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FINE-SCALE PHYLOGEOGRAPHIC CONGRUENCE DESPITE DEMOGRAPHIC INCONGRUENCE IN TWO LOW-MOBILITY SAPROXYLIC SPRINGTAILS

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Evolutionary trajectories of codistributed taxa with comparable ecological preferences and dispersal abilities may be similarly impacted by historical landscape-level processes. Species' responses to changes in a shared biogeographic landscape may be purely concerted, completely independent, or classified as falling within an intermediate part of the continuum bounded by these two extremes. With sufficient molecular data, temporal contrasts of congruence among taxa with respect to these responses can be made. Such contrasts provide insights into the relative influence of ancient versus more recent climatic (and other) impacts on genetic structuring. Using phylogenetic, allele frequency, and genotypic data from two low-mobility, rotting-log-adapted (saproxylic) springtail species (Collembola) from an isolated 100-km-long section of the Great Dividing Range in southeastern Australia, we tested the concerted-response hypothesis over three timescales. Tests of phylogeographic, demographic, and contemporary population-genetic congruence were performed using an integrative approach that draws on both direct (pattern-based) and indirect (scenario-based) analyses. Our data revealed a general pattern of broad-scale similarities in species' responses to the interaction between Pleistocene climatic cycles and landscape setting, overlaid with some species-specific differences on local geographic and more recent temporal scales. This general pattern of phylogeographic congruence was accompanied by evidence for contemporaneous demographic incongruence indicating that, even at relatively small spatial scales, biogeographic context can exert an overarching influence on genetic structuring.

KEY WORDS: **Comparative phylogeography, concerted-response hypothesis, historical demography, population structure, temporal contrasts.**

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Comparative phylogeographic studies are faced with the challenges of objectively assessing congruence among codistributed taxa, and tying common patterns to underlying processes (Bermingham and Moritz 1998; Avise 2000). Concordant phylogeographic structuring usually indicates similar responses to past climatic changes. However, even when species have evolved in a concerted fashion, some discordance is expected owing to stochasticity in the processes of gene coalescence and DNA sequence evolution. This added complexity has promoted scenario-based assessments of congruence, where the fit of empirical data against predictions generated by competing biogeographic hypotheses is quantified using one or several summary statistics, usually in a parametric hypothesis-testing framework (e.g., Carstens et al. 2005; see also Hickerson et al. 2006). These statistical phylogeographic approaches (sensu Knowles and Maddison 2002) can be considered an indirect means of assessing congruence given that a set of well-defined scenarios is necessary for determining whether there are commonalities in the evolutionary histories of two or more taxa. Analytical methods that account for inherent among-locus variance when inferring species' histories represent a paradigm shift from largely descriptive ad hoc explanations of observed phylogeographic patterns. These methods, however, are accompanied by new challenges such as obtaining reliable estimates of effective population size, locus-specific per-generation mutation rate, and/or population divergence times. In most cases, these parameters underpin tests of competing hypotheses (e.g., Knowles 2001; Masta and Maddison 2002; Carstens et al. 2004, 2005; DeChaine and Martin 2006).

Even relatively simple alternative scenarios may not predict sufficient differences in patterns of genetic variation to enable discrimination among them (Knowles 2004). Consequently, the same best-fit biogeographic model may be identified for multiple taxa despite subtle or not-so-subtle species-specific differences in response to landscape-level evolutionary processes. Statistical assessments of congruence that directly compare geographic patterns of genetic variation among taxa (e.g., Sullivan et al. 2000; Lapointe and Rissler 2005) do not rely on a priori identification of a small set of scenarios that presumably include the true history of each organism under study. Thus direct (pattern-based) approaches can be used to test further those inferences drawn from scenario-based analyses. These two conceptually distinct but complementary analytical frameworks are well suited to testing the concerted-response hypothesis (sensu Sullivan et al. 2000), which postulates that two or more codistributed species have evolved in a lock-step manner in response to past changes in the biogeographic landscape. In assuming that all genealogical discordance is due to stochasticity alone, the concerted-response hypothesis represents one extreme of a continuum. Nonetheless, the predictions of congruence from this hypothesis with respect to phylogeny, demography, and contemporary population structure lend themselves to quantitative statistical analyses. However, to date, indirect and direct approaches to comparative phylogeography have not been integrated fully.

Different, although interrelated, temporal components of species' evolutionary histories can be inferred by exploiting phylogeographic signal carried by multilocus genotypes, allelic frequencies, and phylogenetic information (Sunnucks 2000). Whereas genotype and allele frequencies can change over few generations in diploid sexual species, DNA sequence mutations accumulate and spread throughout a population relatively slowly. A combined three-tiered approach permits explicit assessments of congruence at various temporal depths, but has rarely been applied in phylogeographic studies (Garrick and Sunnucks 2006). Indeed, tests of the concerted-response hypothesis have typically focused on a single tier. Separating components of ancient, intermediate, and recent population history should provide a more complete understanding of the temporal and spatial scales over which organisms respond to changes in the biogeographic landscape.

In the present study, we integrate direct and indirect assessments of congruence to test the concerted-response hypothesis in two low-mobility springtail species, over three relative timescales. We conducted these analyses using phylogenetic, allelic, and genotypic data from multiple loci per species. Our study landscape system includes Tallaganda State Forest, Tallaganda National Park, Badja State Forest, and parts of Gourock National Park and Deua National Park (collectively "Tallaganda" herein), which are adjacent on the Gourock Range. This region is a physiographically isolated, topographically heterogeneous, linear section (100 km north-south by 3–17 km east-west) of the Great Dividing Range in southeastern Australia. Tallaganda was not glaciated during the Pleistocene (Bowler 1982) and has remained geologically stable for $\geq$ 50–70 million years, resulting in the preservation of ancient drainage systems (Frakes et al. 1987). Although eucalypt forest is currently continuous, five discrete catchment-based microgeographic regions that are arguably of considerable antiquity have been identified a priori (Harolds Cross Region, HCR; Eastern Slopes Region, ESR; Anembo Region, AR; Pikes Saddle Region, PSR; and Badja Region, BR; Fig. 1). In addition to water catchment divisions, delimitation of these regions is based on topography and likely paleoclimatic impacts on moist forest distributions (Garrick et al. 2004). As part of a community-level comparative population structure program centered at Tallaganda (Scott and Rowell 1991; Sunnucks and Wilson 1999; Barclay et al. 2000a,b; Sunnucks et al. 2000a; Sunnucks and Tait 2001; Reinhard and Rowell 2005; Beavis and Rowell 2006; Sunnucks et al. 2006; Woodman et al. 2006; Hodges et al. 2007), these five regions have served as the basis for generating phylogeographic hypotheses. The wet-adapted, ecologically specialized saproxylic invertebrates on which we focus (i.e., springtails, velvet-worms, terrestrial flatworms, and funnelweb spiders) typically have

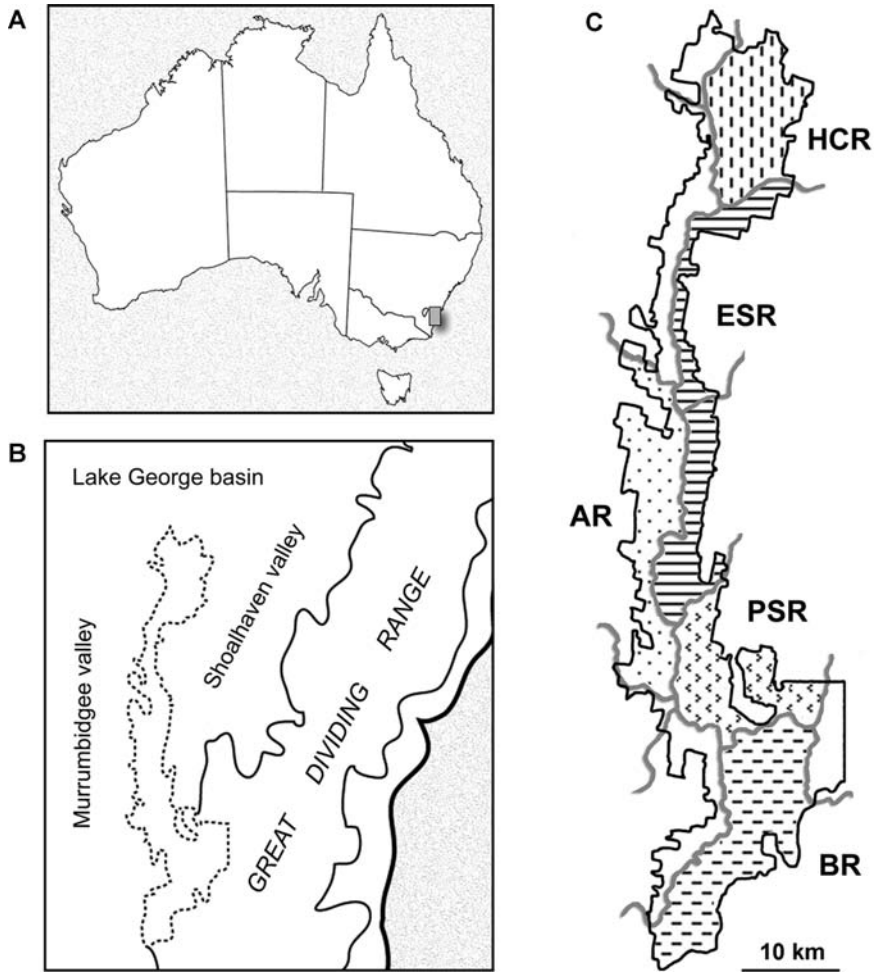

Figure 1. **(A) Map of Australia showing the location and (B) physiogeographic context of the study site. (C) Five catchment-based microgeographic regions of Tallaganda: HCR, Harolds Cross Region; ESR, Eastern Slopes Region; AR, Anembo Region; PSR, Pikes Saddle Region; and BR, Badja Region. Affiliations of areas in white were not explicitly determined in the original landscape model. Major and minor drainage divisions are represented by thick gray lines extending beyond the perimeter of the study area.**

limited dispersal abilities, in part owing to considerable desiccation susceptibility. They can potentially maintain viable populations in small forest refuges, making them well suited for recovering fine-scale phylogeographic signal of long-acting processes such as Pleistocene climatic cycles (Moritz et al. 2001; Hugall et al. 2002).

Qualitatively, spatial-genetic patterns even in some of the most distantly related saproxylic invertebrate species mentioned above show a marked similarity that is also consistent with catchment-based hypotheses. Here, we focus on the two springtail species for which we have generated multilocus datasets containing genotypic, allelic, and phylogenetic information. In this article we test the concerted-response hypothesis via statistical analyses of phylogeographic, demographic, and contemporary population-genetic congruence, using a novel, integrative scenario- and pattern-based framework (Table 1). Of the five a priori regions at Tallaganda, the Eastern Slopes Region is predicted to contain the most high-quality Pleistocene moist forest refuges. Furthermore, topography is expected to exert a strong influence (particularly through high-elevation catchment divisions) on phylogeographic structure in low-mobility saproxylic invertebrates (Garrick et al. 2004, 2007; Sunnucks et al. 2006). These two geographically explicit hypotheses are used to facilitate the scenario-based analyses of congruence employed here. Our study illustrates how the concerted-response hypothesis can be tested over serial temporal scales, both directly and indirectly.

Materials and Methods

TAXA AND SAMPLING

The two new species considered here, Pseudachorutinae Gen. nov. sp. nov. (Pseudachorutinae sp. herein) and *Acanthanura* sp. nov. (Subfamily Uchidanurinae; *Acanthanura* sp. herein), are clearly morphologically distinct from other described neanurid springtails, and appear to be restricted to Tallaganda. Both species

Table 1. Summary of analytical methods used to test the concerted-response hypothesis, the temporal scale addressed by each method, and associated null hypotheses and test statistics ("TS" = tree score). Microgeographic region abbreviations follow Fig. 1.

Analytical method	Temporal period	Null hypothesis		Test statistic	Program used
		Direct (pattern)	Indirect (scenario)		
Parametric bootstrapping	Ancient	Selected populations monophyletic	N/A	$TS_{(constrained-unconstrained)}$	PAUP*
Nested clade analysis	Short/intermediate	Geographic and temporal discordance	Different primary inferences	Qualitative	TCS and GEODIS
Migration matrix estimation	Short/intermediate	Equal migration	Zero migration, ESR or PSR not a major source	Likelihood-ratio	MIGRATE
Boundary overlap	Contemporary	Random association of contact zones	Contact zones not influenced by ridgelines	O_1 or O_{12}	BOUNDARYSEER

prefer cool, humid microclimates inside large-diameter rotting *Eucalyptus* logs, and are poor dispersers due to susceptibility to rapid water loss (Greenslade 1991). Tallaganda's five a priori microgeographic regions (Fig. 1) were represented in the geographical sampling of both species, with 380 Pseudachorutinae sp. and 206 *Acanthanura* sp. collected from 87 and 72 rotting logs, respectively.

DATASETS

Herein, the prefix "*Pseud-*" or "*Acanth-*" denotes Pseudachorutinae sp. or *Acanthanura* sp. loci, respectively (the notation "Sm" or "Uc" used by Garrick et al. 2004, 2007, and Garrick and Sunnucks 2006 has also been retained). The Pseudachorutinae sp. DNA sequence dataset comprised fragments from three genes: mitochondrial cytochrome oxidase I (mt*COI*, 522-bp), *Elongation factor-1*α intron (*EF-1*α, 232-bp), and anonymous locus *Pseud-Sm2* (189-bp). Phase of segregating sites at codominant nuclear DNA (nDNA) loci was determined by isolating alleles from diploid PCR products via single-stranded conformation polymorphism, followed by direct sequencing (Sunnucks et al. 2000b; Garrick and Sunnucks 2006). Four additional nuclear loci (*Pseud-Sm4*, *Pseud-Sm6*, *Pseud-Sm8*, and *Pseud-Sm150*) were genotyped via insertion/deletion (indel) mutations or restriction fragment length polymorphisms. The *Acanthanura* sp. dataset of Garrick et al. (2007) was included in this analysis. The DNA sequence component comprised 570-bp of the same mt*COI* region above, *EF-1*α (266-bp) and two anonymous nuclear loci *Acanth-Uc3* (150-bp) and *Acanth-Uc180* (96-bp). This dataset also consisted of three indel or restriction fragment length polymorphism nuclear markers (*Adenine Nucleotide Transporter* intron, *ANT*; *wingless*, *Wnt*; and anonymous locus *Acanth-Uc44*). Amplification and single-stranded conformation polymorphism screening of mt*COI* followed Garrick et al. (2004). Nuclear marker development and genotyping procedures, including GenBank accessions, are given in Garrick and Sunnucks (2006). Nuclear loci appeared to be noncoding (except *Acanth-UcWnt* for which only silent polymorphisms were resolved), and sequence markers showed no evidence of selection, and little or no evidence of recombination (Garrick and Sunnucks 2006).

CONTEMPORARY POPULATION STRUCTURE AND BOUNDARY OVERLAP ANALYSIS

As a necessary precursor to subsequent analyses, biologically meaningful genetic units were inferred from genotypic data. For each species, the number of populations (K) and membership of individuals in each population were estimated via Bayesian clustering of from six nuclear loci using STRUCTURE version 2.1 (Pritchard et al. 2000). Likelihood estimates were obtained for $K = 1$ to 8 strata under "correlated allele frequency" and "admixture ancestry" models. Five replicate runs per dataset were

performed with 10^5 and 10^6 MCMC generations for burn-in and run length, respectively. The smallest K that captured the major structure in the data and identified geographically cohesive populations (e.g., individual membership coefficients $Q \geq 0.90$) was accepted. Where subtle within-group substructure was evident from mt*COI*, clusters were subdivided further (as in Garrick and Sunnucks 2006; Garrick et al. 2007). A potential bias in cluster identification is introduced if individuals share alleles due to kin structure rather than population-level processes. To avoid sampling kin-groups, we conservatively assumed kin structure existed when a log contained $\geq$ 10 individuals with the same mt*COI* haplotype and their nuclear multilocus genotypes were consistent with expectations for full siblings such that these individuals could have potentially come from a single female. Only two of nine Pseudachorutinae sp. candidate logs, but no *Acanthanura* sp. logs, fulfilled these criteria. For putatively identified kin-group logs, we used only one randomly selected individual for subsequent analyses.

Spatially explicit comparative analyses of contemporary population structure are an important component of our assessment of support for the concerted-response hypothesis at the most recent temporal scale. Boundary overlap statistics (Jacquez 1995) were used to test cooccurrence of the two species' population-genetic contact zones (pattern-based, direct congruence), and to address the hypothesis that longstanding microgeographic regional divisions maintain intraspecific genetic discontinuities (scenario-based, indirect congruence). In the latter case, directional overlap of species-specific contact zones with catchment boundaries that define Tallaganda's five a priori regions was tested. Only east-west oriented catchment boundaries were considered owing to the linear north-south arrangement of springtail population ranges (see Results).

The following description of boundary overlap analysis uses terminology that is more extensively defined in online Supplementary Table S1. To detect difference boundaries (zones of rapid change in the value of a variable, such as local allele frequencies) we used categorical wombling (Oden et al. 1993), implemented in BOUNDARYSEER version 1.2.0 (TerraSeer Inc., Ann Arbor, MI). Each rotting log was assigned to a population based on STRUCTURE Q-values of resident individuals using a majority-rule approach, and to its associated catchment based on geographic location. Rotting logs on ridgelines were randomly assigned to a proximate catchment. Adjacent logs were linked using Delaunay triangulation, and for each linkage, the spatial rate of change in the value of the categorical variable under consideration, such as genetic population membership (measured via a Boundary Likelihood Value) was calculated. Each Boundary Likelihood Value is associated with a candidate Boundary Element (line segments drawn perpendicular to Delaunay linkages, equidistant from the two linked geographic sampling locations). Natural breaks in the frequency distribution of Boundary Likelihood Values were used to determine threshold values. Candidate Boundary Elements associated with above-threshold Boundary Likelihood Values were elevated in status to Boundary Elements, then adjacent Boundary Elements were connected to form spatially contiguous population or catchment boundary sets.

Boundary overlap analysis was used to test the hypothesis of spatially congruent population genetic contact zones in the two species (cooccurrence), and the hypothesis of catchment-driven locations of contact zones in Pseudachorutinae sp. and *Acanthanura* sp. (directional overlap, assessed one species at a time). In all cases, the null hypothesis of no boundary associations (which includes cooccurrence and directional overlap) was tested using the Complete Spatial Randomness permutation procedure. This includes the following steps: (1) calculation of an overlap statistic (see below) from the empirical data, (2) randomizations of the empirical observations among spatial locations and the construction of new boundary sets, (3) repeated recalculation of the chosen overlap statistic to generate a frequency distribution, and (4) the assessment of significance of the empirical value obtained in step 1. When testing whether two boundary sets (i.e., contact zone vs. contact zone, or catchment ridgeline vs. contact zone) overlap more than expected by chance, one of two null spatial models was used, depending on the nature of the null hypothesis. For tests using the boundary cooccurrence spatial model, population boundary sets for both species were randomized. In these cases, the overlap statistic O_{12} (mean geographic distance from a Boundary Element in either boundary set to the nearest Boundary Element in the other boundary set) was calculated. Under the scenario in which catchment boundaries influence locations of genetic contact zones (i.e., directional overlap), only the population boundary set was randomized. For this spatial model the statistic O_1 (mean geographic distance from a Boundary Element within boundary set 1 to the nearest Boundary Element within boundary set 2) was used. Null distributions of O_{12} and O_1 were generated via 10,000 Monte Carlo randomizations of the empirical data, and significance was assessed at the lower 0.05 level.

GENETIC ISOLATION, SOURCE-SINK POPULATION RELATIONSHIPS AND DEMOGRAPHIC CONGRUENCE

In this analysis we focused on one major component of "demography"—historical gene flow/migration. For each species, migration matrices for geographic populations identified by STRUCTURE were estimated using MIGRATE version 2.1.3 (Beerli and Felsenstein 2001). Matrices were compared indirectly via a set of alternative historical scenarios, and directly via relative migration parameter values themselves, using likelihood-ratio tests. This series of tests focuses on short-to-intermediate temporal scales in the history of the two springtails, given that DNA sequence plus frequency information was exploited.

We tested three alternative demographic hypotheses, including one null hypothesis specifically designed to test our prior expectation that parts of the ESR repeatedly served as the most important Pleistocene forest refuge. The three null demographic scenarios were : (1) Complete Genetic Isolation (CGI), representing a history where all a priori microgeographic regions retained refuges, and drift-induced reproductive incompatibilities and/or longstanding impermeable topographic barriers prevented subsequent gene flow; (2) Transient ESR Refuges ($T_{ESR}R$), whereby ESR was not a major refugial source of migrants to neighboring regions following recurrent cycles of Pleistocene climatic amelioration (cf. prior expectations, see Introduction); and (3) Transient PSR Refuges ($T_{PSR}R$), which postulates an absence of major refuges in PSR. Here, $T_{ESR}R$ represents a null model for testing the proposed importance of ESR in harboring saproxylic invertebrate diversity during periglaciations of Tallaganda (Garrick et al. 2004). Conversely, $T_{PSR}R$ serves as an alternative scenario that, although not predicted a priori, is biologically plausible based on the current distribution of tall wet forests throughout PSR (State Forests of New South Wales 1995). Under these models, null expectations for M (migration rate per generation divided by μ) are zero for all population pairs (CGI), or symmetrical M between the focal population and its nearest neighbors ($T_{ESR}R$ and $T_{PSR}R$). In the latter two models, expectations for M reflect no significant source–sink relationships that would otherwise be evident if a particular population had served as a major source of postglacial recolonization of adjacent uninhabited microgeographic regions.

To test demographic congruence directly, the null hypothesis of Equal Relative Migration (ERM) was assessed by using the migration matrices estimated for each species to parameterize that of the other. To define the constraint model, partitioning of maximum-likelihood (ML) point estimates of $M_{observed}$ between Pseudachorutinae sp. population pairs determined values of $M_{constraint}$ between corresponding populations in the *Acanthanura* sp. matrix. Scaling of absolute values of each $M_{constraint}$ was performed by repartitioning the sum of all $M_{observed}$ from the latter matrix. Likelihood-ratio tests determined whether the constraint model was significantly worse than the unconstrained model for *Acanthanura* sp. (a reciprocal test was also performed). For all tests, significance was assessed at the 0.05 level.

The computational burden of full matrix estimation was reduced by implementing a one-dimensional stepping stone migration model, and by pruning datasets. Where necessary, mt*COI* datasets were reduced to 40 randomly selected individuals per population. Nuclear datasets were then drawn from those individuals by randomly selecting one allele per diploid genotype. We discarded any sample with recombinant *Pseud-SmEF-1*α alleles (see Garrick and Sunnucks 2006). MIGRATE search settings were: 10 short MCMC chains (30,000 steps), two long chains (300,000 steps) recording every 100th genealogy, 30,000-genealogy burn-in per chain, adaptive heating (temperatures 0.0, 1.0, 1.2, 1.5, and 3.0), UPGMA starting trees, empirical base frequencies and transition/transversion ratio of 2.0. Initial values for Θ and M were set using F_{ST}. Mt*COI* and multilocus nDNA sequence datasets were analyzed separately, five times each. For the direct assessment of demographic congruence (ERM), likelihood-ratio tests were performed on paired summary files from comparable loci.

PHYLOGEOGRAPHIC CONGRUENCE WITHIN A NESTED CLADE ANALYSIS FRAMEWORK

When working with genetic datasets from continuously distributed species, assumptions that some model-based analytical approaches make about the existence of crisp, clearly demarcated populations can be restrictive (Garrick et al., in press). Because nested clade analysis (NCA, Templeton et al. 1995) treats individuals as the units of interest, its application in the present study provides an opportunity to compare different, but complementary, quantitative and qualitative methods for inferring population processes and historical events that operated over short-to-intermediate timescales. Accordingly, we analyzed each DNA sequence locus one at a time using NCA, and then integrated inferences across loci as a means of accommodating coalescent stochasticity.

Statistical parsimony cladograms (Templeton et al. 1992) were estimated in TCS version 1.21 (Clement et al. 2000) with the 95% confidence criterion enforced. Contiguous alignment gaps in nuclear genes were treated as a single-event fifth character, and ambiguous connections were resolved following Pfenninger and Posada (2002). Hierarchical nesting categories were assigned manually (recombinant *Pseud-SmEF-1*α alleles were omitted as in MIGRATE analysis). Contingency χ^2 tests of phylogeographic structure, and NCA statistics, were calculated in GEODIS version 2.5 (Posada et al. 2000) with 10,000 permutations. Biological interpretations were made following Templeton's (2004) inference key (updated November 2005, http://darwin.uvigo.es/software/geodis.html). Inferences were integrated across loci using three criteria: concordance of primary inferences, geographical concordance, and no contradiction of the temporal sequence of events / processes indicated by interior-tip contrasts. This approach employs the principal of parsimony by inferring the minimum number of events or processes and temporal "phases" in a species' history. An extension of these criteria was used to integrate inferences across taxa, permitting a qualitative assessment of phylogeographic congruence.

PARAMETRIC BOOTSTRAP TESTS OF PHYLOGEOGRAPHIC CONGRUENCE

We hypothesized that Pseudachorutinae sp. and *Acanthanura* sp. would have concordant phylogeographic patterns owing to shared responses to past changes in the distribution of moist forest

habitats at Tallaganda. At the oldest temporal scales over which our data are informative, a direct, pattern-based assessment of the concerted-response hypothesis was performed by comparing topologies of species' mt*COI* gene trees. Specifically, we tested whether the null hypothesis of phylogeographic congruence could be rejected after accounting for phylogenetic uncertainty. For each dataset, the best-fit model of sequence evolution was selected via AIC in MODELTEST version 3.06 (Posada and Crandall 1998). The optimal ML phylogeny was estimated in PAUP* version 4.0b10 (Swofford 2002) using successive approximations (Swofford et al. 1996). Outgroups were Pseudachorutinae sp. 2 from Victoria, or *Acanthanura* sp. 2 from New South Wales (GenBank accessions EF057733 and DQ518749, respectively). Initial model parameter estimates were generated from heuristic maximum parsimony tree searches (100 random addition sequence replicates, TBR branch-swapping). This was followed by iterative ML searches (heuristic search, 1 random addition sequence, TBR) performed until $-\ln L$ tree score, tree topology, and model parameters converged. Node support was assessed via 1000 ML bootstrap replicates using the same search settings (except NNI branch-swapping for *Acanthanura* sp.), with parameters set from the optimal tree.

Geographically localized monophyletic clades evident for only one species were identified a posteriori, then the null hypothesis of monophyly was tested in the other species using parametric bootstrapping (Huelsenbeck et al. 1996). The topological constraint(s) included only haplotypes restricted to a given geographic population, as defined by STRUCTURE (we also excluded inferred migrants, see Results). For each test, the best ML phylogeny with the constraint(s) enforced was estimated via successive approximations, then 100 DNA sequence datasets were generated using MESQUITE version 1.06 (Maddison and Maddison 2005). The best unconstrained tree and the best tree with the constraint(s) enforced were estimated for each simulated dataset using ML (search settings as above) with model parameters fixed at "true" values. The difference in $-\ln L$ tree score ($TS_{constrained} - TS_{unconstrained}$) formed the null distribution, and the same difference calculated from the empirical data formed the test statistic. Significance of departures from monophyly of the focal clade(s) was assessed at the upper 0.05 level.

Results

CONTEMPORARY POPULATION STRUCTURE AND BOUNDARY OVERLAP ANALYSIS

Contemporary population structures of the two springtail species show marked similarities. STRUCTURE analysis confirmed that microgeographic regions HCR, ESR, PSR, and BR harbor genetically distinct populations (AR is distinct in *Acanthanura* sp. only). In both species, the majority of individuals were strongly assigned ($Q \geq 0.90$) to a single cluster. For comparative purposes, we focused on populations common to both species. Despite close proximities of corresponding population boundaries (Fig. 2), no significant cooccurrence was detected, irrespective of whether contact

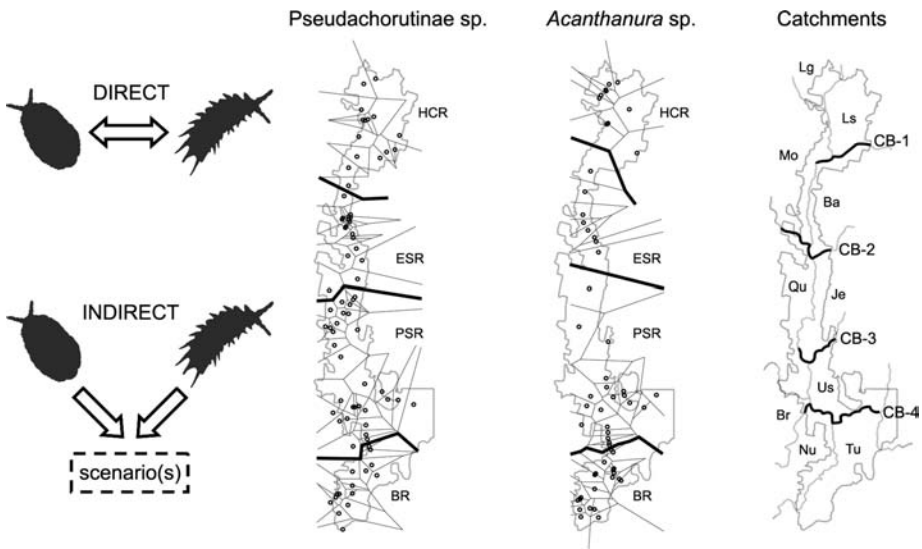

Figure 2. Boundary overlap analysis of congruence using direct (pattern-based) and indirect (scenario-based) approaches. Geographic locations of population contact zones were inferred via categorical wombling, based on genetic clusters identified using STRUCTURE (abbreviations follow Fig. 1). Small circles represent sampling localities, thin lines are candidate Boundary Elements, and thick lines are boundary sets comprised of adjacent Boundary Elements. Catchment boundaries hypothesized to be contact zone-generating landscape features (thick lines) are labeled CB-1 to CB-4. Gray lines extending beyond the perimeter of the study area are major and minor drainage divisions: Lg, Lake George; Ls, Lower Shoalhaven; Mo, Molonglo; Ba, Ballalaba; Qu, Queanbeyan; Je, Jerrabatgulla; Us, Upper Shoalhaven; Br, Bredbow; Nu, Numeralla; Tu, Tuross.

Table 2. Boundary overlap analysis of population contact zones and hypothesized topographic barriers. Geographic population abbreviations for Pseudachorutinae sp. (Pseud) and *Acanthanura* sp. (Acanth) follow Figure 1, and "/" represents the associated contact zone. Spatial locations of contact zones and east-west oriented catchment boundaries (CB-1 to CB-4) are shown in Figure 2. "All" denotes joint analysis of overlap among all population and/or catchment boundaries, for the comparison of interest. *P*-value represents significance of the test statistic at the lower 0.05 level (ns = not significant).

Hypothesis (test statistic)	Boundary set		*P*-value
	Boundary 1	Boundary 2	
Cooccurrence (O_{12})	Pseud HCR/ESR	Acanth HCR/ESR	ns
	Pseud ESR/PSR	Acanth ESR/PSR	ns
	Pseud PSR/BR	Acanth PSR/BR	ns
	Pseud All	Acanth All	ns
Directional overlap (O_1)	Pseud HCR/ESR	CB-1	ns
	Pseud ESR/PSR	CB-2	<0.001
	Pseud PSR/BR	CB-4	ns
	Pseud All	CB-All	ns
Directional overlap (O_1)	Acanth HCR/ESR	CB-1	<0.001
	Acanth ESR/PSR	CB-2	0.005
	Acanth PSR/BR	CB-4	<0.001
	Acanth All	CB-All	<0.001

zones were analyzed individually or jointly (Table 2). For the scenario-based tests, four putative contact zone-generating catchment boundaries were identified (CB-1 to CB-4, Fig. 2). All *Acanthanura* sp. contact zones overlapped significantly with proximate catchment boundaries, but for Pseudachorutinae sp., only the ESR / PSR contact zone versus CB-2 comparison showed significant overlap. Joint analysis of all catchment and population boundaries per species also indicated that high-elevation ridgelines are more permeable to gene flow in Pseudachorutinae sp. than in *Acanthanura* sp. (Table 2).

GENETIC ISOLATION, SOURCE–SINK POPULATION RELATIONSHIPS AND DEMOGRAPHIC CONGRUENCE

MIGRATE produced consistent parameter estimates across replicates from mt*COI*, but not for multilocus nuclear sequence datasets. To assess support for alternative demographic scenarios from migration matrices estimated from nuclear genes, we employed a majority-rule approach (see online Supplementary Tables S2 and S3). The CGI scenario was rejected in all pairwise population comparisons for both mt*COI* and nuclear datasets (Table 3). Thus, if springtail populations persisted in separate

Table 3. Summary of likelihood-ratio tests of demographic congruence (taxon abbreviations follow Table 2). Indirect tests were performed via a set of three scenarios: CGI, Complete Genetic Isolation; $T_{ESR}R$, Transient ESR Refuges; and $T_{PSR}R$, Transient PSR Refuges. Demographic histories were compared directly via the Equal Relative Migration (ERM) null hypothesis. Subscripts of the migration rate parameter, *M*, indicate geographic population pairs (abbreviated following Fig. 1) associated with likelihood-ratio test *P*-values (ns = not significant). Values in parentheses indicate the number of replicates out of five that yielded the same inference.

Null hypothesis (prediction)	Key parameters	mtCOI		Nuclear loci	
		Pseud *P*-value	Acanth *P*-value	Pseud *P*-value	Acanth *P*-value
CGI scenario ($M = 0$)	$M_{HCR\leftrightarrow ESR}$	<0.001 (5)	<0.001 (5)	<0.001 (5)	<0.001 (5)
	$M_{ECR\leftrightarrow PSR}$	<0.001 (5)	<0.001 (5)	<0.001 (5)	<0.001 (5)
	$M_{PSR\leftrightarrow BR}$	<0.001 (5)	<0.001 (5)	<0.001 (5)	<0.001 (5)
$T_{ESR}R$ scenario (*M* symmetrical)	$M_{HCR\leftrightarrow ESR}$	≤0.001 (5)	ns (5)	≤0.001 (5)	ns (3)
	$M_{ESR\leftrightarrow PSR}$	≤0.009 (5)	≤0.013 (5)	≤0.040 (5)	≤0.008 (3)
$T_{PSR}R$ scenario (*M* symmetrical)	$M_{ESR\leftrightarrow PSR}$	≤0.009 (5)	≤0.013 (5)	≤0.040 (5)	≤0.008 (3)
	$M_{PSR\leftrightarrow BR}$	ns (5)	ns (5)	≤0.043 (3)	≤0.004 (4)
ERM pattern (same partioning of *M*)	$M_{HCR\leftrightarrow ESR}$	ns (5)	ns (4)	<0.001 (5)	<0.001 (5)
	$M_{ESR\leftrightarrow PSR}$	<0.001 (5)	<0.001 (5)	≤0.003 (5)	<0.001 (5)
	$M_{PSR\leftrightarrow BR}$	<0.001 (4)	<0.001 (5)	<0.001 (5)	<0.001 (5)

refugia, the nature of that isolation did not promote complete reproductive isolation in either species. However, unambiguous demographic incongruence was evident from mt*COI* tests of refuge-based scenarios. $T_{ESR}R$ was rejected for Pseudachorutinae sp. because gene flow among HCR, ESR, and PSR was significantly asymmetrical with nearly all migration sourced from ESR, consistent with expectations for a major refuge. Conversely, ESR was not a prominent source of migrants in the *Acanthanura* sp. mt*COI* migration matrix (Table 3, Fig. 3). Instead, $T_{PSR}R$ was rejected and PSR inferred as a major refuge based on directionality of the significant asymmetry between ESR and PSR (symmetrical gene flow between PSR and BR is not inconsistent with this conclusion). In the direct comparison of species' mt*COI* migration matrices, the ERM null hypothesis was rejected for two of the three pairwise population comparisons (Table 3). This finding corroborates the species-specific demographic differences inferred from indirect tests.

The Pseudachorutinae sp. nDNA migration matrix indicated a net northward movement of alleles. Accordingly, both refuge-based scenarios were rejected owing to the most southerly population in each pairwise comparison being the inferred source (Table 3, Fig. 3). Thus Pseudachorutinae sp. nuclear data did not support a single major refuge in ESR (cf. mt*COI*), but rather, indicated a southern refuge (or refuges) outside of Tallaganda. This trend was not evident in the *Acanthanura* sp. nDNA migration matrices: there was no consistent signal of northward migration driving significantly asymmetrical gene flow between populations (Table 3, Fig. 3; also see online Supplementary Tables S2 and S3). This demographic incongruence inferred from species' multilocus nuclear datasets was also evident from direct tests, where the ERM hypothesis was rejected in all comparisons (Table 3).

PHYLOGEOGRAPHIC CONGRUENCE WITHIN A NESTED CLADE ANALYSIS FRAMEWORK

Of the 28 nested clades with significant phylogeographic structure in the three-locus Pseudachorutinae sp. dataset, 13 yielded unambiguous biological inferences. Comparable signal was present in the four-locus *Acanthanura* sp. dataset (15 inferences from 25 significant clades, see online Supplementary Table S4 and Fig. S1). For loci that produced multiple disconnected networks, tip / interior status could not be determined at the total cladogram-level, so broad-scale phylogeographic inferences were few. Integrating inferences across loci was unambiguous: for both species, the temporal configuration that minimized the number of events / population processes consisted of four "phases." The chronology of primary inferences indicated recurrent alternation between past fragmentation followed by range expansion, and restricted gene flow (Fig. 4). In most cases, fragmentation events subdivided genetic lineages at or near catchment boundaries. All inferred origins of range expansion, and locations of gene flow restricted by distance, were confined within a priori regions. However, rare long-distance dispersal was inferred from nuclear genes for both springtail species (Fig. 4), indicating that springtail populations were not completely isolated over short-to-intermediate timescales.

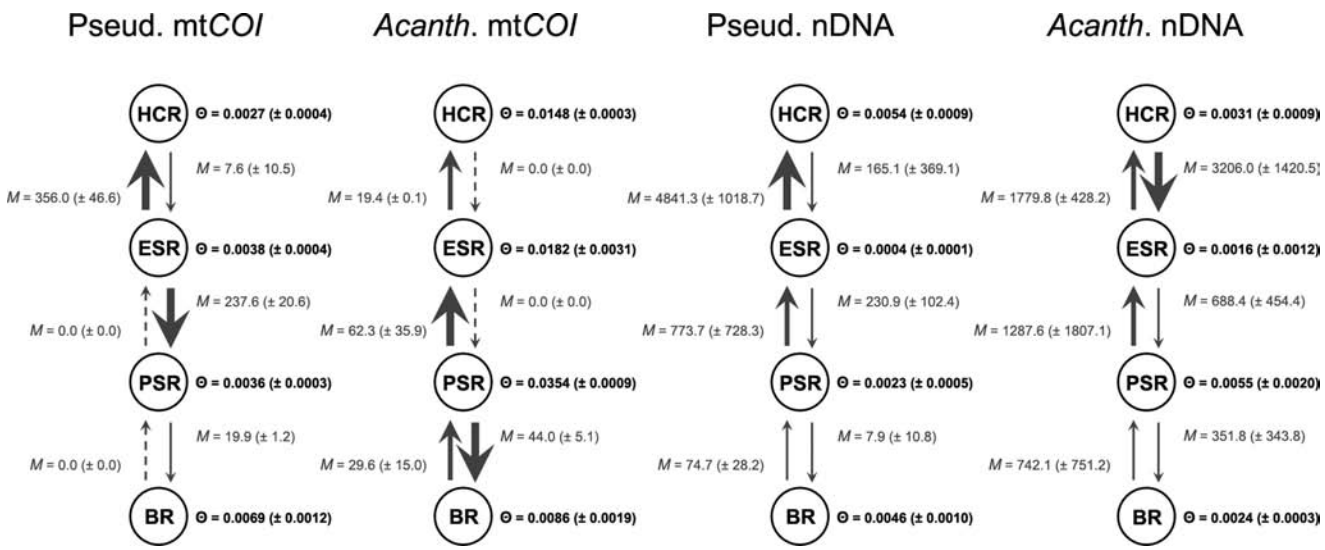

Figure 3. Maximum-likelihood estimates of migration matrix parameters M = migration rate per generation divided by μ, and $\theta = N_e\mu$ for mtDNA, or $4N_e\mu$ for diploid autosomal genes. Pseudachorutinae sp. nuclear loci were *Pseud-SmEF-1*α and *Pseud*-Sm2; *Acanthanura* sp. loci were *Acanth-UcEF-1*α, *Acanth-Uc3*, and *Acanth-Uc180*. Values are presented as the average of five replicate MIGRATE runs per dataset, with standard deviation in parentheses (see online Supplementary Table S1 for point estimates). Arrows show directionality of migration between neighboring geographic populations (abbreviations follow Fig. 1), where arrow size reflects proportion of total migration.

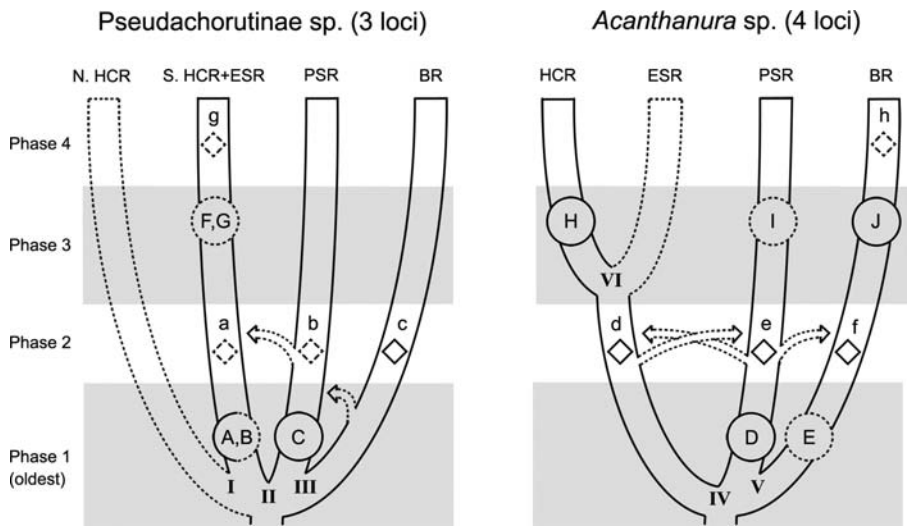

Figure 4. Nested clade analysis reconstructions of population history. The temporal "phases" identified in each species' history do not necessarily coincide with respect to absolute timing of events: they represent the most parsimonious configuration of inferences integrated across loci, and are broadly congruent. Past fragmentation and range expansion events are represented as bifurcations / multifurcations in the species tree (roman numerals), and large circles (uppercase letters), respectively. Restricted gene flow is indicated by small diamonds (lowercase letters) and rare long-distance dispersal by arrows (only *Acanthanura* sp. dispersal events validated by tests of incomplete lineage sorting are shown; see Garrick et al. 2007). Broken lines are inferences that were not cross-validated by $\geq$ 2 loci, either within the same taxon, or across taxa. Abbreviations for microgeographic regions to which inferences relate follow Figure 1, and prefixes "N." and "S." are north and south, respectively (online Supplementary Table S3 identifies loci and nested clade(s) that generated each inference).

Several cross-validated NCA inferences were spatially and temporally congruent between species (Fig. 4). Phase 1 fragmentation separating BR (III and V) and fragmentation near the ESR / PSR catchment division (II and IV) were detected for both springtails, as was range expansion within PSR (C and D). Broadly congruent population processes in phase 2 were restricted gene flow within southern HCR (a and d), PSR (b and e) and BR (c and f), although the precise within-region locations varied. Phase 3 range expansion within HCR was also common to both springtails (F and H), although with different putative geographic origins. Nonetheless, the primary inferences are spatially and temporally concordant. That said, two clear cases of phylogeographic incongruence were evident. Inferences of phase 1 Pseudachorutinae sp. range expansion originating in southern HCR (A), and phase 3 *Acanthanura* sp. range expansion within BR (J), were not detected in the other species. All other apparent discordances were based on single-locus inferences and are therefore considered tentative (Templeton 2002).

PARAMETRIC BOOTSTRAP TESTS OF PHYLOGEOGRAPHIC CONGRUENCE

Thirty-five Pseudachorutinae sp. mt*COI* haplotypes (labeled with the prefix "Hap") were detected from 370 individuals (GenBank accessions: EF057714–EF057732 [Hap01–Hap19], this study; AY694098–AY694113 [HapA–HapP], Garrick et al. 2004). Sixty-nine *Acanthanura* sp. haplotypes (prefix "H") were identified from 203 individuals (DQ518680–DQ518748 [H01–H69], Garrick et al. 2007). Sequences were consistent with Lunt et al.'s (1996) model of insect mt*COI* and displayed open reading frames, indicating recovery of true mtDNA. The estimated *Pseud*-mt*COI* phylogeny ($-\ln L$ 1497.26403, TIM + G model) shows PSR and BR haplotypes each form moderately to strongly supported monophyletic clades (Fig. 5). The *Acanth*-mt*COI* phylogeny ($-\ln L$ 2770.32422, GTR + I + G model) shows more marked phylogeographic structure. Well-supported monophyletic clades distinguish HCR and AR; ESR sequences form three major clades (Fig. 5).

Although *Acanthanura* sp. BR and PSR haplotypes do not each form reciprocally monophyletic clades, they do group into a higher-level monophyletic clade. In contrast, Pseudachorutinae sp. PSR and BR clades are each monophyletic, but not each other's closest relatives. Furthermore, monophyly of HCR haplotypes seen in *Acanthanura* sp. was not reflected by the Pseudachorutinae sp. phylogeny (Fig. 6). Parametric bootstrap tests showed when the constraints of monophyly of PSR + BR haplotypes and monophyly of HCR haplotypes were simultaneously enforced on the Pseudachorutinae sp. tree, the null hypothesis of phylogeographic congruence was accepted (Table 4). Accordingly, phylogenetic uncertainty alone can account for apparent topological differences between species' mt*COI* gene trees—it is not

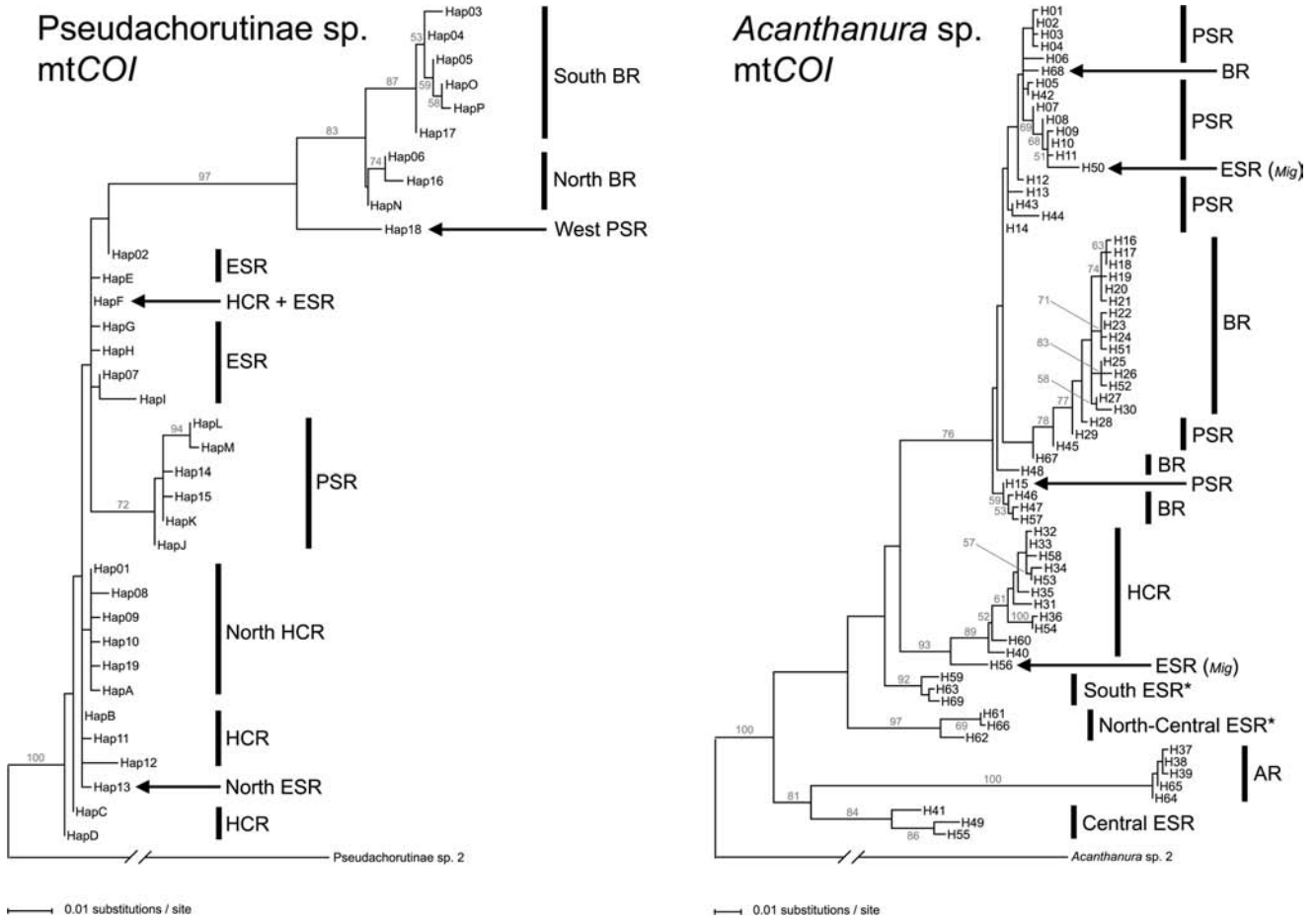

Figure 5. **Maximum-likelihood phylogenies estimated using the best-fit substitution model and the successive approximations procedure. An asterisk marks *Acanthanura* sp. clades that include some PSR-assigned individuals (based on STRUCTURE analysis) with ESR-like *Acanth*-mt*COI* haplotypes; *"Mig"* indicates singleton haplotypes that Garrick et al. 2007 identified as being indicative of past gene flow from a neighboring geographic population. Numbers at nodes are bootstrap values. Abbreviations for geographic populations from which haplotypes were sampled follow Figure 1.**

necessary to invoke different evolutionary histories at the temporal scale under consideration.

Discussion

Despite detectable species-specific demographic differences, our results indicate that the biogeographic context of Tallaganda promoted largely congruent fine-scale phylogeographic structuring in two low-mobility saproxylic springtails. For these species, distinct evolutionary lineages seem to have a long history of association with single catchment-based microgeographic regions, indicating multiple Pleistocene refugia. In this periodically periglaciated montane temperate forest (Bowler 1982; Frakes et al. 1987), concerted evolutionary responses among wet-adapted invertebrates may be driven by their codependence on large-diameter rotting *Eucalyptus* logs. Sheltered gullies and creek lines probably retained moist forest habitats during cool dry periods (Heatwole 1987; Hope 1994), perhaps repeatedly serving as refuges. Indeed, for low-mobility saproxylic organisms, biogeographic context may override differences in species' biology and life history to a greater extent than in other ecological communities. Recent work by Graham et al. (2006) provides some support for this hypothesis.

TESTING CONGRUENCE OVER TIME

Through exploiting the contrasting temporal signals carried by genotypic, allelic, and phylogenetic data, we have attempted to distinguish processes and events that impacted genetic structuring at different points in the evolutionary histories of two springtail species from Tallaganda. Comparative analyses were used to retest the concerted-response hypothesis at each of three relative timescales. In this section, we synthesize results of pattern-based (direct) and scenario-based (indirect) approaches to quantifying congruence.

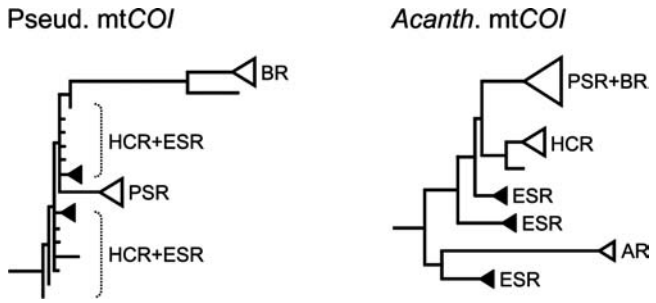

Figure 6. Topological relationships among major clades, simplified from Figure 5. Triangles represent closely related sequences that have been collapsed. White triangles indicate moderately to well-supported monophyletic, geographically localized clades present in one species but apparently absent in the other. Geographic population abbreviations follow Figure 1.

At the oldest timescales over which the present genetic data are informative, only mt*COI* tree topology was considered in our phylogenetic test of the concerted-response hypothesis. Despite apparent discordance relating to monophyly of haplotypes restricted to Harolds Cross Region, and to Pikes Saddle + Badja Regions (Figs. 5, 6), parametric bootstrapping showed that these differences can be attributed to phylogenetic uncertainty in the Pseudachorutinae sp. mt*COI* tree (Table 4). Owing to methodological difficulties associated with simulating DNA sequence data under a model that has no defining constraints, this finding (i.e., failure to reject the null hypothesis) provides only modest support for the concerted-response hypothesis. However, it is consistent with the notion that these species did not exhibit markedly different responses to historical contractions of moist forest habitats throughout the Pleistocene periglaciations.

Table 4. Reciprocal parametric bootstrap tests of phylogeographic congruence. Hypotheses were generated a posteriori from Figure 6. Parentheses denote clades constrained to be monophyletic, the test statistic is the difference in −ln *L* tree score when the null hypothesis of congruence is enforced versus not enforced, and *P*-value indicates whether the null hypothesis was rejected at the 0.05 level (ns = not significant). Taxon and geographic population abbreviations follow Table 2 and Figure 1, respectively. Two alternative delineations of PSR were used to explore the impact of differences between *Acanth*-mt*COI* and nuclear loci relating to the ESR/PSR boundary location: "strict," based solely on nuclear genotype clustering, and "relaxed," where PSR-assigned individuals with ESR-like *Acanth*-mt*COI* haplotypes (see Fig. 5) were omitted from the constraint.

Taxon	Hypothesis	Observed test statistic	*P*-value
Pseud	(HCR)	0.151	ns
	(PSR+BR)	2.139	ns
	(HCR), (PSR+BR)	2.850	ns
Acanth	(PSR)[1]	158.210	<0.01
	(PSR)[2]	83.095	<0.01
	(BR)	58.900	<0.01

[1] "Strict" population delineation
[2] "Relaxed" population delineation

A limitation of applying parametric bootstrapping to test phylogenetic hypotheses at the intraspecific level is that it does not incorporate haplotype frequency information. No distinction is made between high- versus low-frequency haplotypes being shared among (or unique to) particular populations, yet this can carry important phylogeographic signal. Other analyses designed to exploit such information necessarily focus on more recent temporal scales, permitting comparisons of inferences drawn from different periods throughout species' evolutionary histories.

NCA indicated that common population processes and historical events shaped the genetic structures of the two springtails over short-to-intermediate timescales (Fig. 4). Although this assessment of phylogeographic congruence is primarily qualitative, impacts of among-locus stochasticity (Templeton 2002, 2004), and potentially high NCA false positive rates, at least for certain inferences based on single locus datasets (Panchal and Beaumont 2007), were minimized via analysis of multiple independent loci per species. Two consecutive cycles of regional fragmentation followed by range expansion seem to have played a major role in driving the evolutionary trajectories of both species (Fig. 4). This temporal sequence is consistent with repeated contraction and expansion of moist forest habitats across southeastern Australia throughout the ~19 climatic cycles of the Pleistocene (Bowler 1982; Hope 1984; Kershaw et al. 1991). Intervening phases of restricted gene flow likely reflect intrinsic features of species biology—in this case, low dispersal ability. Although NCA does not explicitly test for temporally congruent events, the broad-scale temporal concordance of NCA inferences across taxa, together with considerable fine-scale geographic congruence, indicates a common history of covicariance (Avise 2000). Indeed, there were few clear indications of phylogeographic incongruence: only two cross-validated, species-specific range expansion events were inferred (Fig. 4). In these particular cases, lack of concordance across species cannot be attributed to the low power of NCA in detecting such events, given that several loci were analyzed (see Templeton 2004). Thus, at the whole dataset-level, the concerted-response hypothesis was generally supported.

Although NCA carries the benefit of considering a relatively large suite of historical processes and events (cf. scenario-based statistical approaches), it is still limited by the scope of the inference key. The general-purpose utility of NCA comes at the expense of statistical discrimination among specific a priori hypotheses. Thus, integrating other complementary analyses, including those focused at comparable temporal scales by virtue of the genetic data they exploit, is particularly desirable.

Tests of the concerted-response hypothesis via estimated migration matrices revealed marked demographic incongruence. The two species' mt*COI* datasets were consistent with different refuge-based scenarios. The Eastern Slopes Region, predicted to have harbored the most high-quality moist forest refuges at Tallaganda during Pleistocene periglaciation (Garrick et al. 2004), was the major source of Pseudachorutinae sp. migrants. Conversely, Pikes Saddle Region was the major source of *Acanthanura* sp. migrants (Table 3, Fig. 3). Notably, multilocus nuclear migration matrices did not corroborate a single major refuge for each species. Nuclear datasets also yielded considerably more variability in estimates of *M* across replicate runs relative to mt*COI*. This indicates that amount of data (in terms of number of loci) may be a poor predictor of confidence in estimated parameter values, given that likelihood surfaces may still be quite flat. Nonetheless, the Pseudachorutinae sp. nuclear dataset clearly indicated a northward migration bias, yet there was no consistent directionality of gene flow in the corresponding *Acanthanura* sp. matrix (Table 3, Fig. 3). One plausible explanation is that Pseudachorutinae sp. has been reliant on fewer refuges (thus demographic signal is stronger), at least one of which was presumably located in Badja Region or on the adjoining Great Dividing Range (Fig. 1). Corroborating evidence for species-specific demographic differences came from direct comparisons of migration matrices. These tests showed that the Equal Relative Migration hypothesis was largely rejected (Table 3). Several of Tallaganda's microgeographic regions seem to have retained periglacial forest refuges for wet-adapted fauna (e.g., flatworms, Sunnucks et al. 2006; funnelweb spiders, Beavis and Rowell 2006; water skinks, Hodges et al. 2007). Based on our findings, their relative role as primary sources of postglacial recolonization is likely to be taxon dependent.

Species' responses to physical landscape features that potentially restrict gene flow on ecological timescales can illuminate whether contemporary population processes reinforce or overwrite more ancient phylogeographic patterns. Comparative analyses of present-day population structuring, as evident from genotypic data, also provide a means of extending temporal contrasts of the concerted-response hypothesis. Boundary overlap analysis is commonly used in landscape ecology (Jacquez et al. 2000), but to date, it has not been applied in comparative phylogeography. This spatially explicit approach is well-suited to testing our hypothesis that the locations of genetic contact zones in the two springtail species coincide with high-elevation catchment divisions.

Despite qualitatively similar population range distributions, subtle species-specific differences were evident from scenario-based analyses. East-west oriented high-elevation ridgelines (up to 1400 m) were strongly correlated with the locations of three *Acanthanura* sp. contact zones, yet this association was much less pronounced for Pseudachorutinae sp. Similarly, direct tests showed that cooccurrence of species' corresponding contact zones was not significant (Table 2). Differential reliance on the cool humid microclimates characteristic of large-diameter rotting logs (Grove 2002), which are scarce in the low-canopy subalpine woodlands that dominate ridgelines (State Forests of New South Wales 1995), might underpin such fine-scale spatial discordances. However, it is possible that apparent incongruence is partly a consequence of geographic sampling regime. Because the two springtail species are patchily distributed, their genetic datasets were not drawn from identical locations. Thus, in a comparative phylogeographic context, boundary overlap analysis might be most informative when applied to readily sampled organisms, or to taxon pairs that include one immobile species (e.g., host plants and their parasites or pollinators). Furthermore, the Complete Spatial Randomness permutation procedure represents a conservative test of boundary overlap (Fortin et al. 1996). Greater sensitivity could be achieved by accounting for spatial autocorrelation among adjacent sites. Perhaps the most challenging limitation of our implementation of boundary overlap analysis is that boundaries were necessarily classified as "crisp" (i.e., well-defined, see Jacquez et al. 2000), yet population boundaries are naturally "fuzzy" or imprecise (Schaefer 2006). Indeed, this issue is pertinent to many emerging statistical phylogeographic analyses (e.g., Hey and Machado 2003).

SOURCES OF ERROR IN COMPARATIVE ANALYSES

Scenario-based tests of congruence evaluate several hypotheses independently in codistributed taxa. When using DNA sequence data, an empirical gene tree is treated as just one of many possible realizations of a given evolutionary history (Knowles 2004). Predicted patterns of genetic variation are specified a priori, then an empirical test-statistic is compared to null distributions generated under each alternative hypothesis (Hey and Machado 2003). Conversely, pattern-based assessments of congruence treat the optimal tree topology (or some other empirical parameter value) as being directly informative about organismal history. Although this approach does permit rigorous statistical comparisons of congruence across taxa (e.g., Sullivan et al. 2000), interpretation of process is uncoupled from the statistical test itself.

In some applications, direct tests may be overly stringent because variance in a test-statistic that is attributable to coalescent stochasticity is not taken into account. For example, estimating accurate phylogenies despite incomplete lineage sorting is notoriously difficult (Maddison and Knowles 2006), such that direct comparisons of species' gene tree topologies may fail to illuminate concerted responses, even when species have in fact evolved in a lock-step manner. Sources of error that affect scenario-based tests are primarily analytical, and relate to model misspecification. These tests are dependent on well-defined models of population history (e.g., Knowles 2001; Carstens et al. 2005; DeChaine and

Martin 2006), but critical parameters such as effective population size are usually poorly known. Indeed, when evaluating only the relative fit of several alternative models (e.g., Pfenninger and Posada 2002), the chosen model may nonetheless fit the data quite poorly. Taken together, limitations of the two classes of comparative phylogeographic analysis make a strong case for integrative approaches.

COMMUNITY-LEVEL RESPONSES TO PLEISTOCENE CLIMATIC IMPACTS AT TALLAGANDA

At Tallaganda, geographically concordant transitions between divergent genetic lineages are evident in several taxa dependent on, or associated with, rotting logs. Subdivision across the Pikes Saddle / Badja Region boundary seems to be the most taxonomically pervasive phylogeographic break. This is seen in the present springtail species (see also Garrick et al. 2004, 2007), two terrestrial flatworms (*Artioposthia lucasi* and *Caenoplana coerulea*, Sunnucks et al. 2006), a velvet-worm (*Euperipatoides rowelli*, Sunnucks and Wilson 1999; Sunnucks et al. 2000a; Sunnucks and Tait 2001), and a water skink (*Eulamprus tympanum*, Hodges et al. 2007). In all cases, genetic data are consistent with a narrow contact zone, perhaps just a few hundred meters wide.

Although paleoclimatic effects are often cited as the cause of phylogeographic breaks, the processes maintaining these breaks also deserve consideration. This is especially important in cases in which secondary contact, probably many thousands of years ago, is still evident as narrow contact zones that should have become blurred even in organisms with very limited dispersal. Broadly speaking, stable hybrid zones are generally thought to be maintained by hybrid dysgenesis or ecological gradients (Barton and Hewitt 1985). There is evidence for the former in *E. rowelli*. At the same Eastern Slopes/Pikes Saddle Region break identified for Pseudachorutinae sp., this velvet-worm shows abrupt changes in microsatellite allele and dorsal color pattern frequencies (Sunnucks and Wilson 1999), together with serious physical malformations in hybrid offspring and greatly reduced female fecundity (Sunnucks and Tait 2001).

There are no empirical data on how often or over what timescales partial postmating isolation can be expected to arise between isolated populations. But if it is a random process, phylogeographic signal may be maintained in some areas following secondary contact but lost in others, despite comparable biogeographic histories. Where hybrid zones are maintained by environmental factors, climatic changes may drive geographic movement of these zones (e.g., Kohlmann et al. 1988). However, the location of the Pikes Saddle/Badja Region contact zone is not clearly correlated with abrupt changes in environmental variables (e.g., log moisture content, Woodman et al. 2006) or ecological boundaries (e.g., major forest leagues, State Forests of New South Wales 1995). We suggest that clustering of contact zones at Tallaganda is influenced by paleoclimatic history, where lineages diverged via habitat-contraction-induced vicariance and subsequent long-term isolation in refugia. Current boundaries probably formed on secondary contact following range expansion, contributed to by hybrid dysgenesis.

CONCLUSIONS

Integrative analytical approaches provide a flexible framework for testing congruence of natural genetic–geographic patterns among codistributed biota. In this study, we illustrated the complementarity of pattern-based (direct) and scenario-based (indirect) tests of the concerted-response hypothesis. Moreover, we showed that by employing a variety of molecular data, different time slices of species' evolutionary histories can potentially be separated, allowing temporal contrasts of their responses to changes in the biogeographic landscape. The notion that genotypic, allelic, and phylogenetic data carry signal over different temporal spectra is well known, but rarely exploited in phylogeographic studies. Despite marked differences in demographic histories of the two springtail species considered here, the interaction between topography and Pleistocene climatic cycles elicited similar evolutionary responses, indicating that landscape history can exert an overarching influence on genetic structuring, even at very fine spatial scales. We hypothesize that the saproxylic ecological community may show particularly strong phylogeographic congruence in topographically complex nonglaciated areas, because members are predisposed to tracking the changing distribution of moist forest habitats with unusually high fidelity.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Definitions for terminology used in the description of boundary detection and overlap analysis.

Table S2. Maximum-likelihood point estimates of M and Θ for each replicate MIGRATE run.

Table S3. Likelihood-ratio tests of scenario-based and direct assessments of demographic congruence, for each replicate MIGRATE run.

Table S4. Biological inferences for nested clades with significant phylogeographic structure.

Figure S1. Statistical parsimony cladograms and nesting design.

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