and southern periphery

Our findings describe contrasting genetic patterns toward the latitudinal extremes of the neon damselfish. Contrasting patterns at the latitudinal range peripheries for temperate species may not be surprising given the vastly different environmental conditions organisms are likely to experience (Hoban et al. 2010, Assis et al. 2013, Hasselman et al. 2013). However for a tropical species such as the neon damselfish we expected similar genetic patterns as, presumably, individuals experience similar environmental conditions at both latitudinal range peripheries. Toward the northern periphery we found support for a scenario of historical colonization followed by relative isolation and time for populations to equilibrate and private haplotypes to arise (low  $\pi$ , Table 1, Fig. 4; higher differentiation, i.e.  $\Phi_{ST}$ -based IBD; significant global  $\Phi_{ST}$  and  $F_{ST}$ ). Accordingly, a recent review of marine phylogeographic studies in this northwestern Pacific region suggests that most species have significant population structure and show evidence of a past range expansion (Ni et al. 2014).

The southern periphery was likely colonized on a similar time scale as the north, but migration may be too high for populations to have reached migration–drift equilibrium (as indicated by a lack of IBD, Hutchison and Templeton 1999, Fig. 6). Nonetheless, our results were not completely aligned with our hypotheses pertaining to an asymmetric contemporary migration scenario (higher proportional contribution of  $\beta_{SNE}$  to total  $\beta_{SOR}$ , Fig. 5; positive relationship between pairwise- $\beta_{SNE}$  and geographic distance, Fig. 6; high nestedness according to latitude). In part, this may be due to our population sampling not being exhaustive enough to capture total genetic diversity, including shared genetic diversity with all the potential source populations for the range periphery. The East Australian Current that runs north–south along the coastline is known to drive the recruitment of many organisms including the neon damselfish (Booth et al. 2007, 2011, Figueira et al. 2009). This southeast coast of Australia is a hotspot for marine tropical vagrants and contemporary range shifts (Booth et al. 2011, Baird et al. 2012, Feary et al. 2013). High levels of genetic diversity, such as what we see in the southern periphery of neon damselfish's range, have also been recorded in other organisms that are undergoing range expansion in this region (*Centrostephanus rodgersii*, Banks et al. 2010) and it has been suggested that such admixture of intraspecific lineages at the leading edge of species ranges may be common (Rius and Darling 2014).

The most southerly observed breeding pair of *P. coelestis* is recorded at the Solitary Islands (SLT) beyond which occupancy can be ephemeral, as individuals succumb to harsh over-winter water temperatures in some years (Figueira and Booth 2010). The high levels of genetic diversity in spite of the temporal instability of populations, suggests that immigration into the southern periphery is likely from several source populations and is on-going (termed a 'black-hole sink' habitat, Gomulkiewicz et al. 1999). The genetic diversity of a population that is heavily dependent on immigration (a sink, Holt 1985) should, in theory, have few private genotypes or alleles (Slatkin 1985) and be a sub-sample of that found within the source population(s). Although we

found that the turnover of haplotypes among populations ( $\beta_{SIM}$ ) still contributed the most to the differentiation in this region, there was a larger contribution from shared haplotypes (the nestedness-resultant component,  $\beta_{SNE}$  Fig. 5) in the south than the north. Our analysis focused on the nestedness of the population haplotype compositions relative to latitude (predicting higher nestedness with higher latitude) also confirmed that the populations in the southern periphery were nested 'sub-sets' of those at lower latitudes. Our study, in concert with previous studies (Diniz-Filho et al. 2012, Habel et al. 2013), demonstrates the utility of these methods to understand the organization of genetic diversity along spatial gradients.

## Conservation implications

Genetic diversity is frequently quoted as being informative for developing biodiversity management plans and the conservation of species (Laikre et al. 2010).  $Hd$  and  $\pi$  are the most commonly reported measures of genetic diversity for DNA sequence data, and are thus the most accessible to decision-makers. However, our study indicates instances where using these measures of genetic value alone may be uninformative and misleading. The sympatry of divergent clades within the core of the species range enhanced the perceived levels of genetic diversity in some populations and hence their conservation value. Moreover, the low genetic diversity of the Micronesian clade ( $\pi$  and  $Hd$ ), relative to the widespread Pacific clade had a profound influence on the interpreted range-wide genetic patterns. These two clades have recently been described as separate species and are highly unlikely to interbreed (Liu et al. 2013). Therefore, conservation decisions based on these perceived levels of genetic diversity and range-wide patterns would be inappropriate.

There has been much debate about the conservation worth of peripheral populations (Lesica and Allendorf 1995). The contrasting genetic make-up of peripheral populations found in the north and the south of Pacific clade's range, indicates that this question needs to be addressed independently for each species (Guo 2012), and potentially for each peripheral region within a species' range. Although high levels of immigration increase the genetic variation on which selection can act (Rius and Darling 2014), such genetic swamping can preclude local adaptation (Kirkpatrick and Barton 1997, Kawecki and Holt 2002). In contrast self-recruitment (as might be the case in the northern periphery) can promote the efficacy of local selection, where genotypes suited to local conditions are specifically favored to survive and reproduce (Lenormand 2002). Thus, whereas both peripheral regions of the Pacific clade's range harbor equivalent levels of genetic diversity, the unique genetic diversity of the north periphery may be more valued, and is indicative of demographic conditions conducive to local adaptation. Conclusions such as this are only possible via analyses which move beyond a focus on genetic diversity per se, as illustrated in our study.

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Supplementary material (Appendix ECOG-01398 at <[www.ecography.org/appendix/ecog-01398](http://www.ecography.org/appendix/ecog-01398)>). Appendix 1–4.