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Do lowland habitats represent barriers to dispersal for a rainforest mayfly, *Bungona narilla*, in south-east Queensland?

Alison J. McLean[A,B], *Daniel J. Schmidt*[A] *and Jane M. Hughes*[A]

[A]Australian Rivers Institute, Cooperative Research Centre for Freshwater Ecology, Griffith School of Environment, Griffith University, Nathan, Qld 4111, Australia.
[B]Corresponding author. Email: alison.mclean@griffith.edu.au

Abstract. Long-distance dispersal might be an important mechanism for the maintenance of aquatic insect populations in heterogeneous landscapes. However, these events can be difficult to measure by direct observation because the techniques can be time-consuming, expensive and technically difficult. When dispersal results in gene flow within and between populations, patterns of variation can be detected by genetic methods. The levels of population genetic structuring and the relationship between gene flow and geographical distance were assessed in the mayfly species *Bungona narilla* (Harker, 1957) in rainforest streams in south-east Queensland that are separated by lowland habitats. An analysis of molecular variance based on mitochondrial DNA data, using a fragment of the cytochrome oxidase I gene, revealed significant differentiation between regions, suggesting that maternal gene flow was restricted. A nested clade analysis revealed patterns of historical (contiguous) range expansions and recent restricted gene flow along with some long-distance dispersal events. Our analyses have shown that populations of *B. narilla* are significantly structured throughout the species range in south-east Queensland and that the low elevation habitats separating the northern and southern populations are restricting gene flow to some extent.

Additional keywords: gene flow, genetics, mitochondrial DNA, nested clade, population structure.

Introduction

Dispersal plays an important role in the genetic structuring of natural populations because the extent of gene flow between populations is the cohesive force that holds geographically separated populations into a single evolutionary unit – the species (Templeton 2006; Allendorf and Luikart 2007). When the amount of gene flow between local populations is high, gene flow has the effect of decreasing genetic variation between populations and increasing variation within populations. However, when gene flow is limited between populations, genetic differentiation occurs as a result of genetic drift, selection and mutation (Hedrick 2005; Templeton 2006).

The heterogeneous nature of landscapes can impose potential barriers for dispersal among freshwater species, resulting in populations becoming isolated. For species with an active and passive mode of dispersal (e.g. the mayfly *Bungona narilla*), gene flow occurs within streams via larval drift and within and between streams through adult flight. By comparing genetic differentiation within and among streams it is possible to infer the dominant mode of dispersal for a species (Schultheis *et al*. 2002; Smith *et al*. 2006). Genetic studies of stream insects in North America suggest that some dispersal occurs at small spatial scales, but at larger scales the physical nature of the environment becomes a barrier to dispersal, resulting in high levels of genetic structure at the largest spatial scale and little or no structuring at small spatial scales (Hughes *et al*. 1999; Myers *et al*. 2001; Schultheis *et al*. 2002; Finn *et al*. 2006).

In south-east Queensland, populations of stream insects display an unusual pattern of genetic differentiation in that the greatest genetic structuring of populations is often found at the smallest scale and genetic homogeneity among populations has been observed for species separated by as much as 50 km in different subcatchments/catchments (Trichoptera, Hughes *et al*. 1998; Schultheis and Hughes 2005; Ephemeroptera, Schmidt *et al*. 1995; Hughes *et al*. 2000, 2003*a*; Hemiptera, Bunn and Hughes 1997). This heterogenous pattern at the smallest scale has been explained by the 'patchy recruitment hypothesis' (PRH) (Bunn and Hughes 1997).

Initial genetic studies of stream insects in south-east Queensland used allozyme markers to provide evidence for the PRH (Schmidt *et al*. 1995; Bunn and Hughes 1997). Recently, mitochondrial DNA (mtDNA) was used in conjunction with allozymes to measure population structure in a stone-cased caddisfly (Schultheis and Hughes 2005) and similar results were recorded, that is, the greatest genetic differentiation was observed at the smallest spatial scale. Schultheis and Hughes (2005) found that some sites had many different haplotypes, which appeared contrary to the idea that the larvae were the offspring of only a few adults. However, when the adult lifespan of a species is relatively short (e.g. mayflies), it is possible that females are time-limited in finding suitable oviposition sites and

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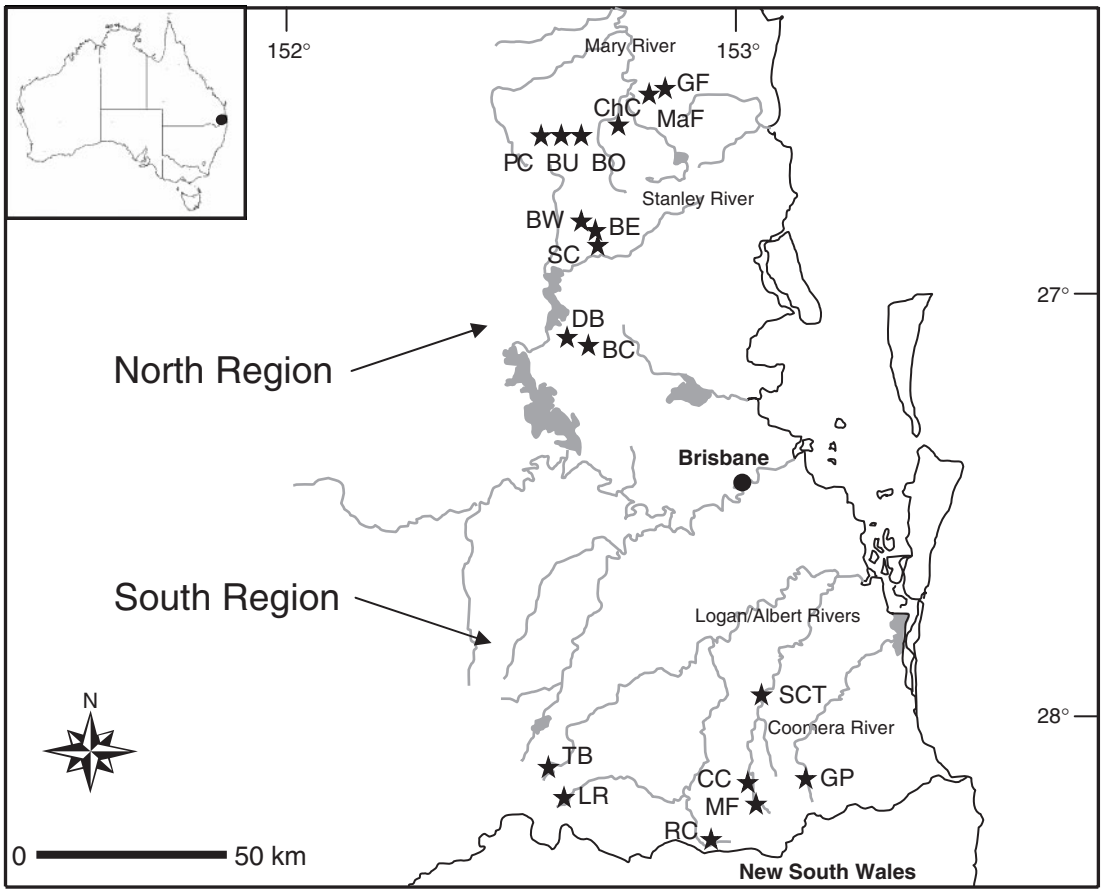

Fig. 1. Map showing the sampling sites for *Bungona narilla* (Baetidae : Ephemeroptera) in south-east Queensland.

this might explain why some sites contain offspring from only a few matings, whereas others are more diverse; thus, the overall significant result might have been the result of a limited number of families at some, but not all, sites (Hughes *et al*. 2003*a*; Petersen *et al*. 2004).

When the spatial scale of a study is increased, it is expected that these small-scale differences will even out as the number of represented adults increases when stream populations are pooled into subcatchments, catchments and regions (Schmidt *et al*. 1995). For example, in a study by Baker *et al*. (2003), no spatial genetic structuring of a hydropsychid caddisfly was found within or between catchments separated by up to 80 km and the authors were able to infer that adult dispersal was not limited in this species. Contrary to this result, previous broad-scale research on freshwater invertebrates in the current study area has identified restricted gene flow among mountain ranges as a consequence of isolation of rainforests separated by lowland areas (*Euastacus* spp., Parastacidae, Ponniah and Hughes 2004, 2006; *Tasimia palpata*, Tasimiidae, Murria and Hughes in press).

Many species of mayfly are known to fly distances of up to 50 km, preventing genetic differentiation among neighbouring populations (Gibbs *et al*. 1998; Smith and Collier 2001; Monaghan *et al*. 2002). The subject of the present study, *B. narilla*, is a locally common mayfly, restricted to rainforest streams in south-east Queensland. Previous studies on this species have shown that adult dispersal is not limited, although these conclusions are limited to small-scale patterns in the rainforest streams of the Conondale, Blackall and D'Aguilar Ranges (Schmidt *et al*. 1995; Bunn and Hughes 1997; Hughes *et al*. 2003*a*). In the present study, we assessed genetic differentiation at a regional level and included sampling sites that were separated by extensive lowland habitats.

As a result of the increase in the scale of the present study, we hypothesised that the lowland habitats separating the northern and southern populations of *B. narilla* would restrict the level of gene flow and the geographic dispersal ability of this species. Population genetic structure was examined using a fragment of the mtDNA cytochrome oxidase I gene. Using mtDNA, we were able to examine the phylogeographic structuring of *B. narilla* populations to determine whether there had been any geographic or historical barriers to gene flow.

Materials and methods

Collections

Samples of *Bungona* spp. nymphs were collected from May 2006 to February 2007 from small rainforest streams in the upper reaches of the Stanley, Logan, Albert and Coomera catchments of south-east Queensland (Fig. 1) (Table 1). *Bungona narilla* is a very small (<10 mm) and abundant baetid mayfly endemic to

Table 1. Description of the sites, coordinates and sample sizes for analysis

Site	Code	Catchment	*n*	Latitude	Longitude
Gherrula Falls	GF	Mary	10	26°36′44″	152°50′17″
Mapleton Falls	MaF	Mary	9	26°37′49″	152°50′25″
Chinaman Creek	ChC	Mary	20	26°26′38″	152°42′54″
Peters Creek	PC	Mary	4	26°41′22″	152°35′10″
Booloumba Creek	BO	Mary	10	26°42′11″	152°37′14″
Bundaroo Creek	BU	Mary	29	26°41′42″	152°36′44″
Dianas Bath	DB	Stanley	8	27°07′54″	152°41′08″
Byron Creek	BC	Stanley	25	27°08′40″	152°42′38″
Branch Creek East	BE	Stanley	8	26°51′28″	152°42′07″
Branch Creek West	BW	Stanley	40	26°51′37″	152°41′34″
Stony Creek	SC	Stanley	10	26°52′31″	152°44′05″
Logan River	LR	Logan	10	28°15′29″	152°44′39″
Running Creek	RC	Logan	3	28°19′19″	152°56′22″
Teviot Brook	TB	Logan	10	28°13′08″	152°31′05″
Cainbable Creek	CC	Albert	10	28°11′19″	153°07′10″
Moran Falls	MF	Albert	14	28°13′56″	153°07′30″
Sandy Creek	SCT	Albert	16	27°54′04″	153°10′01″
Gwongoorool Pool	GP	Coomera	17	28°11′56″	153°11′19″

Australia and it is distinguishable from other members of the Baetidae by an oblique line of long, fine setae on the basal one-third of the tibiae, with the setal length three-quarters that of the tibial length (Dean and Suter 1996). Like all Ephemerophtera, the adults are very short-lived (usually between 1 and 3 days) and during this time they do not feed (Sweeney *et al*. 1986).

All catchments sampled have their headwaters in National Parks and have remained largely untouched by intensive land-use practices, such as agriculture and grazing. In contrast, the lowland habitats separating the northern and southern region (Fig. 1) are a mosaic of hills, valleys, rivers and lakes, and many areas have been extensively cleared for housing and farming practices.

Sampling design

To test predictions about dispersal and gene flow a hierarchical design similar to that of Hughes *et al*. (1995) and Schmidt *et al*. (1995) was used. Two regions were selected (North and South) that were separated by extensive lowland habitat and within these regions small headwater streams were sampled. Genetic variation was analysed for a total of 279 samples, 135 of which were new samples collected for the present study. Additional data were obtained from Hughes *et al*. (2003*a*) and used to represent the northern populations of *B. narilla* for mtDNA analysis. Larvae were collected by sweeping a hand net along the surface of submerged rocks. The larvae were preserved in liquid nitrogen in the field and stored at −80°C on return to the laboratory.

Molecular techniques

A modified hexadecyltrimethylammonium bromide/phenol chloroform DNA extraction protocol (Doyle and Doyle 1987) was used to isolate total genomic DNA from a small portion (<1 mm) of the abdomen and cerci removed from each individual. A 710-bp fragment of the protein-coding gene *cytochrome oxidase 1* (*CO1*) was amplified in a polymerase chain reaction (PCR) using the universal primers LCO1490 and HCO2198 (Folmer *et al.* 1994). The PCR amplifications were carried out in 12.5-μL reaction volumes containing 0.4 μM of each primer, 0.2 mM dNTPs (Bioline, Alexandria, New South Wales, Australia), 2 mM $MgCl_2$ (Fisher), 1× polymerase reaction buffer (Fisher, www.fisherbiotec.com) and 0.3 units *Thermus aquaticus* (*Taq*) polymerase (Fisher). The samples were subjected to the following PCR protocol: an initial denaturation at 94°C for 5 min, followed by 15 cycles of 94°C denaturing for 30 s, 40°C annealing for 30 s and 72°C extension for 1 min. This was followed by 25 cycles of 94°C for 30 s, 55°C annealing for 30 s and 72°C extension for 1 min.

To check for successful amplification of the target fragment, 1% agarose gels (1.0% agarose, 0.5 μL ethidium bromide 100 mL^{-1}) were run. Prior to sequencing, purification of the amplified PCR product was carried out using EXO SAP (Fermentas, www.fermentas.com) in a reaction containing 5 μL of PCR product, 0.2 μL exonuclease I and 1 μL shrimp alkaline phosphate and subject to an incubation period of 37°C for 35 min followed by a heating process of 80°C for 20 min. Sequencing of the PCR product was done using the BigDye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 3031 × 1 automated sequencer.

Data analysis

Mitochondrial DNA sequences were aligned and edited using Sequencher Version 4.1 (Gene Codes Corporation 2000). The sequences obtained from Hughes *et al*. (2003*a*) were included and aligned with the new sequences, reducing the sequence lengths to 397 bp for analysis. Haplotype and nucleotide diversity estimates were calculated in Arlequin Version 3.1 (Excoffier and Schneider 2006). An analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992) was carried out in Arlequin 3.1 to investigate the genetic structure of all populations at three hierarchical levels. The hierarchical levels of analysis and their corresponding *F*-statistics used in the present study were 'among-regions-F_{CT}', 'among sites within

regions-F_{SC}' and 'among all sites-F_{ST}'. When a significant F_{ST} value was returned in the AMOVA at the 'among all sites' level (indicating that panmixia can be rejected because there is some differentiation between at least two of the sites), further pairwise F_{ST} statistics were calculated in Arlequin 3.1. Φ-statistics were also used because Φ_{ST} values include the divergence between haplotypes when calculating population differentiation (Excoffier *et al*. 1992).

Isolation by distance

To assess whether genetic distance was correlated with geographic distance (i.e. isolation by distance (IBD)), Mantel tests were carried out in Arlequin (Mantel 1967). This procedure estimates a non-parametric correlation between F_{ST} values and geographic distance (km) for all pairwise combinations of populations. Geographic distances were obtained from latitude and longitude coordinates using a web-based great circle distance calculator (Geocities 2007).

Nested clade analysis

Nested clade analysis (NCA) was used to infer the historical processes that might have influenced the geographic distribution of mtDNA genetic variation. The aligned sequence data were used to construct a haplotype network in TCS Version 1.21 (Clement *et al*. 2000). TCS uses statistical parsimony to connect haplotypes based on a 95% confidence limit and the resulting network displays the genealogical relationship between the haplotypes. This network was redrawn into a series of nested clades following the nesting rules of Templeton *et al*. (1995). GeoDis Version 2.5 (Posada *et al*. 2000) was used to calculate the NCA distance measures using a randomisation procedure (1000 permutations) to determine the statistical relationship between the haplotypes in the network and geography. Significantly large or small values were interpreted using the latest version of the GEODIS inference key (11 November 2005; Available online at http://darwin.uvigo.es/software/geodis.html).

Neutrality tests and mismatch distributions

To test the hypothesis of selective neutrality and population equilibrium, neutrality tests were carried out in Arlequin Version 3.1 (Excoffier and Schneider 2006). Two neutrality tests were carried out: Tajima's D test (Tajima 1989) and Fu's F_S test (Fu 1997). The basis of Tajima's D test is that under neutrality, the genetic diversity estimated from pairwise differences should be equal to the genetic diversity estimated from segregating sites. Population expansions can cause significant negative departures of Tajima's D from zero (ArisBrosou and Excoffier 1996; Tajima 1996). Fu's F_S test of selective neutrality is based on a comparison of the observed number of alleles in a sample with the number of alleles expected in a neutral sample of equivalent genetic diversity. A significantly negative F_S value indicates that more alleles are present in the sample than expected, and can indicate a sudden population expansion in the past (Fu 1997).

To estimate the timing of a demographic expansion, mismatch analysis was carried out in Arlequin Version 3.1. The mismatch distribution was tested with the statistic of 'raggedness (*r*, Harpending *et al*. 1993), which estimates the smoothness of the mismatch distribution, and has been found to distinguish between stationary (i.e. constant size, $r > 0.05$) and expanding (i.e. growing, $r < 0.05$) populations (Harpending *et al.* 1993; Harpending 1994). From the mismatch analysis, time since expansion can be calculated from Tau ($\tau = 2ut$), where $u = \mu k$ (μ = mutation rate, k = no. base pairs) and t is time since expansion in years. As Tau is obtained from the mismatch analysis, the formula is rearranged to find t ($t = \tau/2(\mu k)$).

Results

A total of 33 haplotypes were analysed (GenBank accession numbers EU789591–EU789623) of which 12 were new haplotypes identified in the present study based on polymorphisms at 36 nucleotide sites, with 13 singleton sites and 23 parsimony informative sites (Table 2). The haplotype (gene) diversity estimates were high and variable (ranging from 0 to 0.96, mean 0.907), reflecting the high number of haplotypes encountered at most sites (Table 2). Nucleotide diversity was reasonably low within all sites and one site had a nucleotide and haplotype diversity of zero because all eight individuals sampled had the same haplotype (haplotype 2). Of the 33 haplotypes, six were shared across regions, with one haplotype shared across 17 of the 18 sites.

The mtDNA data indicated significant genetic differentiation between regions ($F_{CT} = 0.11$; $\Phi_{CT} = 0.17$, $P < 0.001$), suggesting some restriction to gene flow between the northern and southern regions. There was also significant variation among sites within regions ($F_{SC} = 0.10$; $\Phi_{SC} = 0.12$, $P < 0.001$) and among all sites ($F_{ST} = 0.21$; $\Phi_{ST} = 0.274$, $P < 0.001$) (Table 3). Pairwise F_{ST} and Φ_{ST} comparisons between populations detected an array of significant differences between pairs of sites, irrespective of region. Mantel tests for IBD revealed that there was no evidence to indicate that genetic differentiation among populations (Φ_{ST}) increased with geographical distance (km) ($P = 0.23$).

The nested cladogram for *Bungona narilla* consisted of 17 one-step clades, six two-step clades, two three-step clades and the total cladogram (Fig. 2). The contingency analysis revealed significant spatial genetic structuring involving two one-step clades, one two-step clade, one three-step clade and the total cladogram (Table 4). The inference key suggested that the pattern of geographical variation within Clade 1-3 was consistent with restricted gene flow with some long-distance dispersal. Clades 2-4 and 3-1 were also consistent with restricted gene flow, but the variation resulted from IBD.

A contiguous range expansion was inferred for Clade 1-8 and this is evident from the geographic distribution of haplotype 12 away from the ancestral haplotype 33 (Table 4). A contiguous range expansion was also inferred for Clade 2-2. This was indicated by the interior clade's restricted range (Clade 1-6 had a significantly small D_c) and the fact that it was close to the geographic centre of the total cladogram (significantly small D_n). This, together with the apparently unrestricted range (non-significant D_c values) of the tip haplotypes, suggests that the geographic distribution of genetic variation within Clade 2-2 might be the product of a range expansion event.

At the total cladogram level, an inconclusive outcome resulted because neither clade was identified as tip or interior. To continue analysis, Clade 3-2 was designated as the tip clade

Table 2. Table of haplotype frequencies based on *cytochrome oxidase 1* sequence data
See Table 1 for a definition of the site abbreviations

Region:	South												North					
Site:	GP	MF	CC	SCT	RC	LR	TB	GF	MaF	ChC	PC	BO	BU	BE	SC	BW	DB	BC
Sample size:	17	14	10	16	3	10	10	10	9	20	4	10	29	8	10	40	8	24
Haplotype																		
1												1						
2	2		2	2	2	4	3	3	4	10	2	5	18	8	1	27	4	11
3																1	1	
4	1	2																
5		1														1		
6						1	1											
7								1	1							2		
8							1		1	1	1	1	1		1		1	5
9	3	9				1										1		
10			1	1														
11	6																	
12						1												
13	5							1			1	1	1			2		
14												1	5			3		
15				1														
16								2	2	7			1		3	1	1	4
17		2	2	10		2	2								1			1
18			1	2	1		3											
19			1															
20			1															
21			1															
22			1			1												
23													1					
24													1					
25													1					1
26																		1
27								1										
28																1		
29									1							1		
30								1										
31												1						
32										2							1	
33								1							4			1
Nucleotide diversity	0.005 ± 0.003	0.005 ± 0.003	0.011 ± 0.007	0.007 ± 0.005	0.012 ± 0.010	0.009 ± 0.006	0.010 ± 0.006	0.009 ± 0.006	0.009 ± 0.006	0.008 ± 0.005	0.005 ± 0.004	0.007 ± 0.004	0.005 ± 0.003	0.00	0.011 ± 0.007	0.005 ± 0.003	0.007 ± 0.005	0.007 ± 0.004
Haplotype diversity	0.787	0.584	0.956	0.608	0.667	0.844	0.844	0.911	0.806	0.647	0.833	0.778	0.599	0.00	0.800	0.544	0.786	0.743

Table 3. Mitochondrial DNA analysis of molecular variance results for populations of *Bungona narilla* showing *F*-statistics and Φ-statistics

Hierarchical level	Mitochondrial DNA			
	F-statistics	*P*	Φ-statistics	*P*
Among regions	0.11 (F_{CT})	<0.001	0.17 (Φ_{CT})	<0.001
Among sites within each region	0.10 (F_{SC})	<0.001	0.12 (Φ_{SC})	<0.001
Among all sites	0.21 (F_{ST})	<0.001	0.27 (Φ_{ST})	<0.001
Within individual regions				
North	0.06	<0.001	0.05 (Φ_{ST})	0.006
South	0.17	<0.001	0.23 (Φ_{ST})	<0.001

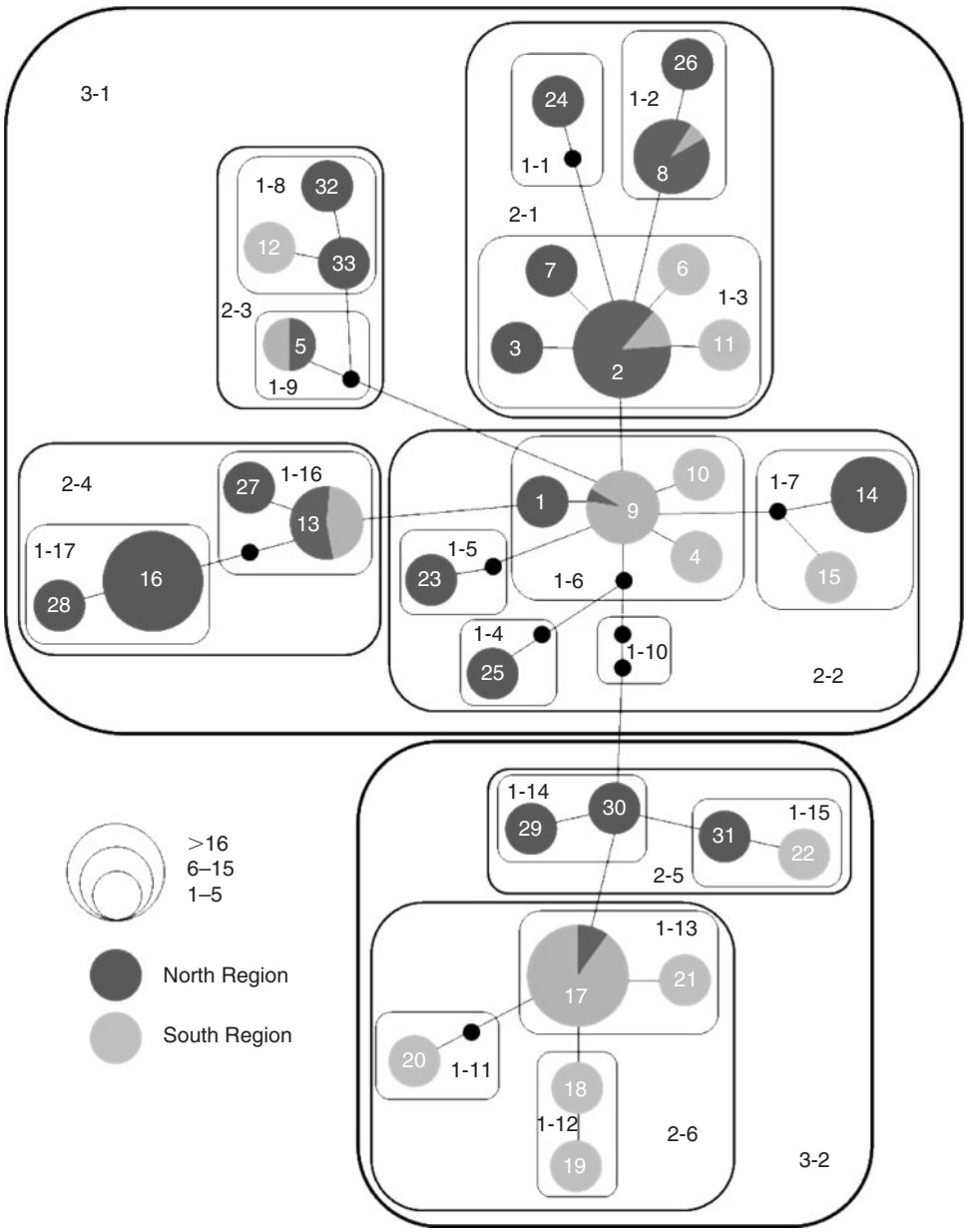

Fig. 2. The network produced by TCS showing the relationships between haplotypes. One-step clades are numbered 1-*n* (*n* = 1–17); two-step clades are numbered 2-*n* (*n* = 1–6); three-step clades are numbered 3-*n* (*n* = 1–2) and the total cladogram. Nesting was carried out according to Templeton *et al.* (1995).

Table 4. Results of the nested clade analysis on *Bungona narilla* populations and their biological inferences
Significantly large (L) or small (S) values for clade (D_c), nested clade (D_n) and interior-to-tip clade (I–T) distances are indicated in superscript. Clades with a nonsignificant association between genetic or geographic variation are excluded

Clade	Nested clades	Position	D_c	D_n	Inference
1-3	Hap2	I	100.22^S	101.14^L	1.2.3.5.6.7.Y
	Hap6	T	11.28^L	113.36^S	Restricted gene flow/ dispersal, but with some long-distance dispersal
	Hap7	T	9.38	70.25	
	Hap3	T	8.38	37.38	
	Hap11	T	0.00	104.59	
		I–T	94.73	14.70	
1-8	Hap33	I	10.75^S	21.84	1.2.11.12.N
	Hap12	T	0.00	138.77	Contiguous range expansion
	Hap32	T	26.77	25.98	
		I–T	−9.33	−32.34	
2-2	1-4	T	3.69^S	119.78^S	1.2.11.12.N
	1-5	T	0.00	122.45^L	Contiguous range expansion
	1-6	I	31.44	59.58^S	
	1-7	T	36.22	103.83	
		I–T	3.02	−48.14	
2-4	1-16	I	76.94^L	68.66^L	1.2.3.4.N
	1-17	T	25.64^S	33.30^S	Restricted gene flow with isolation by distance
		I–T	51.30^L	35.36^L	
3-1	2-1	T	93.79^S	92.89	1.2.3.4.N
	2-2	I	72.49	94.82	Restricted gene flow with isolation by distance
	2-3	T	47.83	60.27	
	2-4	T	41.10	67.29	
		I–T	−8.57	8.79	
Total cladogram	3-1	I	85.75^S	91.44	1.2.11.12.N
	3-2	T	92.18	117.05	Contiguous range expansion
		I–T	−6.42	−25.61	

Table 5. Tests of neutrality and the mismatch distribution results for Clades 3-1 and 3-2 for *Bungona narilla*
*$P < 0.05$; **$P < 0.01$; C.I., confidence interval; kya, thousand years ago

Demographic expansion model	Clade 3-1	Clade 3-2
Tau (τ) (95% C.I.)	4.7 (0.00–9.29)	1.6 (0.00–4.30)
Onset of expansion (95% C.I.)	490 kya (0–968)	167 kya (0–448)
Raggedness index	0.033	0.027
Neutrality tests		
Tajima's D	–1.426*	–1.285
Fu's F_S	–10.330**	–3.827*

owing to its lower genetic diversity, suggesting that it arose from Clade 3-1 (Crandall and Templeton 1993; Posada *et al*. 2000). The significantly small D_c for the interior suggests that Clade 3-1 has a restricted range and the inference indicated a contiguous range expansion from south to north.

From examination of the AMOVA analysis and the haplotype network, there was support for significant genetic differentiation between northern and southern regions and, therefore, mismatch distributions and tests of neutrality were analysed for Clades 3-1 and 3-2 separately (Fig. 2). Clade 3-1 showed an excess of haplotypes that were either rare or recently derived, indicated by significant negative values for Tajima's D and Fu's F_S, consistent with a population expansion (Table 5). Clade 3-2 displayed similar results for Fu's F_S, but was non-significant for Tajima's D ($P = 0.08$). Pairwise differences within each of the two three-step clade mismatch distributions appeared to fit a model of sudden demographic growth (Clade 3-1, $r = 0.033$; Clade 3-2, $r = 0.027$), further supporting a history of population expansion (Fig. 3).

Based on the pairwise sequence divergence rate of 2.3% for arthropods (Brower 1994) and a generation time of 12 months, tau was estimated separately for each clade and time since expansion was calculated to be ~490 000 years before present (ybp) for Clade 3-1 and 167 000 ybp for Clade 3-2. The 95% confidence intervals are very broad, both encompassing zero, but the upper limits for both are still well within the Pleistocene (Table 5).

Discussion

Population structure and dispersal: regional patterns

Selection, IBD and random drift might lead to considerable genetic heterogeneity among *Bungona narilla* populations occupying the northern and southern regions sampled in the present study. However, low pairwise Φ_{ST} values for a few sites in the northern and southern regions indicated that gene flow has been sufficient to prevent these forces from causing extensive differentiation of populations either side of the lowland area.

Sites in the southern region showed large and significant F_{ST} and Φ_{ST} values, indicating that gene flow was restricted in this area. Further investigation revealed that the sites along the Logan/Albert Rivers contributed significantly to the large

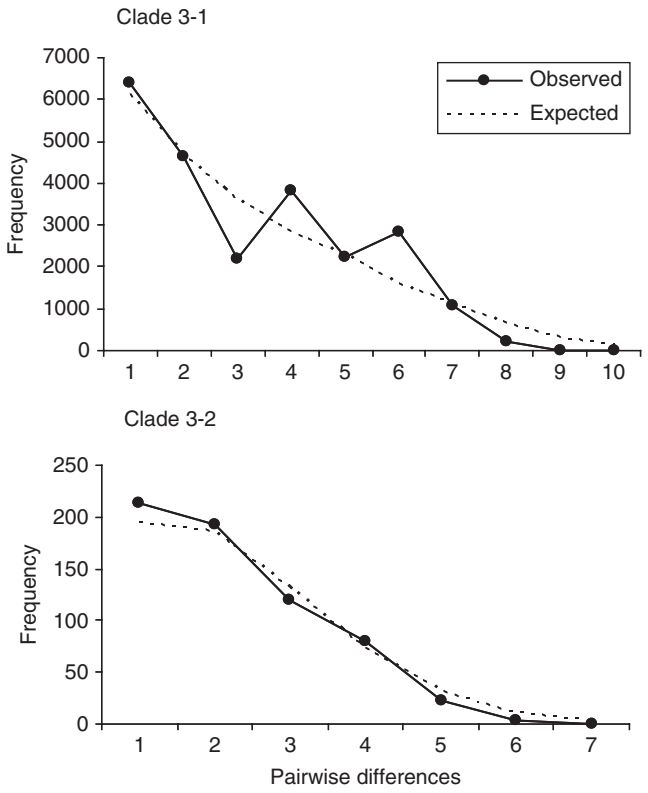

Fig. 3. Mismatch distributions for *Bungona narilla* for Clade 3-1 and Clade 3-2. Solid lines represent the observed distribution and dashed lines represent the expected distribution according to the sudden expansion model.

values observed. Pairwise differences based on haplotype frequencies alone (F_{ST}) were significant between one site (Moran Falls (MF)) and all other sites in the catchment ($n = 6$). However, when nucleotide differences were included, MF was only significantly different from all sites along the Albert River ($n = 3$; Fig. 1). One explanation for this might be that physical barriers exist to restrict larval movement from this site. The MF site is situated at the top of an 80-m waterfall that flows into the left branch of the Albert River ~3 km downstream. Studies of the movement of stream insects indicate a general trend for upstream dispersal by adults and downstream dispersal via larval drift (Müller 1982). However, the presence of waterfalls can restrict larval movement via drift and can result in an accumulation of individuals with distinct haplotype frequencies (Hughes *et al*. 2003*b*). This is concordant with the result for MF because there is a high frequency of one haplotype (haplotype 9), and also the presence of another haplotype (haplotype 4) that does not appear elsewhere in this catchment.

It is interesting to note that both haplotypes do occur in the neighbouring river (Gwongoorool Pool (GP), Coomera), suggesting that some adult dispersal might have taken place via the rainforest corridor that exists between these two river systems. However, when GP and MF were grouped together with Cainbable Creek (CC) into a hierarchical analysis of mountain ranges (Lamington National Park), significantly higher F_{ST} and Φ_{ST} values were observed (results not shown). The sequence divergence for Lamington National Park was the highest recorded from all the analyses, indicating that the molecular differences are largest for pairs of sites drawn from this region and that significant geographical subdivision exists within this national park. Alternatively, the results observed might be indicative of patchy recruitment because MF and GP had the lowest number of haplotypes in this catchment ($n = 4$, sample size 14 and $n = 5$, sample size 17 respectively). Therefore, patchy recruitment and/or physical barriers restricting gene flow might be responsible for the significant differentiation observed within this area.

Substructuring is evident for the northern populations of *B. narilla*, and one site (Stony Creek (SC)) was significantly different from all sites in the Stanley River ($n = 5$). No obvious barriers to dispersal occur between these sites, although fixed differences in mtDNA haplotypes have been observed for a freshwater crayfish (Ponniah and Hughes 2006) and a freshwater shrimp (Hurwood *et al*. 2003) in the same area. As the sites along the Stanley River are no more geographically isolated than the others in the study, the significant values observed might reflect the effects of patchy recruitment.

Previous small-scale patterns of genetic variation have shown similar results for *B. narilla* (Schmidt *et al*. 1995; Hughes *et al*. 2000, 2003*a*) and several species of caddisfly (Hughes *et al*. 1998; Schultheis and Hughes 2005) from streams in the same area, and according to Hughes *et al*. (2000), the asynchronous emergence of adults might limit the number of matings in a particular reach within a stream. In a study by Bunn and Hughes (1997), a stone-cased caddisfly displayed limited recruitment in reaches within a stream. Larval densities were recorded and it was estimated that between 3 and 12 females could account for the number of larvae observed. Although *B. narilla* larval densities were not recorded in the present study, it is possible that limited recruitment might be occurring because, on average, five haplotypes were observed at each site, and according to Williams and Williams (1993) only a few randomly flying females would be required to re-populate a section of stream.

In general, the results observed for *B. narilla* at local scales are comparable with studies of stream insect population structure that have suggested a strong differentiation of populations among sites (Schmidt *et al*. 1995; Bunn and Hughes 1997; Schultheis *et al*. 2002; Hughes *et al*. 2003*a*). Further sampling at the reach scale in the southern populations of *B. narilla* might help to resolve the differences observed among sites in the Albert River.

Evidence of historical gene flow between populations

The haplotype network of *B. narilla* revealed that a few haplotypes were shared between the regions (haplotypes 2, 8, 9, 13 and 17) and occurred in relatively high frequencies across sites, and this could be interpreted as gene flow. However, these haplotypes represent internal nodes of the haplotype network (Fig. 2) and are likely to be ancestral to the tip haplotypes, indicating that the observed pattern of haplotype sharing is possibly a result of historical gene flow with the retention of ancestral alleles (Templeton 1998).

Contrary to this suggestion, the network revealed one tip haplotype (5) that was shared across regional boundaries. As tip

haplotypes, by definition, are younger than the interior haplotypes to which they are connected, it is possible that the widespread distribution of haplotype 5 across the low elevation area is a result of recent dispersal.

When geographical distance between sampling sites was included in the analysis of the haplotype network, the null hypothesis of no geographical association was rejected for 6 of the 26 clades tested. From these results and from the results of the mismatch distribution and neutrality tests of *B. narilla* populations, the following scenario is suggested.

The oldest demographic event inferred in the NCA was identified as a contiguous expansion event. As Clade 3-1 was designated as the interior clade, the assumption is that the expansion originated within the southern region and moved into the northern region. At this time, *B. narilla* populations not only expanded in size, but also in geographical range. A significant Fu's F_S and mismatch distribution support an expansion hypothesis for Clade 3-1 and the estimated date of expansion was ~490 000 ybp (Table 5). If this time estimate is realistic, the expansion occurred during the late Pleistocene.

According to the NCA, another two contiguous expansion events occurred, indicating historical gene flow and secondary contact as populations expanded from refugial areas created during subsequent glacial periods. The fact that populations north and south share the two most common haplotypes might be a result of either secondary contact or sharing of ancestral haplotypes that were present before isolation of the two regions. Since the inferred expansion, there has apparently been restricted gene flow between populations north and south, although rare long-distance dispersal events might have occurred.

In summary, the AMOVA and NCA showed that populations of *B. narilla* were significantly structured throughout the species range in south-east Queensland and that the low elevation areas separating the north and south populations were restricting gene flow to some extent. Restricted gene flow was also observed among sites within each region and although there were sufficiently small amounts of contemporary gene flow, some substructuring was also evident. The small migration rate observed from the sharing of haplotypes across regions has been sufficient to prevent phylogeographic divergence of populations in this area of south-east Queensland.

The long-distance dispersal events that are inferred might be the result of two factors: the distribution of stepping-stone habitats capable of sustaining populations in areas between the two main rainforest habitats and the dispersal capabilities of *B. narilla*. Detailed investigations of *B. narilla* populations in the Conondale Ranges in south-east Queensland have found no evidence to suggest that adult dispersal is limited, and in fact adult flight is suggested to be an important dispersal mechanism for this species (Hughes *et al*. 2003*a*).

Effects of Pleistocene glaciations on B. narilla *populations*

Climate oscillations, particularly during the Pleistocene, have influenced the current distribution and population genetics of many plants and animals (Hewitt 2000; Burns *et al*. 2007; Toon *et al*. 2007). In Australia, complex patterns of habitat fragmentation have been caused by fluctuations in sea level, rainfall, humidity and temperature (Schauble and Moritz 2001), and dry corridors like the Burdekin Gap in northern Queensland are known to constitute a biogeographical barrier for several species (frogs, James and Moritz 2000; birds, Joseph and Moritz 1994; fish, Unmack 2001; Wong *et al*. 2004).

Regressions of populations because of changing climate can lead to genetic divergence of isolated refugial populations (Taberlet *et al*. 1998) and if restrictions to gene flow are maintained, as a result of geographic isolation or limited dispersal abilities, speciation can occur (Hewitt 2000). An example from south-east Queensland is that of the freshwater crayfish genus *Euastacus* (Ponniah and Hughes 2004, 2006), where taxa speciated during the Pliocene and remained on isolated mountains throughout the climatic fluctuations of the Pleistocene. Contrary to this, many taxa have undergone Pleistocene range expansions. During interglacial periods they were able to extend their ranges, and this can often be traced by detection of secondary contact and/or hybridisation between previously isolated lineages (Hewitt 2000; Pfenninger and Posada 2002).

In south-east Queensland, the lowland area separating the northern and southern regions in the present study has been found to constitute an ancient barrier to dispersal for another freshwater insect species (the caddisfly *Tasimia palpata*; Murria and Hughes in press) as a result of the contraction of rainforests during Pleistocene glaciations. *Tasimia palpata* is restricted to rainforest habitats similar to those of *B. narilla*; assuming that both species occupied small rainforest streams over evolutionary time, their distributions would have contracted during the periods of cyclic aridity that occurred in Australia during the Pleistocene. During the warmer interglacial periods, populations were able to disperse as rainforests expanded allowing secondary contact between once isolated populations. Our results are consistent with the findings of Murria and Hughes (in press) and suggest that the lowland area separating the northern and southern populations was an ancient barrier to dispersal for these rainforest specialists.

The responsiveness of *B. narilla* populations to past climatic conditions documented here highlights the possibility that future anthropogenic climate change could also induce local extinctions (Thomas *et al*. 2004) by reducing the already fragmented rainforest habitats of these populations even further. As we have shown, the lowland area separating the northern and southern populations of *B. narilla* in south-east Queensland acts as a barrier to dispersal and further habitat fragmentation will reduce the chances for recolonisation from nearby source populations.

To corroborate the conclusion that lowland habitat restricts the movement of *B. narilla* on a regional scale, additional sampling from areas west of the Conondale Ranges and north of Main Range is required. This might indicate whether suitable habitats are available in this region to sustain populations of *B. narilla* and potentially provide a stepping-stone connection for the distribution of the haplotypes observed. Sampling was attempted in these areas during the current study, but because of low rainfall before and during the period the upper reaches were in poor condition with little or no water.

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