Spatial scales of genetic patchiness in the western rock lobster (*Panulirus cygnus*)

W. J. KENNINGTON[A], O. BERRY[A,B], D. M. GROTH[C], M. S. JOHNSON[A] and R. MELVILLE-SMITH[D]

[A]*School of Animal Biology, The University of Western Australia, Crawley, WA 6009, Australia*

[B]*CSIRO Division of Marine and Atmospheric Research, Underwood Avenue, Floreat, WA 6014, Australia*

[C]*Curtin Health Innovation Research Institute, Biomedical Research Institute, Curtin University, GPO Box U1987, Perth, WA 6845, Australia*

[D]*Department of Environment and Agriculture, Curtin University, GPO Box U1987, Perth WA 6845, Australia*

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Corresponding author: Jason Kennington, School of Animal Biology, The University of Western Australia, Crawley WA 6009, Australia.

Tel.: +61 8 6488 3233

Fax: +61 8 6488 1029

E-mail: jason.kennington@uwa.edu.au

Abstract

In planktonic dispersers, impediments to dispersal, local selection or large variance in the reproductive success among individuals (sweepstakes reproductive success) can create genetic heterogeneity at local scales. While these processes are well recognized, relatively few studies have investigated the spatial scales over which genetic heterogeneity occurs and how it is distributed across species' ranges. We investigate population structure in the western rock lobster (*Panulirus cygnus*), a commercially exploited species found in shallow and deep water reef habitats along the Western Australia coastline. We screened 631 individuals from nine locations across the species' range for genetic variation at 22 microsatellite loci. Consistent with expectations of extensive larval mixing during an extended planktonic stage, we found no significant genetic differentiation among the nine locations (F_{ST} = 0.003, G''_{ST} = 0.007). Despite the lack of large-scale geographic structure, small but significant positive spatial autocorrelation (SA) was detected over distances up to 40 km. Two-dimensional local SA analysis confirmed that fine-scale genetic heterogeneity was common throughout the species' range. An intriguing aspect of these results was that they were based on juvenile and adult lobsters, suggesting restricted movement or spatial cohesion of individuals after settlement.

Introduction

The spatial extent of genetic structure is largely dependent on the dispersal capacity of individuals (Bohonak 1999). This is especially apparent in the marine environment, where species with planktotrophic larvae that spend months in the water column tend to maintain low levels of genetic structure across large geographic scales, while those with short planktotrophic larval phases or direct developers usually have much higher levels of subdivision (Waples 1987, Palumbi 1994, Johnson & Black 2006a, Lee & Boulding 2009). However, the role of dispersal capacity in structuring marine populations is complex, and can be strongly influenced by other factors. For example, in spiny lobsters, most species are characterized by a lack of genetic differentiation among localities, consistent with their high dispersal capability during an extended planktonic larval stage (e.g., Ovenden et al. 1992, Silberman et al. 1994, Tolley et al. 2005, García-Rodrguez & Perez-Enriquez 2008, Naro-Maciel et al. 2011), but in some species, barriers to dispersal created by topographic or oceanographic features can lead to moderate to high levels of population structure (Perez-Enriquez et al. 2001, Gopal et al. 2006, Palero et al. 2008).

Impediments to dispersal are not always obvious. For example, Johnson and Black (2006b) showed that over short distances (< 2 km) genetic subdivision increased fivefold between populations on different islands compared to different populations on the same island in both a direct developing snail and a planktonic disperser. Such genetic heterogeneity at local scales can occur even when there is little genetic subdivision over large distances (e.g., Hedgecock 1986, Benzie & Stoddart 1992, Johnson et al. 1993, Ayre & Hughes 2000). Adaptation to local environments (e.g. low salinity or temperature) can also lead to genetic differentiation at selected and linked neutral loci, despite high levels of gene flow (see Nielsen et al. 2009). The extent of population structure can therefore vary considerably among species and is not always determined by life-history characteristics alone. For

commercially exploited species, failure to detect underlying population structure is a concern, because it may result in overexploitation and depletion of localized subpopulations, with a corresponding loss of genetic variation (Carvalho & Hauser 1994, Begg et al. 1999).

The western rock lobster *Panulirus cygnus* (Decapoda: Palinuridae) is found in shallow and deep water reef habitats along the Western Australia coastline, from Cape Leeuwin (34° 22' S) to North West Cape (21° 45' S). It supports one of the most economically important single species fisheries in Australia, with until recently, an annual commercial catch of between 8 000 and 14 500 *t* (Fletcher et al. 2005). A key assumption underlying the management of *P. cygnus* is that the breeding stock comprises a single, demographically united population. This assumption is based on the extended pelagic larval stage of *P. cygnus*, which is thought to ensure high dispersal throughout the species' range. Larvae hatch in spring and early summer, and spend the next nine to eleven months in the plankton, with mid stages being found up to 1500 km offshore. The late-stage larvae metamorphose into pueruli and swim inshore to start the juvenile stage of their life-cycle (Phillips et al. 1979). Allozyme studies also suggest *P. cygnus* is a single panmictic population, but with ephemeral genetic patchiness (small-scale genetic heterogeneity among local populations) caused by temporal variation in allele frequencies of recruits (Thompson et al. 1996, Johnson & Wernham 1999). It therefore represents an extreme model for testing for subtle fine-scale genetic structure over a large geographic range. The aim of this study was to investigate the spatial scale of genetic patchiness in juvenile and adult *P. cygnus* across the main geographic distribution of the species. To achieve a resolution beyond previous genetic studies, we sampled at finer spatial scales, and used 22 microsatellite loci for our study. Microsatellites have proven to be a powerful tool for detecting genetic subdivision within marine species with high larval dispersal capabilities (e.g., Knutsen et al. 2003, Riccioni et al. 2010, White et al. 2010) and

have revealed spatial genetic structure on finer scales than found with allozymes and mtDNA (e.g., Ruzzante et al. 1996, Jørgensen et al. 2005).

Materials and methods

Sample collection

In 2009, tissue samples were collected from juvenile and adult *P. cygnus* (carapace length > 45 mm) at nine locations spanning nearly 660 km along the Western Australian coastline (Fig. 1). A total of 631 individuals were captured using commercial lobster pots set over distances up to 27.4 km apart within each location. Sample sizes at each location ranged between 19 and 64 individuals. To allow investigation of fine-scale patterns within the Houtman Abrolhos Islands, samples were collected from an additional seven sites (eight sites in total) between four and 82 km apart (sample sizes ranged between 40 and 68 individuals). The spatial coordinates for each individual were recorded at the time of capture.

DNA extraction and microsatellite genotyping

DNA was extracted from the middle lobe of the tail fan stored in 100% ethanol, using a QIAGEN Dneasy Blood and Tissue kit, following the manufacturer's recommendations. After the DNA was extracted, each sample was analysed using a NanoDrop ND-1000 spectrophotometer to determine the concentration and quality of the DNA. All DNA samples were stored at –20 ºC until genotyping. Genotypes at 22 microsatellite loci (S3, S8, S28, S50, W25, Pcyg1 – 9 and 11– 18) were determined for each individual using primers and PCR running conditions described in Groth et al. (2009) and Kennington et al. (2010). PCR products were analyzed on an ABI 3700 sequencer using a GeneScan-500 LIZ internal size standard and scored using GENEMARKER (SoftGenetics, State College, PA, USA) software.

123 *Data analysis*

124 Microsatellite variation at each location was quantified by calculating allelic richness (a
125 measure of the number of alleles independent of sample size) and Nei's (1987) estimator of
126 gene diversity. The presence of null alleles was tested for each locus using MICROCHECKER
127 (van Oosterhout et al. 2004). Tests for a deficit or excess in heterozygotes at each location
128 were carried out using randomisation tests, and the results were characterized using the F_{IS}
129 statistic. Significantly positive F_{IS} values indicate a deficit of heterozygotes relative to
130 random mating, and negative values indicate an excess of heterozygotes. Linkage
131 disequilibrium between each pair of loci was assessed by testing the significance of
132 association between genotypes. Corrections for multiple comparisons were carried out using
133 the Sequential Bonferroni method (Rice 1989).

134 Genetic differentiation among locations was assessed by calculating Weir and
135 Cockerham's (1984) estimator of F_{ST} and G''_{ST}, a version of Hedrick's (2005) standardized
136 G_{ST} corrected for bias when the number of populations is small (Meirmans & Hedrick 2011).
137 Microsatellite R_{ST} values (Slatkin 1995) were also calculated, but were qualitatively similar
138 to F_{ST} values so are not reported. Tests for genetic differentiation were performed by
139 permuting genotypes among samples. Estimates of allelic richness, gene diversity, F_{IS},
140 deficits in heterozygotes and linkage disequilibrium were calculated using the FSTAT version
141 2.9.3 software package (Goudet 2001). Estimates of F_{ST}, G''_{ST} and tests for genetic
142 differentiation using were performed using the software package GENALEX version 6 (Peakall
143 & Smouse 2005). Differences in estimates of genetic variation and F_{IS} among locations were
144 tested using Friedman's ANOVA. To test for a relationship between genetic and geographical
145 distance, we compared a matrix of G''_{ST} with a matrix of geographical distance (ln km),
146 using a Mantel test with 10 000 permutations.

147 Spatial genetic structure was also investigated using two Bayesian clustering methods,
148 implemented with the software packages STRUCTURE (Pritchard et al. 2000) and GENELAND
149 (Guillot et al. 2005). Both these programs group individuals into the most likely number of
150 clusters (*K*) that maximizes the within cluster Hardy-Weinberg and linkage equilibria.
151 However, GENELAND differs from STRUCTURE in that geographical information can be
152 incorporated to produce more accurate inferences of population structure based on the spatial
153 distribution of individuals. Analyses involving STRUCTURE were based on an ancestry model
154 that assumed admixture and correlated allele frequencies. No prior information about the
155 origin of the samples was used. Ten independent runs were performed for each value of *K* (1–
156 10), with a burnin of 10 000 followed by 100 000 MCMC iterations. The most likely number
157 of clusters was assessed by comparing the likelihood of the data for different values of *K* and
158 using the ΔK method of Evanno et al. (2005). For the GENELAND analysis, the spatial
159 coordinates (latitude and longitude) of each individual were used to run the spatial model.
160 The uncertainty of coordinates was set at zero. Ten independent runs were performed for
161 each value of *K* (1–10) using the uncorrelated and null allele models. Each run consisted of
162 100 000 MCMC iterations with a thinning of 100 and a burnin of 200. The most likely
163 number of clusters was chosen as the modal *K* (from each independent run) with the highest
164 posterior probability.
165 We also carried out spatial autocorrelation (SA) analysis to evaluate the genetic
166 similarity of individuals over varying spatial scales. We used GENALEX to calculate a spatial
167 autocorrelation (*r*) coefficient for a range of distance classes. The results from the SA
168 analysis were presented in two ways. Firstly, *r* was plotted as a function of distance class to
169 produce a spatial genetic autocorrelogram. Secondly, because estimates of spatial
170 autocorrelation are influenced by the size of distance classes (see Peakall et al. 2003) , *r* was
171 calculated for series of increasing distance class sizes. When significant positive spatial

structure is present, *r* will decrease with increasing distance class sizes. The distance class where *r* no longer differs significantly from zero provides an approximation of the extent of detectable positive spatial genetic structure (Peakall et al. 2003). Tests for statistical significance were performed by random permutation and calculating the bootstrap 95% confidence limits (CL) of *r*, using 1000 replicates in each case. We also performed a two-dimensional local spatial autocorrelation analysis using GENALEX. With this analysis, the local autocorrelation (*lr*) is estimated by comparing an individual with its *n* nearest neighbours, allowing investigation of local patterns of spatial autocorrelation within the two dimensional landscape (Double et al. 2005). Calculations of *lr* were made using the nearest five, 10, 20 and 50 individuals. As with the global autocorrelation analysis, statistical significance was determined using permutation tests.

Finally, tests for selection acting on marker loci were carried out using the F_{ST} outlier approach (Beaumont & Nichols 1996, Beaumont 2005), implemented with the LOSITAN software package (Antoa et al. 2008). The method evaluates the relationship between F_{ST} and expected heterozygosity in an island model of migration with neutral markers. This distribution is used to identify loci with excessively high or low F_{ST} values compared to neutral expectations. These loci are candidates for being subject to directional and balancing selection respectively. Simulations were run using 10 000 replications, 99% confidence intervals and the neutral and forced mean options. For this analysis, individuals were grouped by location and both the stepwise and infinite allele mutation models were performed.

Results

Thirteen loci (S3, S8, S50, Pcyg02, Pcyg04, Pcyg06, Pcyg07, Pcyg09, Pcyg12, Pcyg13, Pcyg14, Pcyg16 and Pcyg17) were identified as having null alleles in at least one location using MICROCHECKER and excluded from further analyses unless specified otherwise. The

197 remaining loci showed high levels of genetic diversity at each location (Table 1). There were
198 no significant differences in allelic richness (χ^2 = 11.57, P = 0.172), gene diversity (χ^2 = 6.89,
199 P = 0.548) or F_{IS} (χ^2 = 3.11, P = 0.927) among locations. Nor was there genotypic
200 disequilibrium between pairs of loci after adjusting for multiple comparisons or deviations
201 from Hardy-Weinberg Equilibrium (HWE).

202 There was no significant genetic differentiation among the nine sampling locations (F_{ST}
203 = 0.003, G''_{ST} = 0.007, P = 0.249). Most tests of population differentiation between pairs of
204 locations were non-significant (Table 2), and divergences between most locations were
205 comparable to those observed between sampling sites within the Houtman Abrolhos Islands
206 (pairwise G''_{ST} ranged from –0.015 to 0.020), which were separated by much smaller
207 geographical distances. There was no evidence for isolation-by-distance using pairwise G''_{ST}
208 values calculated between locations (broad-scale) or between sampling sites within the
209 Houtman Abrolhos Islands (local-scale) (Mantel tests, P = 0.170 and 0.111 respectively). No
210 significant genetic divergences among locations were also found when analyses were
211 performed using all 22 loci (F_{ST} = 0.000, G''_{ST} = 0.000, P = 0.595). We also failed to detect
212 isolation-by-distance when analyses were performed using all 22 loci (Mantel tests, P = 0.170
213 and 0.453 for broad and local spatial scales respectively).

214 No genetic subdivision was found using Bayesian clustering analysis. The STRUCTURE
215 analysis revealed decreasing log probability estimates with increasing values of K and there
216 were no large fluctuations in ΔK, suggesting that the probable number of clusters was one.
217 Further, when K > 1, the proportion of individuals assigned to each cluster was fairly even
218 and most individuals were admixed, consistent with inferred population structure not being
219 real (Pritchard et al. 2010). The analysis involving GENELAND gave a similar result, with
220 posterior distributions of the estimated number of populations indicating a clear mode at K =
221 1 in nine out of 10 replicates. Similar results were obtained when clustering analysis were

performed using all 22 loci, with the STRUCTURE analysis revealing only slight increases in log probability estimates with increasing values of K and no large fluctuations in ΔK, while the GENELAND analysis indicated a clear mode at $K = 1$ in all 10 replicates.

In contrast to the Mantel tests, spatial genetic structure was detected with the SA analyses. Significantly positive r values were found within the first four distance classes (0–10, 11–20, 21–30 and 31–40 km), after which r decreased and oscillated between being non-significantly different from zero and significantly negative (Fig. 2A). This pattern is indicative of fine-scale spatial genetic structure generated by discrete patches of similar multilocus genotypes (Smouse & Peakall 1999, Diniz-Filho & Telles 2002). Positive spatial genetic structure at local geographical scales was confirmed when estimates of r were calculated with increasing distance class sizes. Figure 2B shows little change in r between 10 and 100 km, after which r decreased, but remained significant until 150 km. It also appears that positive genetic structure was not confined to one geographical area. Two-dimensional local spatial autocorrelation analysis revealed clusters of positive lr at most sampling locations (Fig. 3). The close proximity of significantly positive and non-significant values suggests that local patches were not genetically uniform. A similar number and distribution of positive lr values were obtained when calculations were based on sampling the nearest five, 10, 20 and 50 individuals, confirming the consistency of the result. Again, similar results to these were found when all 22 loci were used. The only exceptions being that in the SA analysis positive r values were found within the first two distance classes only and none of the remaining distance classes were significantly negative. The outlier analyses failed to detect any loci with higher than expected F_{ST} values.

Discussion

The major finding of this study was the significant genetic heterogeneity among local populations in *P. cygnus*, over very short spatial scales, without the presence of large-scale geographic structure. We found extremely low levels of differentiation among locations sampled across the species' range (F_{ST} = 0.003, G''_{ST} = 0.007), consistent with extensive gene flow over large geographic distances. The lack of geographic pattern was emphasized by genetic divergences between locations separated by distances over 650 km being no larger than the divergences between sites at the Houtman Abrolhos Islands, which are separated by distances less than 85 km. We also found no evidence of isolation-by-distance using pairwise G''_{ST} estimates and no genetic subdivision using Bayesian clustering analysis. Our results, therefore, add weight to the findings of previous allozyme (Thompson et al. 1996, Johnson & Wernham 1999) and microsatellite studies (Kennington et al. 2013), which suggest that *P. cygnus* is a single, panmictic population.

Fine-scale population structure in *P. cygnus* was most clearly evident with spatial autocorrelation analysis. Significant genetic structure was observed when lobsters were sampled over distances up to 40 km, with detectable positive spatial genetic structure extending out to 150 km when distance classes were pooled. Further, two-dimensional local SA analysis indicates that these patterns were not driven by the strong influence of one region alone, but were a common feature throughout the species' range. Such microgeographic genetic patchiness has been demonstrated in other marine species with planktonic larvae (e.g., Hedgecock 1994b, Knutsen et al. 2003, Pujolar et al. 2006), including several species along the Western Australian coast (Johnson & Black 1982, Watts et al. 1990, Johnson et al. 1993, Johnson et al. 2001). Genetic patchiness has also been observed in *P. cygnus* using allozymes (Johnson & Wernham 1999), but the scale of the genetic heterogeneity reported

here is much smaller than shown previously. This likely reflects the increased genetic sensitivity and fine-scale geographic information of this study.

Spatial genetic patchiness in some species is due to temporal variation in the genetic composition of recruits (Johnson & Black 1984, Watts et al. 1990, Hedgecock 1994a, Pujolar et al. 2006). This also seems to be the case for *P. cygnus*, in which a combination of temporal variation in allele frequencies and contrasting patterns of recruitment resulted in genetically different cohorts of *P. cygnus* at two sites (Johnson & Wernham 1999). Furthermore, this pattern was ephemeral, as it was not repeated in the subsequent two years. Under the 'sweepstakes reproductive success' hypothesis (Hedgecock 1994a), temporal genetic variance in recruits might be a by-product of large variance in the reproductive success of individuals, owing to chance matching of reproductive activity with oceanographic conditions conducive for larval survival. Other possible explanations for temporal genetic variation in *P. cygnus* recruits are (1) origin from different source populations, (2) limited mixing of larvae in the plankton, or (3) natural selection on larvae prior to settlement. Given the low geographic structure in *P. cygnus*, it is unlikely that temporal genetic variation arises from different source populations. The finding that *P. cygnus* larvae settling at the same time at locations 350 km apart shared the same allele frequencies (Johnson & Wernham 1999) also argues against temporal genetic variation being due to the cohesion of larvae in the plankton, though this result was based on only three allozyme loci.

If a combination of temporal variation in allele frequencies and contrasting patterns of recruitment is responsible for the genetic patchiness observed in this study, it would require juvenile *P. cygnus* to be relatively sedentary. This appears to be the case. Studies on foraging movements suggest juvenile *P. cygnus* forage over relatively small areas (~150 m radius), though the extent of movement is variable (Jernakoff et al. 1987, Jernakoff & Phillips 1988). The life-cycle of *P. cygnus* also includes a migratory phase, which occurs between four and

six years of age, just after many lobsters undergo a synchronised moult that changes their normal red shell to a paler colour (Morgan et al. 1982). During this migration, lobsters leave the coastal reefs and move into deeper water breeding grounds, where they become sedentary again on deeper reefs. Because the lobsters we collected were predominantly from shallow water locations, it is unlikely that they had undertaken these migratory movements. Nevertheless, tag and release experiments have shown that while large movements (>200 km) do occur, most lobsters (>87%) are recaptured within 10 km of their release site (Chubb et al. 1999), which is within the distance range we detected positive population structure. More recently, a study using acoustic telemetry found that only a small proportion (13.6%) of migratory phase lobsters emigrated from their resident reef, suggesting that a mass offshore migration may not hold for all inshore reefs (MacArthur et al. 2008).

Another explanation for spatial genetic patchiness is natural selection acting after settlement (Larson & Julian 1999). Given the broad latitudinal range of *P. cygnus* (> 1200 km), local populations are likely to experience highly varied environmental conditions, providing the opportunity for local adaptations to develop across populations (Kawecki & Ebert 2004). Indeed, several studies have found evidence for local adaptation in widely distributed marine fish (see Nielsen et al. 2009). While we found no clear evidence of directional selection using outlier analysis, genome scans involving many more neutral markers, candidate genes or population transcriptomics would be needed to exclude confidently this possibility. A study monitoring the genetic composition of cohorts of recruits as they develop into adults would also yield valuable insights on post-settlement processes.

Implications for fisheries management

The implications of genetic patchiness for fisheries management have been discussed by Larson and Julian (1999). If genetic patchiness is due to selection after settlement, they

319 suggest that the implications for fisheries management are minor, unless a fishery is
320 concentrated on one particular habitat or location, which might disproportionately affect a
321 certain portion of the gene pool. By contrast, factors affecting the genetic composition of
322 recruits prior to settlement may have greater consequences. The most relevant of these to *P.*
323 *cygnus* is the effect of stochastic spatial variation in the sources of successful larvae
324 (sweepstakes reproductive success). This effect implies that the sources of successful larvae
325 vary unpredictably over time. Fisheries management should therefore ensure that both the
326 distribution as well as the total spawning potential of the exploited population is protected.
327 Further, spatial stochasticity of successful spawning argues for either the spatial dispersion of
328 reserves (if they are involved in managing the exploitation), or suitably low exploitation rates
329 across the fishery, thereby increasing the chance that at least some larvae will be released into
330 conditions favourable for their survival. Local genetic patchiness also suggests juvenile and
331 adult lobsters are comparatively sedentary, so they may be more susceptible to
332 environmental/anthropogenic impacts at a finer scale than previously thought.
333

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495
496

497 **Table 1**. Genetic variation at each location.

Site	Sample size	A_R (SE)	H (SE)	F_{IS}
Kalbarri	39.4 (0.3)	7.1 (1.9)	0.58 (0.11)	-0.05^{NS}
HA1	38.9 (0.4)	7.8 (2.0)	0.62 (0.10)	0.01^{NS}
Dongara	38.2 (0.7)	7.2 (1.8)	0.62 (0.10)	-0.01^{NS}
Jurien Bay	38.3 (0.6)	7.3 (2.0)	0.60 (0.10)	-0.01^{NS}
North Lancelin	17.7 (0.6)	7.2 (2.0)	0.56 (0.11)	-0.04^{NS}
Lancelin	35.9 (0.7)	7.0 (1.8)	0.59 (0.11)	0.03^{NS}
Rottnest Island	63.0 (0.2)	7.5 (1.9)	0.64 (0.11)	-0.03^{NS}
Fremantle	20.4 (0.2)	6.8 (1.8)	0.61 (0.10)	-0.01^{NS}
Mandurah	21.4 (0.2)	7.3 (1.8)	0.61 (0.10)	0.00^{NS}

498 A_R: allelic richness (based on a sample size of 14 individuals); H: gene diversity. ^{NS}
499 designates no significant deviation from Hardy-Weinberg equilibrium.

500

Table 2. Pairwise G''_{ST} estimates (below diagonal) and *P*-values from tests of differentiation (above diagonal) between geographic locations. The adjusted significance level for multiple comparisons is 0.0014. Significant divergences are highlighted in bold text.

	Kalbarri	HA1	Dongara	Jurien Bay	N. Lancelin	Lancelin	Rottnest Is.	Fremantle	Mandurah
Kalbarri	–	**0.000**	0.005	0.157	0.065	0.120	0.845	0.176	0.308
HA1	**0.045**	–	0.128	0.137	0.252	0.205	0.002	0.022	0.099
Dongara	0.023	0.010	–	0.333	0.006	0.157	0.591	0.201	0.650
Jurien Bay	0.007	0.009	0.003	–	0.413	0.661	0.705	0.341	0.922
North Lancelin	0.017	0.007	0.037	0.002	–	0.431	0.025	0.352	0.251
Lancelin	0.009	0.006	0.008	–0.004	0.001	–	0.433	0.110	0.603
Rottnest Island	–0.005	0.026	–0.002	–0.003	0.023	0.001	–	0.278	0.789
Fremantle	0.009	0.026	0.009	0.004	0.005	0.015	0.005	–	0.888
Mandurah	0.004	0.015	–0.005	–0.013	0.008	–0.004	–0.007	–0.015	–

507 **Figure legends**

508

509 **Fig. 1** Locations where *P. cygnus* samples were collected.

510

511 **Fig. 2** Spatial autocorrelation analyses. (A) Correlogram plot of the genetic correlation
512 coefficient (*r*) as a function of distance. (B) Multiple distance class plot, showing the
513 influence of different distance class sizes on genetic correlation. Permuted 95% confidence
514 interval (dashed lines) and the bootstrap 95% confidence error bars are shown.

515

516 **Fig. 3** Plot of two-dimensional local spatial autocorrelation analyses. Symbols represent
517 geographical coordinates with significantly positive (red circles) or non-significant (crosses)
518 *lr* values. Calculations of *lr* were based on sampling the nearest 20 individuals. For clarity
519 significantly positive values were offset by –0.1° longitude.

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521

522 Fig. 1

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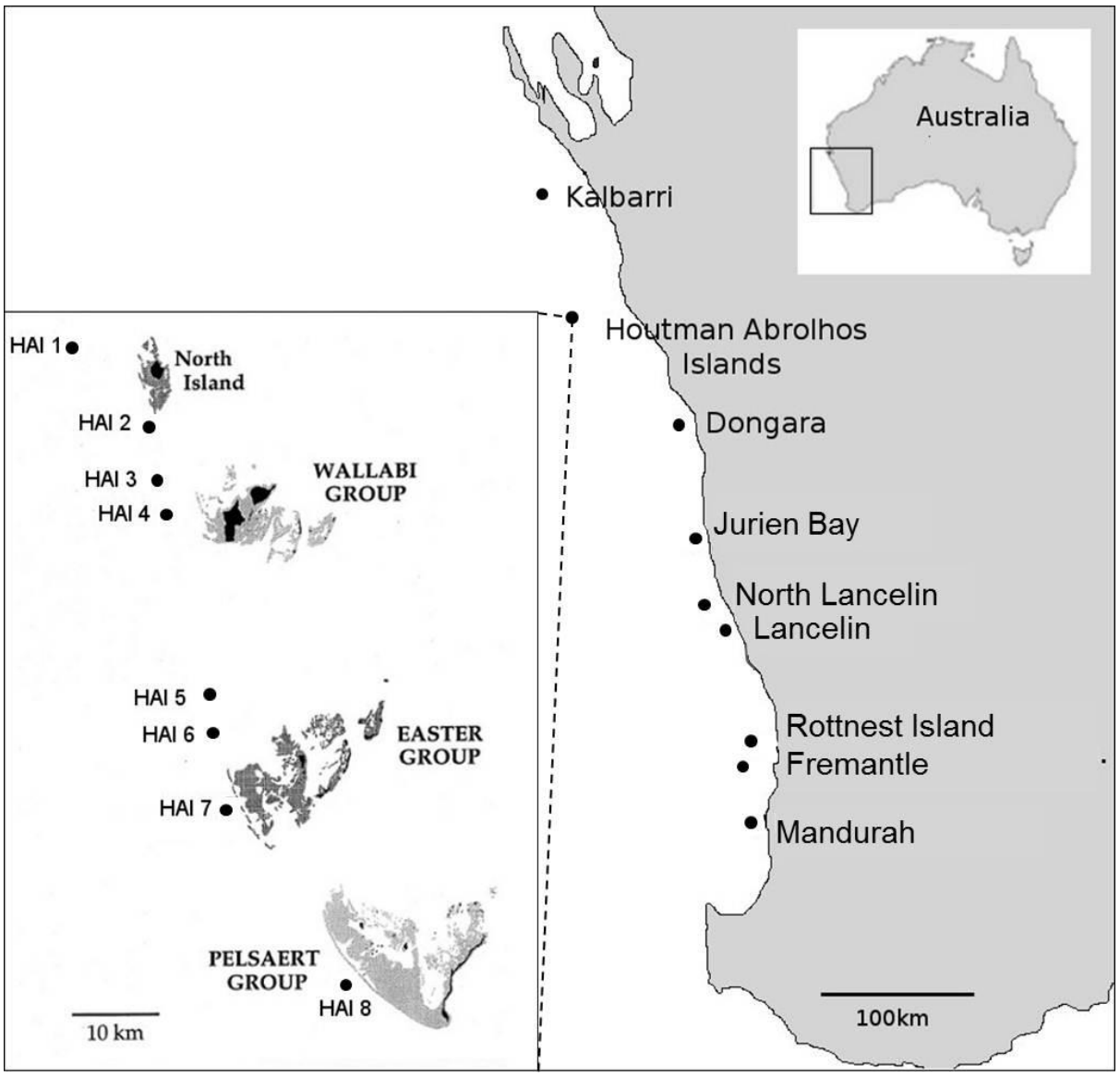

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528 Fig. 2

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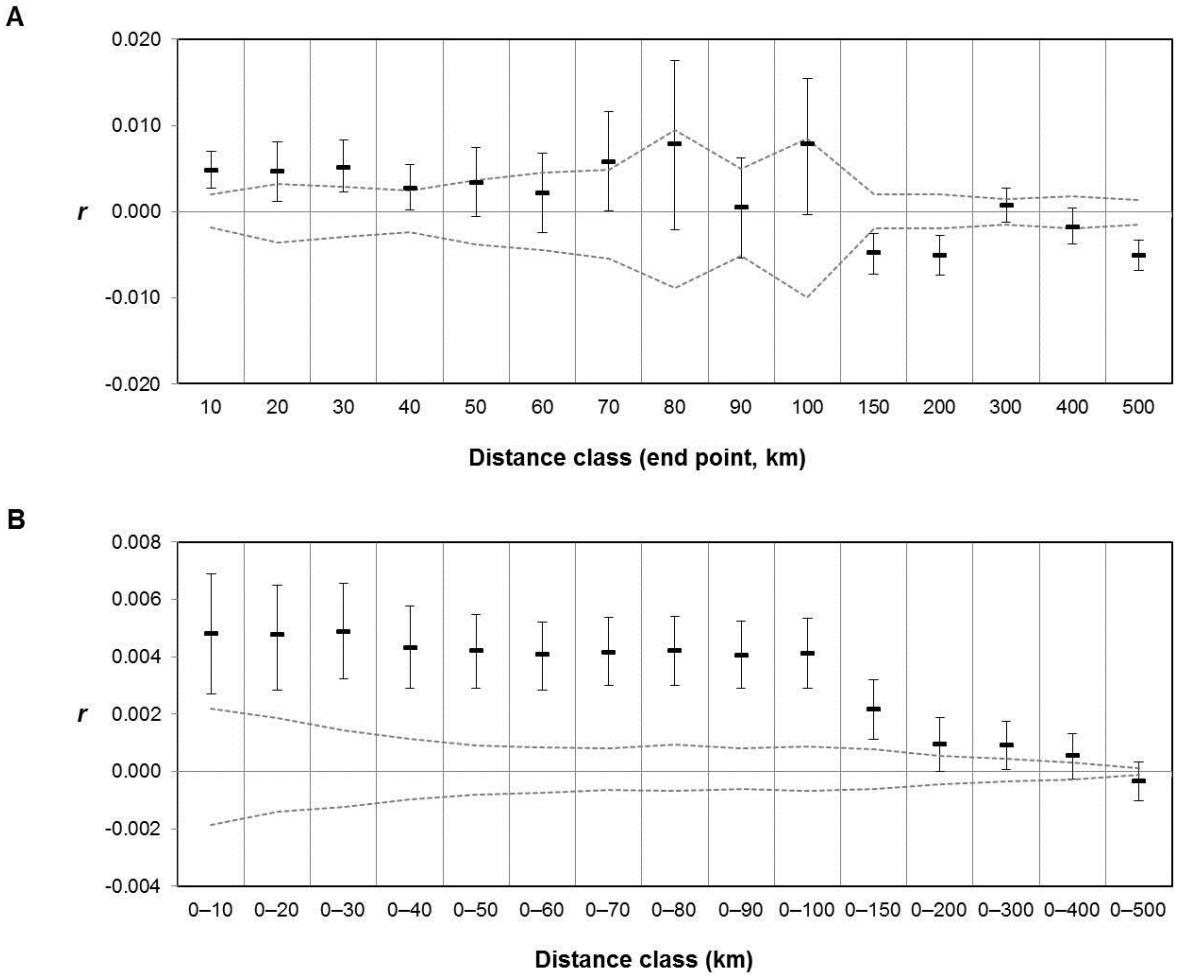

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534 Fig. 3

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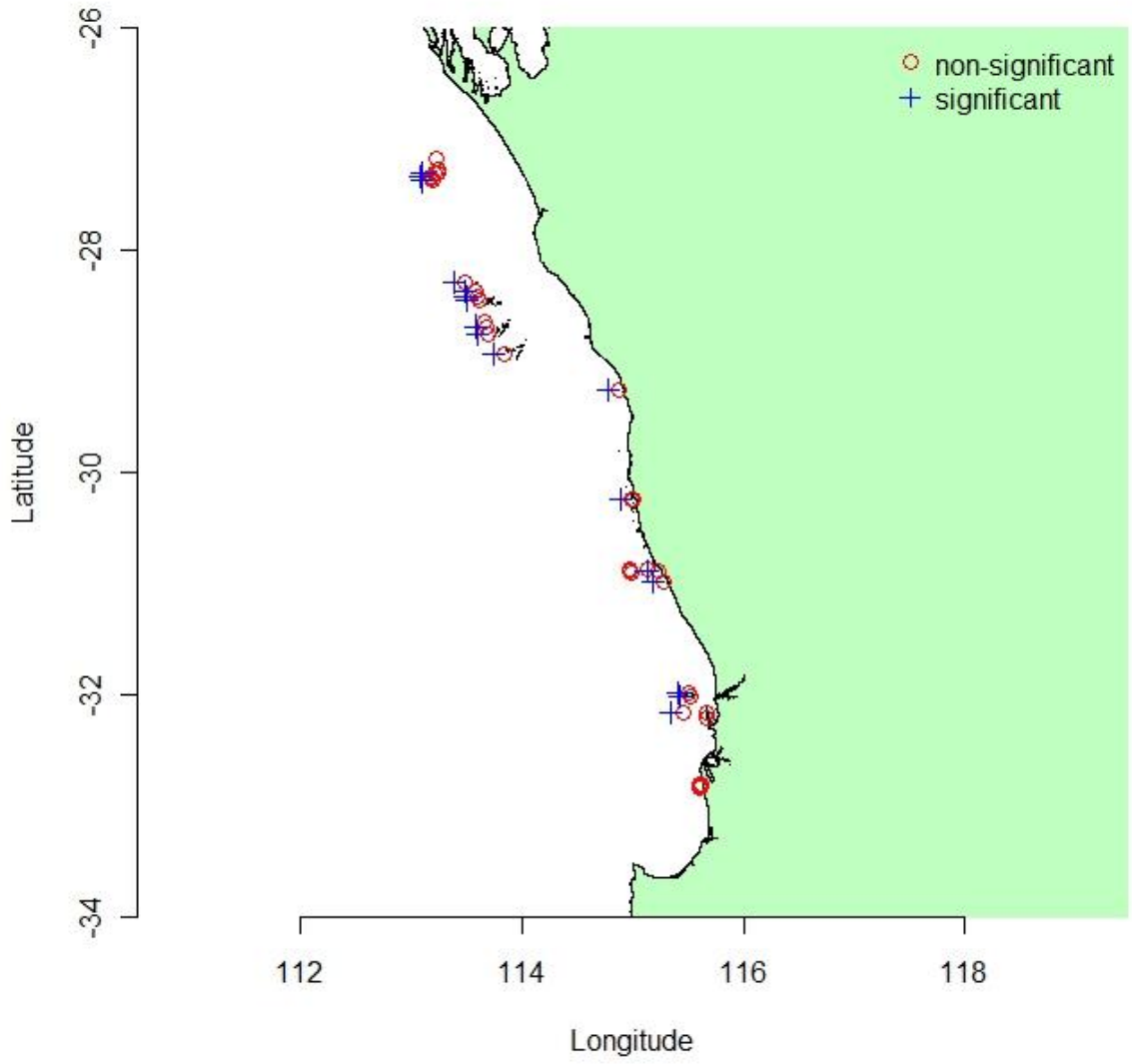

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