APPLIED ISSUE

The world in a grain of sand: evolutionarily relevant, small-scale freshwater bioregions on subtropical dune islands

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SUMMARY

1. Conservation plans are required to safeguard freshwater biodiversity in the face of increasing threats. Traditionally plans have used surrogates for biodiversity that do not account for the evolutionary process, but genetic data in the form of comparative phylogeography can fulfil this role.
2. Comparative phylogeographic analyses of multiple freshwater fish and decapod crustacean species were carried out with specimens from two model systems, namely the sand dune islands of Fraser and North Stradbroke in eastern Australia.
3. Almost all of the species studied from both islands displayed an intraspecific evolutionary split between sides of the island (east/west on North Stradbroke Island, and north/south on Fraser Island), indicating that each side of each island hosts its own distinct community of populations of freshwater animals.
4. The probable process responsible for both of these divergent communities is different source populations for each side of each island.
5. This study shows that biodiversity will not always follow obvious geography and that significant diversity may exist at small scales within multiple species. These evolutionarily relevant units of biodiversity should be incorporated at the beginning of the conservation and resource management planning process.

Keywords: Australia, comparative phylogeography, conservation genetics, conservation planning, Fraser Island, Stradbroke Island

Introduction

Conservation planning is vital to ensure fully functioning freshwater ecosystems and the persistence of unique biota in the face of many pressures (Turak & Linke, 2011). Conservation plans come in many guises, from breeding programmes for an endangered species (Frankham, 2010) to deciding where to locate protected areas with limited funds (Linke, Turak & Nel, 2011). Systematic conservation planning uses clearly defined processes in which the precise assets to be conserved are identified and the effects of various conservation regimes quantified (see Linke et al., 2011).

The raw material for any conservation plan is a thorough knowledge of the geographic location and level of uniqueness of the relevant biota. Effective conservation actions rely on accurate biological information used in the planning process. As ‘complementary’ areas do not share much of the same biota, a higher percentage of the local biodiversity is protected if distinct, complementary sites are selected (Cook, Page & Hughes, 2008). However, this form of protection is only effective if community composition is known precisely, but unclear taxonomy and the development of molecular genetic techniques (Arbogast & Kenagy, 2001) have challenged many of these assumptions around complementarity. A recent study of complementarity of freshwater invertebrates (Cook et al., 2008) found that undescribed ‘cryptic’ species and deep within-species genetic differences mean that the definition of areas as complementary can change when evolutionary
data are integrated and that endemic freshwater biodiversity may exist at much smaller scales than expected.

An important element missing from complementarity and bioregions is an appreciation of the evolutionary processes that have generated patterns of biodiversity. Generally, ‘surrogates’ are used to represent an area’s genetic diversity, such as endemism or species distributions (Carvalho et al., 2011). For large-scale studies, the collection of molecular genetic data is probably not practical, but using a surrogate is not an alternative when molecular data are available (Carvalho et al., 2011). Indeed, the use of molecular data is more feasible at the smaller scales where conservation plans are often actually applied (e.g. national park, catchment or local area) (Pérez-Losada et al., 2009). Preserving evolutionary processes has emerged as an important theme in planning protected areas (Nel et al., 2009; Linke et al., 2011), as well as being an important element in breeding programmes (Frankham, 2010; Olden et al., 2011).

Phylogeography is the overlaying of genetic (generally within-species) and geographic patterns, much like biogeography but at smaller geographic and evolutionary scales (Arbogast & Kenagy, 2001). The two are closely linked because similar processes generate both population- and species-level diversity (Hickerson et al., 2010). Bioregions and complementary areas are generally based on species or family level distributions (Marshall, Steward & Harch, 2006c). This may be appropriate at large scales, but can be too coarse at the actual scale at which many plans are required. A strong phylogeographic pattern among populations of a single species gives a good idea of the evolutionary and biogeographic history of that species, and whether important conservation units of biodiversity may lurk beneath the species level (Frankham, 2010), which is of particular interest for breeding or reintroduction programmes (Olden et al., 2011).

However, the pattern from a single species may be of only limited wider conservation interest if its pattern is idiosyncratic, as it may not be an effective ‘umbrella’ species (Simberloff, 1998) or serve well as a planning surrogate for unsampled species (Growns, 2009). However, strongly congruent phylogeographic patterns across many co-distributed species (‘comparative phylogeography’) can not only help define truly complementary units (Bermingham & Moritz, 1998) but also get at the very root of the processes that generated, and continue to generate, these patterns of biodiversity (Carvalho et al., 2011).

How to integrate molecular data into systematic planning is challenging (Carvalho et al., 2011), but there are some obvious implications. Intraspecific genetic data can be used to map ‘biodiversity features’ (Nel et al., 2009) at the start of the planning process, and ‘biotic elements’ (groups of co-occurring species, Carvalho et al., 2011) can be more easily identified. The use of fine-scale population data in complementarity-based algorithms (called for in Cook et al., 2008; Turak et al., 2011) can give more precise results that reflect evolutionary processes. This is because a ‘best solution’ by a planning algorithm of a minimum set of selected sites would likely need to include areas that represent structured populations of species rather than just the species as a whole, with resultant shifting irreplacibility values for some planning units.

A ‘natural laboratory’ (Carvalho et al., 2011) to explore many of these issues exists off Australia’s subtropical Queensland coast on vegetated, sand dune islands. These are good model systems for other areas where conservation planning is being considered because these islands are relatively small, self-contained areas, with fairly simple freshwater food webs in which many species can be included in molecular analyses. Comparative phylogeography has rarely, if ever, been done on this scale with many species. The types of analyses presented here can be easily scaled up to larger areas in different ecosystems as the methods are transferable.

The combination of sandy soils and acidic waterbodies on these islands has lead to the development of a fragile, restricted coastal heathland known as wallum, which contains a specialised community of flora and fauna (Marshall et al., 2006a). The freshwater biota contains two IUCN Red Listed fish species, *Nannoperca oxleyana* Whitley, 1940 and *Pseudomugil mellis* Allen & Ivantsoff, 1982 (Pusey, Kennard & Arthington, 2004). This freshwater community relies on a complex mix of groundwater-fed lakes, swamps, creeks and unusual ‘perched’ lakes and wetlands (Horton, 1983), which sit isolated above the local watertable in their own mini-aquifers, and for which this area is the world centre (Hadwen, Arthington & Mosisch, 2003). This delicate environment is so special that Fraser Island (the world’s largest sand island at 120 km long) is mostly national park and has been declared a UNESCO natural World Heritage site (Hadwen et al., 2003). North Stradbroke Island (NSI) has been more impacted, with sand mining (Laycock, 1975) and water extraction, but will soon also be largely national park (Queensland Government, 2010).

The present study grew out of a multi-stranded risk analysis undertaken by the Queensland State government to document the ecological assets of NSI that depend on ground water (see Marshall, McGregor & Negus, 2006b). Existing data from two freshwater crustaceans on the island (Page & Hughes, 2007a; Bentley, Schmidt & Hughes, 2010) has suggested that there could be different communities on the eastern and western sides, meaning
they could be complements and thus with potentially important implications for risk assessments and conservation plans. Data from the same two species (and studies) also implied that Fraser Island may also be bisected by a currently unappreciated border between freshwater communities, but this one between north and south.

The inclusion of evolutionary processes in conservation planning has happened rarely (Carvalho et al., 2011), and so we aim to show that this information can provide appropriately scaled biodiversity information for future conservation planning. Specifically, the aims of this study are to (i) uncover the geography of genetic patterns in the freshwater fish and decapod crustacean species of North Stradbroke and Fraser Islands, so as to (ii) understand the processes responsible for this diversity.

Methods

Sampling strategy

A systematic survey of every major, and nearly every minor, freshwater body on NSI was carried out in numerous sampling trips between 2003 and 2010, with 14 sites providing specimens of freshwater fish and decapod crustaceans (Fig. 1, Table 1 for all sites on both islands). Fraser Island was sampled more opportunistically, but still amounted to 21 sites. These included perched lakes, watertable lakes, perched wetlands, watertable swamps and creeks.

Fish and decapod species (Table 2) were targeted because of their reliance on the aquatic environment and thus a small likelihood of terrestrial dispersal, while aquatic insects were avoided due to common flighted dispersal across barriers (Hughes, 2007). Specimens were caught with a seine, dip-net, baited trap or with an electrofisher, with fin clips or whole individuals preserved in liquid nitrogen or 95% ethanol.

Specimens of four of the six decapod and 10 of the 16 fish species reported from NSI freshwaters (Marshall et al., 2011) were included in this study. Those not included were either diadromous and so unlikely to provide small-scale genetic divergence across barriers (Hughes, 2007). Specimens were caught with a seine, dip-net, baited trap or with an electrofisher, with fin clips or whole individuals preserved in liquid nitrogen or 95% ethanol.

Each site on both islands was assigned to an island ‘side’ (‘East’ or ‘West’ on NSI, and ‘North’ or ‘South’ on Fraser Island) based on the similar geographic results from Caridina indistincta C (Page & Hughes, 2007a) and Cherax dispar (Bentley et al., 2010). On Fraser Island, all sites south of Bogimbah Creek (the southernmost ‘northern’ site from Bentley et al., 2010) were deemed to be ‘South’. On NSI, all sites to the west of the 18-mile swamp complex were deemed ‘West’ (see Fig. 1 for map and Table 1 for site information).

Molecular methods and data set construction

Genomic DNA was extracted, gene fragments amplified and sequences produced at the DNA Sequencing Facility at Griffith University as per Page & Hughes (2010). Where possible, published sequences were integrated into our data sets, and so mitochondrial genes and relevant PCR primers were chosen that would align with these data (see Table 2, and Table S1). The four genes used in this study are ATPase (ATP), cytochrome b (CytB), cytochrome oxidase subunit I (COI), and control region (CR). The first three of these genes diverge at broadly similar rates, while CR can be more variable (Page & Hughes, 2010).

Published sequence data from specimens of the relevant species that had also been sampled from NSI or Fraser Island were downloaded from GenBank and aligned with data generated by this study (Table 1). Separate data sets were created for each species from each island that had data from both sides of the respective island.

Phylogeographic analyses

The population structure of all species was assessed so as to discover the geographic distribution of genetic diversity across the islands. Haplotype networks were constructed for each data set using TCS version 1.21 (Clement, Posada & Crandall, 2000). For species with a high level of genetic divergence, for which TCS was unable to construct a network, a minimum evolution tree was instead created using PAUP* version 4.0 b10 (Swofford, 2002) with a P-distance model.

Pairwise \( \Phi_{ST} \) was calculated between the two sides of each island for each species using Arlequin version 3.5 (Schneider, Roessli & Excoffier, 2000), with 1000 permutations for significance testing, to assess the amount of inter-site variation explained by island side. Negative \( \Phi_{ST} \) values were set to zero. Arlequin was also used to calculate the corrected (to account for within-clade polymorphism) average pairwise sequence divergence.
between populations on each side of the island for each species (P-distance).

Multi-dimensional scaling (MDS) plots were created in Primer version 5.2.8 (Primer-E Ltd., Plymouth, UK) (1000 restarts, Euclidean distance) using P-distance matrices exported from PAUP for all NSI data sets. This allowed visualisation of genetic groupings within each species and a comparison with a more traditional multi-species MDS constructed from the comprehensive NSI species presence/absence data from Marshall et al. (2011) (1000 restarts, Bray–Curtis similarity).

To obtain some idea of the timescales involved and therefore infer the potential processes behind the generation of biodiversity, a number of molecular divergence rates (1, 2 and 3% per million years) were applied to the calculated sequence divergence (±SE). An additional extreme rate of 20% per million years was also calculated for control region because of possible very fast rates in this fragment (Page & Hughes, 2010). For closely related island ‘sides’ with sequence divergences of less than 1%, the coalescent method of Nielsen & Wakeley (2001) as implemented in MDIV (model = finite sites [HKY], cycles = 5 000 000, burn-in = 10%, Mmax & Tmax = various) was also used with all divergence rates. As molecular rates are difficult to estimate, these calculations were combined to give a wide range of possible dates, allowing each divergence to be assigned to a broad geological epoch, rather than a precise date.

Results

One hundred and seventy-one specimens from 12 fish and three decapod species were sequenced (GenBank accession numbers of haplotypes JF487940–JF487990). These
<table>
<thead>
<tr>
<th>Island/site</th>
<th>Site code</th>
<th>Site type</th>
<th>Lat./long.</th>
<th>Fish species</th>
<th>Crustacean species</th>
<th>Total specimens per species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraser Island</td>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>GA</td>
<td>HC</td>
</tr>
<tr>
<td>N. Bogimbah Ck.</td>
<td>BG</td>
<td>Ck</td>
<td>-25.303, 153.056</td>
<td>2</td>
<td>5</td>
<td>5</td>
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<tr>
<td>Boool Ck.</td>
<td>BK</td>
<td>Ck</td>
<td>-24.746, 153.176</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Bowarrady Ck.</td>
<td>BW</td>
<td>Ck</td>
<td>-25.133, 153.165</td>
<td>4</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Coongul Ck.</td>
<td>CG</td>
<td>Ck</td>
<td>-25.197, 153.111</td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>L. Allom</td>
<td>LA</td>
<td>PL</td>
<td>-25.198, 153.211</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L. Bowarrady</td>
<td>LB</td>
<td>PL</td>
<td>-25.146, 153.215</td>
<td>4</td>
<td>4</td>
<td>4, 6</td>
</tr>
<tr>
<td>Ocean L.</td>
<td>OL</td>
<td>WT</td>
<td>-24.926, 153.278</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Unnamed Ck.</td>
<td>UN</td>
<td>Ck</td>
<td>-24.744, 153.177</td>
<td>1</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>White L.</td>
<td>WL</td>
<td>PL</td>
<td>-25.118, 153.203</td>
<td>3</td>
<td>3</td>
<td>4, 6</td>
</tr>
<tr>
<td>Woralie Ck.</td>
<td>WO</td>
<td>Ck</td>
<td>-25.181, 153.148</td>
<td>4</td>
<td>4</td>
<td>4, 6</td>
</tr>
<tr>
<td>S. Alligator Ck.</td>
<td>AC</td>
<td>Ck</td>
<td>-25.490, 152.997</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Fig Tree Ck.</td>
<td>FT</td>
<td>Ck</td>
<td>-25.660, 152.985</td>
<td>9</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Gerowweea Ck.</td>
<td>GW</td>
<td>Ck</td>
<td>-25.599, 153.084</td>
<td>4</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Govi Ck.</td>
<td>GO</td>
<td>Ck</td>
<td>-25.599, 153.093</td>
<td>13</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>L. Birrabeen</td>
<td>BI</td>
<td>PL</td>
<td>-25.503, 153.050</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>L. Boomanjin</td>
<td>BO</td>
<td>PL</td>
<td>-25.558, 153.069</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>L. McKenzie</td>
<td>LM</td>
<td>PL</td>
<td>-25.443, 153.058</td>
<td>2</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>L. Wabby</td>
<td>LW</td>
<td>WT</td>
<td>-25.457, 153.129</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rocky Ck.</td>
<td>RC</td>
<td>Ck</td>
<td>-25.473, 153.010</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Tooloora Ck.</td>
<td>TC</td>
<td>Ck</td>
<td>-25.710, 153.078</td>
<td>13</td>
<td>14</td>
<td>27</td>
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<td>Wanggoobda Ck.</td>
<td>WA</td>
<td>Ck</td>
<td>-25.455, 153.009</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Stradbroke Island</td>
<td></td>
<td></td>
<td></td>
<td>AA</td>
<td>GA</td>
<td>HC</td>
</tr>
<tr>
<td>E. 18-Mile Swamp causeway</td>
<td>18S</td>
<td>WT</td>
<td>-27.522, 153.498</td>
<td>7</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Blue L.</td>
<td>BL</td>
<td>WT</td>
<td>-27.529, 153.477</td>
<td>9</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Blue L. Ck. outflow</td>
<td>BC</td>
<td>Ck</td>
<td>-27.535, 153.489</td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Blue L. Ck. overflow</td>
<td>BV</td>
<td>Ck</td>
<td>-27.534, 153.481</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Hering Lagoon</td>
<td>HL</td>
<td>WT</td>
<td>-27.576, 153.469</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Keyholes</td>
<td>KH</td>
<td>WT</td>
<td>-27.846, 153.512</td>
<td>3</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Palm Lagoon</td>
<td>PL</td>
<td>WT</td>
<td>-27.674, 153.441</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Yarraman Lagoons</td>
<td>YL</td>
<td>WT</td>
<td>-27.687, 153.510</td>
<td>5</td>
<td>5</td>
<td>1, 4</td>
</tr>
<tr>
<td>W. Aranarawai Ck.</td>
<td>AR</td>
<td>Ck</td>
<td>-27.854, 153.451</td>
<td>3</td>
<td>6</td>
<td>10</td>
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<tr>
<td>Brown L.</td>
<td>BR</td>
<td>PL</td>
<td>-27.493, 153.430</td>
<td>5</td>
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<td>19</td>
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<tr>
<td>Capemba Ck.</td>
<td>CM</td>
<td>Ck</td>
<td>-27.469, 153.425</td>
<td>2</td>
<td>1</td>
<td>8</td>
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<tr>
<td>Flinders Beach Wetland</td>
<td>FL</td>
<td>CS</td>
<td>-27.408, 153.471</td>
<td>2</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Little Canalpin Ck.</td>
<td>LC</td>
<td>Ck</td>
<td>-27.623, 153.419</td>
<td>35</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Yerrol Ck.</td>
<td>YC</td>
<td>Ck</td>
<td>-27.479, 153.414</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

Island/site abbreviations: Ck., Creek; E., East; Is., Island; L., Lake; N., North; S., South; W., West.
Site type abbreviations: Ck., Creek; CS, coastal swamp; PL, Perched Lake; WT, Watertable lake.
Sequence sources: 1, this study; 2, Bentley, Schmidt & Hughes (2010); 3, Knight et al. (2009); 4, Page & Hughes (2007a); 5, Page, Sharma & Hughes (2004); 6, Page, Choy & Hughes (2005); 7, Sharma & Hughes (2009); 8, Sharma & Hughes (2011); 9, Wong, Keogh & McGlashan (2004).
## Table 2 Freshwater species included in this study (see Table S1 for primer sequences)

<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Descriptor</th>
<th>Common name</th>
<th>Species code</th>
<th>Gene</th>
<th>Basepairs (FI/NSI)</th>
<th>Primers (forward/reverse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td><strong>Chandidae</strong></td>
<td><em>Ambassis agassizi</em></td>
<td>Steindachner, 1867</td>
<td>Agassizi’s Glassfish</td>
<td>AA</td>
<td>CytB</td>
<td>632</td>
</tr>
<tr>
<td></td>
<td><strong>Eleotridae</strong></td>
<td><em>Gobiomorphus australis</em></td>
<td>(Krefft, 1864)</td>
<td>Striped Gudgeon</td>
<td>GA</td>
<td>CytB</td>
<td>594</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Hyseleotris compressa</em></td>
<td>(Krefft, 1864)</td>
<td>Empire Gudgeon</td>
<td>HC</td>
<td>ATP</td>
<td>408</td>
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<tr>
<td></td>
<td><strong>Eleotridae</strong></td>
<td><em>Hyseleotris galii</em></td>
<td>(Ogilby, 1898)</td>
<td>Firetail Gudgeon</td>
<td>HG</td>
<td>CytB</td>
<td>601</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Mogurnda adspera</em></td>
<td>(Castelnau, 1878)</td>
<td>Purple-spotted Gudgeon</td>
<td>MA</td>
<td>ATP</td>
<td>715</td>
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<tr>
<td></td>
<td><strong>Eleotridae</strong></td>
<td><em>Mogurnda galii</em></td>
<td>(Castelnau, 1878)</td>
<td>Purple-spotted Gudgeon</td>
<td>MA</td>
<td>CytB</td>
<td>561</td>
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<tr>
<td></td>
<td><strong>Melanotaenidae</strong></td>
<td><em>Melanotaenia duboulayi</em></td>
<td>(Castelnau, 1878)</td>
<td>Crimson-spotted Rainbowfish</td>
<td>MD</td>
<td>CytB</td>
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<td></td>
<td><strong>Melanotaenidae</strong></td>
<td><em>Rhinocentrus ornatus</em></td>
<td>Regan, 1914</td>
<td>Ornate Rainbowfish</td>
<td>RO</td>
<td>ATP</td>
<td>296/495</td>
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<td></td>
<td><strong>Percichthyidae</strong></td>
<td><em>Nannoperca oxleyana</em></td>
<td>Whitley, 1940</td>
<td>Oxleyan Pygmy Perch</td>
<td>NO</td>
<td>CR</td>
<td>364</td>
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<tr>
<td></td>
<td><strong>Plotosidae</strong></td>
<td><em>Porochilus renti</em></td>
<td>(Whitley, 1928)</td>
<td>Rendahl’s Catfish</td>
<td>PR</td>
<td>CR</td>
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<td></td>
<td><strong>Plotosidae</strong></td>
<td><em>Tandanus tandanus</em></td>
<td>Mitchell, 1838</td>
<td>Freshwater catfish</td>
<td>TT</td>
<td>CR</td>
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<td><strong>Pseudomugilidae</strong></td>
<td><em>Pseudomugil melli</em></td>
<td>Allen &amp; Ivantsoff, 1982</td>
<td>Honey Blue Eye</td>
<td>PM</td>
<td>ATP</td>
<td>633</td>
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<td><strong>Pseudomugilidae</strong></td>
<td><em>Pseudomugil signifer</em></td>
<td>Kner, 1865</td>
<td>Pacific Blue Eye</td>
<td>PS</td>
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<td>Crustaceans</td>
<td><strong>Atyidae</strong></td>
<td><em>Caridina indistincta “A”</em></td>
<td>Calman, 1926</td>
<td>Freshwater shrimp</td>
<td>CA</td>
<td>COI</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Caridina indistincta “C”</em></td>
<td>Calman, 1926</td>
<td>Freshwater shrimp</td>
<td>CC</td>
<td>COI</td>
<td>450</td>
</tr>
<tr>
<td></td>
<td><strong>Palaemonidae</strong></td>
<td><em>Macrobrachium tolmerum</em></td>
<td>Riek, 1951</td>
<td>Leichhardtian</td>
<td>MT</td>
<td>COI</td>
<td>392</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Cherax dispar</em></td>
<td>Riek, 1951</td>
<td>Slender yabby</td>
<td>CD</td>
<td>COI</td>
<td>422/566</td>
</tr>
</tbody>
</table>

Genes: ATP, ATPase; CytB, cytochrome b; COI, cytochrome oxidase subunit I; CR, control region.
FL, Fraser Island; NSI, North Stradbroke Island.

*Possibility of cryptic species (see Page et al., 2004).
†See Page et al. (2005) for informal species differentiation.
‡Data from Bentley et al. (2010).

Comparative phylogeography for conservation planning.
were added to published data from a further 324 specimens (with one additional species, Cherax dispar) (see Tables 1 and S2) to create the multiple data sets detailed above.

There were five general categories of phylogeographic structure found within each species on each island: (i) ‘non-significant structure’ (non-significant pairwise $\Phi_{ST}$ between island sides); (ii) ‘shallow’ (<1% sequence divergence, significant pairwise $\Phi_{ST}$); (iii) ‘medium’ (1–4% divergence, significant pairwise $\Phi_{ST}$); (iv) ‘deep’ (>4% divergence, significant pairwise $\Phi_{ST}$); and (v) ‘endemic’ (described species only found on one side of island; only done for NSI as Fraser Island has not yet been as well surveyed).

**Non-significant structure**

Only five data sets (of 25) did not show significant phylogeographic structuring, including Gobiomorphus australis, Hypseleotris compressa and Macrobrachium tomentum (Table 3). These species have high salinity tolerances (Pusey et al., 2004; Sharma & Hughes, 2009) and thus a presumed ease of near-shore dispersal and gene flow, making a lack of phylogeographic structure in line with expectations and previous studies (Sharma & Hughes, 2009, 2011). For H. compressa, very few specimens were found on one side of each island (Table 1) and so there was low statistical power to detect structure. Macrobrachium tomentum was only sampled on the western side of NSI in this study, but is reported in Marshall et al. (2011) on the eastern side as well and so it is not considered endemic to the west and would likely fall into the non-significant category (as it does on Fraser Island).

The only freshwater species to show a non-significant phylogeographic structure between island sides in this study was Hypseleotris galii on NSI. Interestingly, this may well reflect the uncertain origin of the fish sampled

| Table 3 Phylogeographic measures between island sides |
|-----------------|---|---|---|---|---|
| Species | Gene | $\Phi_{ST}$ | Per cent sequence difference ($P$-distance) | Divergence at 2%/mya (mya) | Coalescent at 2%/mya (mya) | Range of all calculations (mya) |
| Fraser Island – North vs. South | | | | | |
| Non-significant structure | | | | | |
| Hypseleotris compressa | ATP | 0.000 | | | |
| Macrobachium tomentum | COI | 0.001 | | | |
| Shallow | | | | | |
| Rhadinocentrus ornatus | ATP | 0.277* | 0.16% | 0.079 | 0.133 | 0.053–0.265 |
| Hypseleotris galii Allom | CytB | 1.000* | 0.50% | 0.250 | 0.230 | 0.154–0.499 |
| Medium | | | | | |
| Pseudomugil signifer | ATP | 0.797* | 1.26% | 0.630 | | 0.420–1.260 |
| Pseudomugil meliss | ATP | 0.786* | 1.67% | 0.835 | 0.654–1.961 |
| Melanotaenia duboulayi | CytB | 0.948* | 1.96% | 0.980 | | 0.736–2.207 |
| Caridina indistincta ‘C’ | COI | 0.913* | 2.21% | 1.104 | | 0.841–2.524 |
| Hypseleotris galii Ocean | CytB | 0.978* | 2.52% | 1.262 | | 0.981–2.944 |
| Caridina indistincta ‘A’ FI-A | COI | 0.961* | 2.94% | 1.472 | | 1.056–3.167 |
| Caridina indistincta ‘A’ FI-B | COI | 0.953* | 3.17% | 1.583 | | |
| Deep | | | | | |
| Cherax dispar | COI | 0.949* | 8.40% | 4.201 | | 2.801–8.403 |
| North Stradbroke Island – East vs. West | | | | | |
| Non-significant structure | | | | | |
| Gobiomorphus australis | CytB | 0.000 | | | | |
| Hypseleotris compressa | ATP | 0.128 | | | | |
| Hypseleotris galii | CytB | 0.189 | | | | |
| Shallow | | | | | |
| Cherax dispar | COI | 0.363* | 0.16% | 0.081 | 0.326 | 0.054–0.652 |
| Nanupercia oxleyana | CR | 1.000* | 0.55% | 0.275 | 0.111 | 0.017–0.549 |
| Rhadinocentrus ornatus | ATP | 0.862* | 0.65% | 0.323 | 0.203 | 0.135–0.646 |
| Deep | | | | | |
| Caridina indistincta ‘C’ | COI | 0.984* | 6.49% | 3.247 | | 2.164–6.493 |

*Significant.

mya, millions of years ago.
in Brown Lake on the western side of NSI. This isolated, perched lake has always been described as fish-less (Lee-Manwar, Arthington & Timms, 1980) and yet fish began to be reported there a few years ago, presumably introduced, possibly from the eastern side of the island.

**Shallow to deep significant divergences**

Most data sets (14) displayed significant phylogeographic patterns between sides of each island (Table 3); five being ‘shallow’; seven, ‘medium’; and two, ‘deep’. Haplotype networks of these data sets from Fraser Island (Fig. 2 and Fig. S1) and NSI (Fig. 3) show clearly the difference between the sides of each island and imply little or no gene flow between them.

*Caridina indistincta* `A` has two deep lineages within the northern part of Fraser Island, FI-A and FI-B (see Page & Hughes, 2007a), and so each was compared separately with the southern Fraser Island *Caridina indistincta* `A`. *Hypseleotris galii* also had two lineages in the north of Fraser Island (Allom, Ocean), and each was also separately compared to southern Fraser *H. galii*.

**Endemic**

Five fish species were only found on one side of NSI (*Ambassis agassizi* and *Pseudomugil signifier* on the west side; *Mogurnda adspersa*, *Porochilus rendalhi* and *Tandanus tandanus* on the east side). The distribution of freshwater species on NSI is now well documented (Marshall *et al.*, 2011) and so the absence of a species from one side of the island in our data may well represent a real absence (with the exception of *M. tolmerum* mentioned above).

**Multi-dimensional scaling**

The multi-species presence/absence MDS (Fig. 4a) appears to show a differentiation between the freshwater communities of eastern and western NSI, but this is not significant (one-way ANOSIM *P* = 0.661, Global *R* = −0.062), despite more than half of the species being restricted to one side or the other (12 of 22 species; Marshall *et al.*, 2011). However, when one looks at MDS plots of genetic distances within each species found across NSI, there is significant structuring within many species, as evident in the *Φ* subscripts above. Examples of the different levels of genetic structuring are in Fig. 4: ‘deep’ (Fig. 4b, *Caridina indistincta* C, ANOSIM *P* < 0.01, *R* = 1.000); ‘shallow’ (Fig. 4c, *Rhadinocentrus ornatus*, *P* < 0.01, *R* = 1.000; Fig. 4d, *Cherax dispar*, *P* < 0.01, *R* = 0.466) and ‘non-significant structure’ (Fig. 4e, *Gobiomorphus australis*, *P* = 0.108, *R* = −0.144).

**Molecular clock calculations**

Of the ten species with significant divergences between the north and south of Fraser Island, four probably diverged during the Pleistocene (approximately 2 million to 11000 years ago), a further five sometime in the Pleistocene or Pliocene (approximately 5–2 Mya), and one in the Pliocene or earlier (Table 3, Fig. 5 for both islands). Three of the four significantly structured species on NSI probably diverged in the Pleistocene, and one in the Pliocene or earlier.

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Fig. 2 Haplotype networks of four fish and one decapod crustacean species on Fraser Island showing north–south genetic breaks. (a) *Pseudomugil mells*, (b) *Caridina indistincta* C, (c) *Melanotaenia duboulayi*, (d) *Hypseleotris galii*, (e) *Pseudomugil signifier*. Fish drawings by Pusey *et al.* (2004) (used with permission) and shrimp photograph by TJP. Each circle represents a DNA sequence; each line between them is a single mutation, and dots are unsampled haplotypes.

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Discussion

Biological and ecological relevance for conservation

Our genetic data strongly suggest that each side of each island hosts its own distinct community of populations. Are these intraspecific-level differences biologically and ecologically significant, warranting planning for their conservation? Most species exist as a series of populations, which are often phenotypically and ecologically diverse across a landscape (Bolnick et al., 2011). It is often difficult to uncover significant within-species populations without genetic data (Carvalho et al., 2011).

Phylogeographic diversity identified with mtDNA is usually ‘neutral’, resulting from genetic drift and time rather than selection (Frankham, 2010). In effect, one uses random variation as a surrogate for adaptive variation. Ideally, planning should account for both components of the evolutionary process, but adaptive variation is hard to quantify at small scales (Carvalho et al., 2011).

A knowledge of population structures is vital to plan species-specific conservation plans, in particular for restocking, relocating or breeding programmes (Olden et al., 2010, 2011). Divergent populations will not have met naturally for a long time, and the risks of breeding between populations/units is higher than within them (Frankham, 2010; Olden et al., 2011). For example, the translocation of a divergent population of a freshwater shrimp caused local extinction of the resident form in only seven generations (Hughes et al., 2003).

Most conservation legislation is at the species level, but within-species variation can be significant, particularly when it is shared across many species. The gold standard for assessing population distinctiveness is reproductive isolation, or a partial incompatibility, but this can be difficult and expensive to test (Frankham, 2010). Different mitochondrial groups in freshwater fish have sometimes been shown to interbreed successfully, but may also possess distinct local adaptations (Lajbner et al., 2010).

While mitochondrial data are not evidence of reproductive isolation or adaptive divergence, divergent mitochondrial clusters provide strong evidence for independently evolving populations with very little gene flow (Papadopoulou et al., 2008) and can serve as a surrogate until more detailed and expensive adaptive data are available. Local extinction, whether of species or deep lineages, will likely not be counteracted naturally due to the low level of recolonisation in freshwater species (Hughes, 2007), especially for those on islands.

Aquatic food webs can be altered with unpredictable, cascading ecological effects if distinct populations play
unappreciated roles, and these populations become extinct or are moved between areas (Olden et al., 2011). For example, if there were extinctions on one side of Stradbroke Island, the local ecosystem would become more depauperate and ecological changes difficult to predict. All of these argue for the conservation (and accurate delineation) of multiple, genetically distinct populations (Olden et al., 2010).

While the results presented here represent only fairly small geographic scales, we believe that the ideas we propose here can be applied more broadly. The basis for our approach is that it is important to identify regions that have been isolated for significant lengths of evolutionary time. For many groups of aquatic taxa, populations in these regions will represent evolutionarily significant units (ESU; Moritz, 1994). We predict that small-scale patterns similar to those reported here are likely to be very widespread. They are most likely in obligate freshwater species that inhabit isolated waterbodies, especially those that have not been connected during Pleistocene ice ages (Hughes, Schmidt & Finn, 2009). Streams in upland areas are even more likely to show these patterns (Hughes, 2007), unless there have been drainage changes during the Pleistocene (Burridge, Craw & Waters, 2006).

Fig. 4 Comparative multi-dimensional scaling plots from N. Stradbroke Island (a) species presence/absence (all data from Marshall et al., 2011). Each triangle represents multiple species at a single site; (b–e) pairwise genetic distances within species (each triangle represents a gene sequence). All triangles assigned to east or west sides of island based on Page & Hughes (2007a) and Bentley et al., 2010.
Scales of bioregions

The scale of freshwater bioregions varies greatly. Southeast Queensland forms a single region in Whiting et al. (2000) and Unmack (2001), whereas Marshall et al. (2006a) consider Fraser Island, the nearby coast and NSI as contained within the ‘Wallum’ province. The nature of both geographic and biological units is nested (Cook et al., 2008), and so there is often important phylogeographic variation within regions (Abell et al., 2008). Regions can be both too large, so inappropriate for a particular plan, and too small, and thus complex and expensive (Marshall et al., 2006a). This is key because cost is one of the important metrics employed to come to a ‘best solution’ in systematic conservation planning (Nel et al., 2009).

Freshwater managers often assume that a catchment (Hughes et al., 2009), or even sub-catchment (Linke et al., 2011), is the most relevant geographic unit for planning, but freshwater taxa often exist over very small ranges (Olden et al., 2010), especially when genetic data are integrated (Cook et al., 2008). The relevant scale will depend on the scope of the conservation objective (Nel et al., 2009). Fine-scale ‘bottom-up’ information (Hale & Butcher, 2008) from real-world data (Linke et al., 2011), such as those from the sand islands in the current study, are required for many plans to meet their objectives. Now that there are data available at these scales, managers and planners can start to do cost-benefit analyses to work out at what level to pitch their efforts. The mindset of ‘scale’ itself may be a problem, because this study shows that different types of scales are not necessarily equivalent. While the geographic scales may be moderate, the scales of biodiversity and evolutionary history are large, and therefore if one of the objectives of a plan is to conserve diversity, then the appropriate levels of that diversity needs to be recognised a priori.

The implication for a bioregional approach to the sand islands is that the separate sides of each island may be deemed distinct ‘freshwater focal areas’ (Linke et al., 2011) with complementary communities. Most of both islands will soon be within national parks, but this does not mean that these areas are now fully protected unless there is an acknowledgment of the distinct small-scale bioregions within each park; after all, ‘management within protected areas should aim to protect and improve river biodiversity’ (Turak et al., 2011). The evolutionary nature of these small-scale bioregions may even warrant some new terminology along the lines of ‘evoregion’.

Uncovering the actual processes behind the observed patterns

Conservation planning often seeks to preserve biodiversity patterns rather than the evolutionary processes that formed them (Nel et al., 2009). This is because patterns are easily observed, but processes not so easily inferred. Nearly all the species in this study from both islands show similar phylogeographic patterns, namely an evolutionary
split between different sides of each island. The most parsimonious explanation for shared patterns across multiple species is a shared biogeographic history due to a shared earth history (Arbogast & Kenagy, 2001) that has shaped not only a single species but a whole biotic community and ecosystem (Avise, 2009).

The landscape evolution of south-east Queensland has contributed directly to the evolution of biological diversity of the freshwater fauna of the sand islands, and so must be understood to uncover the relevant processes involved. Extensive dune fields formed during nine major glacial periods of lower sea level over the last 730,000 years (Tejan-Kella et al., 1990; Longmore & Heijnis, 1999), with both islands forming part of the mainland for most of their existence (Stephens, 1992; Longmore & Heijnis, 1999). A series of climate oscillations roughly every 100 thousand years changed sea levels, allowing dunes to be laid down in a chronological sequence (Lees, 2006).

North Stradbroke Island

Relating the phylogeographic patterns with NSI’s geography and geomorphology, it is apparent that the northwest arrayed high central dunes (up to 229 m a.s.l.), which separate east from west, form a barrier between different freshwater communities that is rarely crossed (Fig. 3). The three species that show a ‘shallow’ pattern (Fig. 5) diverged within a similar time frame to the approximate age of the island (approximately 500,000 years, Ward, 2006) and so could have diverged on the island (Bentley et al., 2010), while the older divergence of the shrimp relates to a more ancient mainland landscape (Page & Hughes, 2007a).

The different levels of divergence in co-distributed species are likely due to a temporary barrier (i.e. the sea) being regularly removed and allowing dispersal during multiple different periods along separate sides of a persistent barrier (high dunes), and then reinstated in the same place (by sea-level change) (Hughes et al., 2009). This regular phylogeographic pattern in response to past climate change is seen within many species in the face of cyclical glaciation (Avise, 2009).

The east/west pattern implies that the different sides of the island, bisected by high dunes, have been colonised by many species separately from the mainland along distinct freshwater pathways at times of lower sea level, when a broad plain, criss-crossed by a number of different river systems, linked the island to the mainland (Page & Hughes, 2007a). The absence of 12 fish and macrocrustacean species from one or other side (Marshall et al., 2011) suggests that either there are significant habitat differences or these species never colonised (or crossed to) one side of the island, or else subsequently became extinct once there. This explains why small-scale phylogeographic patterns within a single species can match large-scale biogeographic patterns among many species (see Fig. 4) on either side of a persistent barrier (Avise, 2009). This is because the completely separated populations of most species are on a continuum from recent divergence to extinction or speciation.

The implication for conservation plans of NSI is that freshwater populations from the east and west sides should be treated as distinct biodiversity features in future conservation planning processes and that breeding programmes, such as for the endangered Nannoperca oxleyana (Knight et al., 2009), should separate individuals from different sides until specific biological information can be collected.

Fraser Island

Fraser Island also provides a strong general phylogeographic pattern (north/south) across all ten fully freshwater species tested, but its cause is less immediately clear as there is not an obvious barrier. Fraser’s dunes are aligned north–south following the prevalent onshore wind direction (Tejan-Kella et al., 1990), although transgressive dunes can form during intense aeolian activity (Levin, 2011). There are also no obvious physicochemical differences between the freshwater bodies between north and south (Hadwen et al., 2003). Most of the divergences are as old, or older, than the island itself (Fig. 5), hinting that any process is likely to be at the larger scale. Considering Fraser within the context of the nearby mainland, remembering that for most of its history it was attached (Longmore & Heijnis, 1999), it becomes clear that the mouth of the largest mainland river in the region, the Mary River, aligns exactly with the divide between north and south on Fraser (see Fig. 2).

The Mary River is a known area of phylogeographic breaks in many species of mainland freshwater fauna between the Mary River and catchments to the south. This is seen in fishes (Hughes et al., 1999; Page, Sharma & Hughes, 2004; Wong, Keogh & McGlashan, 2004) and decapod crustaceans (Page & Hughes, 2007b; Sharma & Hughes, 2009; Bentley et al., 2010). This major freshwater frontier of genetic diversity continues across to Fraser Island, implying that the northern part of the island was colonised by fauna from the Mary River and further north during times of lower sea levels, while the south was populated by fauna from rivers to the south (Bentley et al., 2010). Dispersal corridors opened and closed between
Fraser and the mainland as sea levels fell and rose again, reflected in the low levels of genetic divergence between each part of Fraser and its related section of the mainland seen in many species (Bentley et al., 2010; Sharma & Hughes, 2011; T.J. Page & J.M. Hughes, unpubl. data). The deep split across Fraser is even evident in other species not as intimately tied to water, such as frogs (James, 1996) and freshwater turtles (K. Hodges, A. Georges & S.C. Donnellan, unpubl. data).

One of the criteria that Fraser Island satisfied to attain world heritage status (as per Hadwen et al., 2003) is that it represents ‘significant ongoing ecological and biological processes in the evolution and development of terrestrial, fresh water, coastal and marine ecosystems and communities of plants and animals’. This study shows that one of the significant processes that should be protected is the different source populations of the two sides of the island. The implications for conservation plans that consider Fraser Island are similar to that for NSI, namely that populations from the two sides should be considered separately in planning processes. For example, the strong population structure of the endangered fish *Pseudomugil mells* suggests that any translocations or breeding programmes should be within each half of Fraser Island and not between.

**Conclusion**

Comparative phylogeography can help define more targeted community-level evolutionary units of biodiversity and complementarity for systematic conservation planning at the relevant scale for particular plans. This is of special relevance to freshwater species that often display a high level of population structure even over small areas (Olden et al., 2011) and often have very small ranges (Abell et al., 2008). The results of this study show that one cannot assume that biological diversity will necessarily follow obvious geography (such as an island) and that important community-level diversity may exist at smaller scales (such as one side of an island) that should be incorporated into conservation and resource management planning processes. The results of any analyses are only as good as the data and assumptions input into them (‘GIGO: garbage in, garbage out’; Feynman, 2001).

The inclusion of indirect surrogates for the evolutionary process can significantly change the results of conservation plans (Carvalho et al., 2011). There is a rapid growth in the amount of publicly available molecular data, and new technologies mean that many species in a community can now be sequenced quickly (Page & Hughes, 2010). Therefore, direct evolutionary information (DNA sequences) can now be feasibly included in the planning process, initially at the small scale as in this study, but soon at larger and larger scales. While here molecular data are most useful in defining biodiversity units at the beginning of the planning process, the growth of the availability of molecular data argues for the future development of ‘a comprehensive methodology of how to spatially optimize conservation areas using molecular data for multiple species’ (Carvalho et al., 2011).

**Acknowledgments**

Many people helped with fieldwork, including Andrew Bentley, Ben Cook, James Fawcett, Kaye Stuart (Griffith University); Amanda Carter, Dean Holloway, Jaye Lobegeiger, Glenn McGregor, David Moffatt, Stephen Moore, Peter Negus, Jessica Pettitt, Alisha Steward (Queensland Department of Environment and Resource Management); Paul Smith, Scott Whitney (Sibelco Australia Ltd/Consolidated Rutile Ltd). Brad Pusey (Griffith University) allowed us to use his beautiful fish drawings, and Peter Unmack (NESCent) gave us some unpublished primers. Arlene Wheatley and Katherine Real did a great deal of laboratory work. Specimens were sampled under National Parks and Wildlife Service and Department of Primary Industries permits and with the following ethics approvals: AES/13/02/AEC, ENV/26/08/AEC. We also thank Wade Hadwen, Ben Cook and Simon Linke (Griffith University) for their comments on earlier versions of this manuscript, and three anonymous reviewers for suggesting substantial improvements. Funding was provided by the Australian Rivers Institute, Griffith University, and the Queensland Department of Environment and Resource Management.

**References**


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

- **Figure S1.** Fraser Island Genetic Relationships.

- **Table S1.** Primer sequences and sources.
- **Table S2.** All specimens sequenced for this study or downloaded from GenBank with relevant accession numbers.

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(Manuscript accepted 14 October 2011)