Within-drainage population genetic structure of the freshwater fish *Pseudomugil signifer* (Pseudomugilidae) in northern Australia

D.J. McGlashan, J.M. Hughes, and S.E. Bunn

Abstract: Dendritic channel patterns have the potential to isolate populations within drainages, depending on the relative position within the stream hierarchy of the populations. We investigated the extent of genetic subdivision in the Australian freshwater fish *Pseudomugil signifer* (Kner) (Pseudomugilidae) from two drainages in northern Queensland, Australia, using allozyme techniques. The drainages were adjacent and had similar channel patterns each with two major subcatchments coalesced to an estuarine confluence. Analysis of 30 sites across the two drainages revealed that although there was significant genetic variation among sites in both drainages, this was not between the two subcatchments in either case. This result did not support predictions of the stream hierarchy model (SHM), which would predict higher levels of variation among subcatchments than within them, nor did it suggest that estuarine conditions represent a significant barrier to dispersal in this species. More variation was among sites within each subcatchment. Multidimensional scaling plots revealed that, although most sites within a drainage were similar to one another, outlier sites occurred in each drainage, so correlations between genetic distance and geographic distance were weak. We suggest that the distance between sites and the probability of connectivity between sites may better explain the observed distribution of genetic diversity.

Résumé : Selon leur position relative dans la hiérarchie d'un cours d'eau, les populations peuvent potentiellement être isolées dans le bassin versant par la structure dendritique des chenaux. À l'aide de techniques allozymatiques, nous avons étudié l'étendue de la subdivision génétique chez le poisson d'eau douce australien *Pseudomugil signifer* (Kner) (Pseudomugilidae) dans deux bassins versants du nord du Queensland en Australie. Les bassins sont adjacents et possèdent des arrangements de chenaux similaires; chacun comporte deux sous-bassins majeurs qui se fusionnent en une confluence estuarienne. L'analyse de 30 sites répartis dans les deux bassins indique que, bien qu'il y ait une variation génétique significative d'un site à l'autre dans les deux bassins, la variation ne s'établit dans aucun des deux cas entre les deux sous-bassins. Ce résultat ne s'accorde pas avec les prédictions du modèle hiérarchique des cours d'eau (SHM) qui prédit l'existence de variations plus grandes d'un sous-bassin à un autre qu'à l'intérieur d'un même sous-bassin; il ne laisse pas non plus croire que les conditions estuariennes forment une barrière sérieuse à la dispersion chez cette espèce. Il y a plus de variation entre les sites à l'intérieur de chaque sous-bassin. Des cadrages multidimensionnels révèlent que, bien que la plupart des sites d'un même bassin soient semblables, il y a des sites aberrants dans chaque bassin et donc les corrélations entre les distances génétique et géographique sont faibles. La distance entre les sites et la probabilité de connectivité entre les sites peuvent, selon nous, mieux expliquer la distribution de la diversité génétique que nous avons observée.

[Traduit par la Rédaction]

Introduction

There are many studies that demonstrate that genetic differentiation is greatest among populations of freshwater organisms that inhabit streams in different drainages (see Ward et al. 1994). However, there are fewer studies that have explored the levels of genetic differentiation among populations within drainages to assess the influence of stream structure, distance, and historical processes on population structure (Lu et al. 1997).

The structure of drainage networks isolates populations that inhabit different drainages but also partitions populations within a drainage. Meffe and Vrijenhoek (1988) proposed the stream hierarchy model (SHM) to account for the influence of the hierarchical nature of streams on the distribution of genetic diversity. If the isolation of populations is a function of their relative position in the drainage that they inhabit, then at equilibrium, one would expect differentiation to be greatest among drainages, decreasing to the least differentiation among populations within streams.

The probability of connectedness between two populations within a drainage is also likely to be influenced by other extrinsic factors such as the unidirectional flow of streams and distance. In studies of the Trinidadian guppy, reduced levels of

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polymorphism in headwater streams were attributed to founder effects, while downstream sites were influenced by isolation by distance and physical barriers (Shaw et al. 1994). A bias towards downstream over upstream gene flow in stream systems has been suggested to result in fewer alleles, greater differentiation, and lower heterozygosity within upstream populations relative to within downstream populations (Hernandez-Martich and Smith 1997). Slatkin (1993) showed that at genetic drift – gene flow equilibrium, a log linear relationship between geographic distance and genetic differentiation exists, assuming a one- or two-dimensional stepping stone model of gene flow (also see Hutchison and Templeton 1999).

Finally, intrinsic demographic dispersal and extinction/colonisation processes at the localised within-drainage scale may be important in levels of genetic differentiation among populations. Habitat size, and by inference population size, presumably diminishes with distance upstream. Smaller upstream populations may be more prone to extinction and recolonisation events. Whitlock and McCauley (1990) revealed that these metapopulation processes could increase genetic differentiation among populations. Also, as drainage patterns change through geological time, patterns of connectivity may be altered. There may be a shift in the drift – gene flow equilibrium between populations depending on the frequency of the demographic or environmental perturbation and the time elapsed.

We investigated the levels of genetic differentiation among populations within drainages of a freshwater fish, the Pacific blue eye *Pseudomugil signifer* (Kner) (Pseudomugilidae). Our first aim was to partition the distribution of genetic diversity within drainages to test the SHM. As a corollary, we also addressed whether estuarine confluences isolate populations from different subcatchments. Second, we examined the relationship between geographic distance and genetic differentiation among populations within drainages as an alternative to the SHM. Finally, we attempted to construct a conceptual model of within-drainage population structure that integrated the distribution of genetic diversity.

Materials and methods

Study species

Pseudomugil signifer is a small, abundant fish that is continuously distributed from Cooktown, Queensland, to Narooma, New South Wales, along the eastern coast of Australia (Allen 1989) as well as the western coast of Cape York Peninsula (Saeed et al. 1989) (Fig. 1). Semple (1986, 1991) suggested that spawning occurred year-round with approximately 100 eggs produced. Eggs are demersal and adhesive and range from 1.1 to 1.8 mm in diameter, and larvae are 4–5 mm at hatching. Age at maturity is approximately 6 months, and longevity is 1 year. *Pseudomugil signifer* can tolerate brackish to fully marine conditions, being found in mangrove areas (e.g., Blaber 1980) and several offshore islands such as Lizard Island (Saeed et al. 1989) and in mangrove habitat on the Low Isles (Hadfield et al. 1979). Accordingly, this species appears to have a moderate dispersal potential.

Sampling strategy

The Mulgrave-Russell and Johnstone rivers were selected to assess patterns of variation among populations within drainages (Fig. 1). They share similar dendritic channel patterns, in that two major subcatchments coalesce at an estuarine confluence and then empty to the ocean. We collected tributary and main-channel samples from throughout the lowland sections of the two drainages. In addition, we were interested in the amount of variation within a reach of stream, so we collected two samples within Behana Creek (BHA and BHB), which were 3 km distant, and two samples from the Little Mulgrave River and a tributary (LMA and LMB), which were approximately 500 m apart (Fig. 1). Replicates from three sites (BHB, HYC, and SJR) were collected in 1996 and 1997 to examine temporal changes in allele frequency over approximately two generations. Sample site information is given in Fig. 1 and sample sizes are given in Appendix Tables A1 and A2.

Laboratory procedures

Samples were prepared for allozyme electrophoresis and 18 enzyme systems screened following the procedures of McGlashan and Hughes (2000) based on protocols in Richardson et al. (1986). After screening, six loci encoding five enzyme systems were reliably polymorphic. The enzymes used were glucose-6-phosphate isomerase (EC 5.3.1.9; *GPI-1** and *GPI-2** loci), phosphoglucomutase (EC 5.4.2.2; *PGM** locus), alcohol dehydrogenase (EC 1.1.1.1, *ADH** locus), tripeptide aminopeptidase (EC 3.4.11.4; *PEPB** locus), and phosphogluconate dehydrogenase (EC 1.1.1.44; *PGDH** locus). Allozymes were used as the marker of choice, as six polymophic loci had already been identified from a broad geographic study. These loci, although they varied in their levels of variability, all showed highly significant F_{ST} values on the broad scale ranging from 0.193 to 0.814 (D.J. McGlashan and J.M. Hughes, unpublished data).

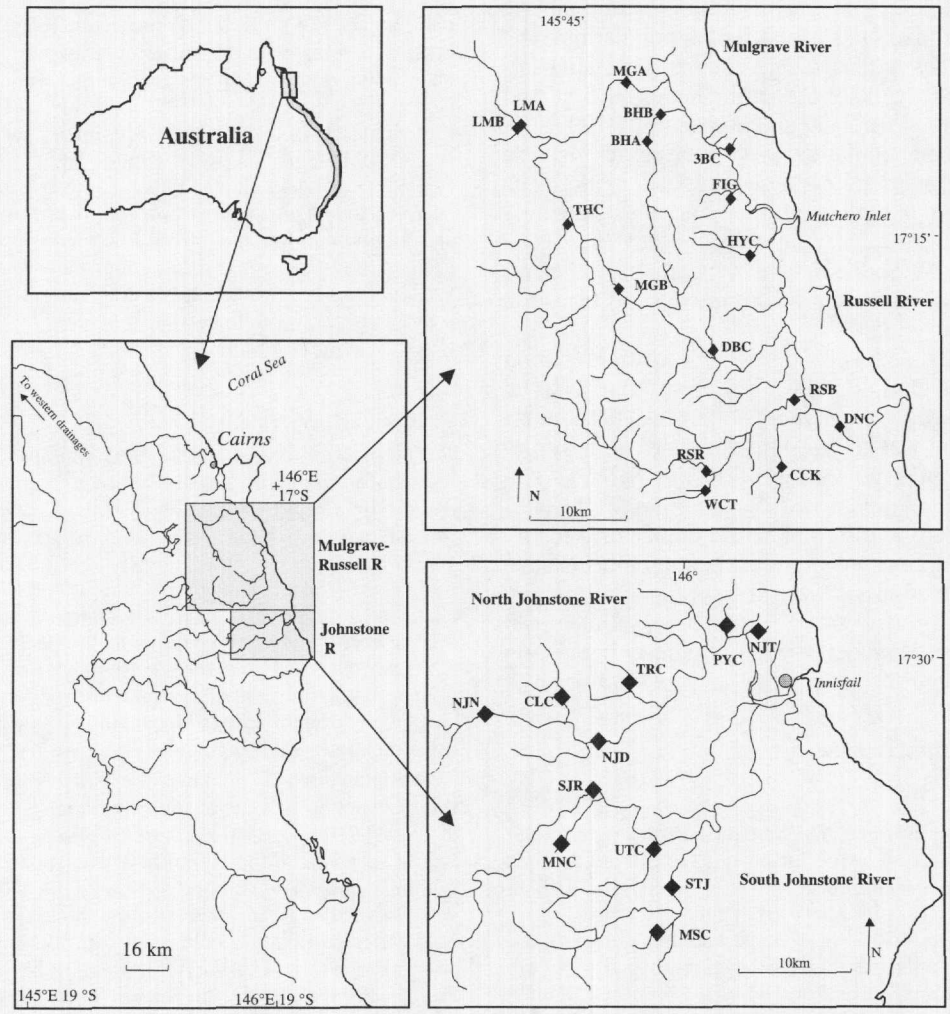
Statistical analysis

The probability test of Guo and Thompson (1992) was used to test conformity with Hardy–Weinberg proportions of populations using GENEPOP (Raymond and Rousset 1995) by testing for both the alternative hypotheses of heterozygote deficiency or excess. Exact *P* tests for detecting linkage disequilibrium were used to test the independence of allozyme loci, as performed by GENEPOP. Exact tests of population differentiation were used to assess the heterogeneity between geographically near sites (i.e., BHA and BHB96, and LMA and LMB), among all sites within a drainage, and between the temporal replicates. Within each subcatchment, sites were categorised as upstream or downstream sites and the mean heterozysity (H) calculated.

We used analysis of molecular variance techniques to partition the distribution of genetic diversity among populations (Schneider et al. 1997) using 10 000 permutations to derive *P* values. There were two levels to the hierarchical analysis: among subcatchments within drainages and among sites within a subcatchment. Each site was assigned to its respective subcatchment to form the hierarchies as detailed in notation below. For the Mulgrave-Russell drainage, sites were assigned to the Mulgrave (MGA, LMB, BHB96, 3BC, FIG, THC, and MGB) or Russell subcatchment (HYC96, DBC, RSB, DNC, RSR, CCK, and WCT). For the Johnstone drainage, sites were assigned to either the North Johnstone (PYC, NJT, CLC, NJN, TRC, NJD) or South Johnstone subcatchment (SJR96, MNC, UTC, STJ, MSC).

Qualitative patterns of population relationships were summarised by multidimensional scaling (MDS) of Rogers' (1972) genetic distance, a metric measure suitable for MDS. We used the methods of Slatkin (1993) to investigate the relationship between levels of genetic differentiation ($\hat{M}$) and geographic (stream) distance. To quantify $\hat{M}$, we used Nei's (1973) measure, G_{ST}, to avoid negative pairwise estimates. Stream distances were measured from 1 : 50 000 or 1 : 100 000 topographical maps using Summagraphics SummaSketch Plus software.

Fig. 1. Map illustrating sampling sites (◆) for *P. signifer* populations in the Mulgrave-Russell and Johnstone drainages. Site abbreviation (bold), site name, and subcatchment status: **MGA**, Mulgrave River A, Mulgrave; **LMB**, No Name Creek, Mulgrave; **LMA**, Little Mulgrave River A, Mulgrave; **BHA**, Behana Creek A, Mulgrave; **BHB96**, Behana Creek B (1996 sample), Mulgrave; **BHB97**, Behana Creek B (1997 sample), Mulgrave; **3BC**, 3rd Bridge Creek, Mulgrave; **FIG**, Figtree Creek, Mulgrave; **THC**, Toohey Creek, Mulgrave; **MGB**, Mulgrave River B, Mulgrave; **HYC96**, Harvey Creek (1996 sample), Russell; **HYC97**, Harvey Creek (1997 sample), Russell; **DBC**, Double Barrell Creek, Russell; **RSB**, Russell River B, Russell; **DNC**, Dinner Creek, Russell; **RSR**, Russell River, Russell; **CCK**, Canal Creek, Russell; **WCT**, Woopen Creek Tributary, Russell; **PYC**, Polly Creek, North Johnstone; **NJT**, North Johnstone Tibutary, North Johnstone; **CLC**, Culla Creek, North Johnstone; **NJN**, North Johnstone River at Nerada, North Johnstone; **TRC**, Tregonthanaan Creek, North Johnstone; **NJD**, North Johnstone River at Dickah Hill, North Johnstone; **SJR96**, South Johnstone River (1996 sample), South Johnstone; **SJR97**, South Johnstone River (1997 sample), South Johnstone; **MNC**, Meingan Creek, South Johnstone; **UTC**, Utchee Creek, South Johnstone; **STJ**, Stewart Creek SJ, South Johnstone; **MSC**, Miskin Creek, South Johnstone. Sites LMA and LMB were pooled to form LMR and BHA and BHB96 were pooled to form BHC after exact tests of population differentiation revealed no significant differences between these sites. Sample sizes are listed in Appendices 1 and 2. Shading in top left map represents the approximate distribution of *P. signifer*.



Results

Variation within populations

In total, 30 sites were sampled from the Mulgrave-Russell and Johnstone drainages. Two sites showed significant heterozygote deficient multilocus departures from Hardy–Weinberg expectations after adjusting the level of significance for multiple tests using the sequential Bonferroni method. The sites were Culla Creek in the North Johnstone subcatchment (CLC, $P = 0.0001$) and Woopen Creek tributary in the Russell subcatchment (WCT, $P = 0.0003$). Tests for linkage disequilibrium between loci revealed no significant results after the sequential Bonferroni adjustment of significance levels.

Population differentiation

At the smallest spatial scale, there was no evidence of significant heterogeneity at any of the six allozyme loci using exact tests of differentiation (Raymond and Rousset 1995) for LMA/LMB (overall $P = 0.75$) or BHA/BHB (overall $P = 0.65$). Data were therefore combined from sites LMA and LMB (forming site LMR) and BHA and BHB96 (forming site BHC) in all subsequent analyses. After applying the

Bonferroni adjustment, there were no significant differences between temporal replicates of site BHB (overall loci P = 0.13), HYC (P = 0.31), or SJR (P = 0.41) as determined by exact tests. They were also combined.

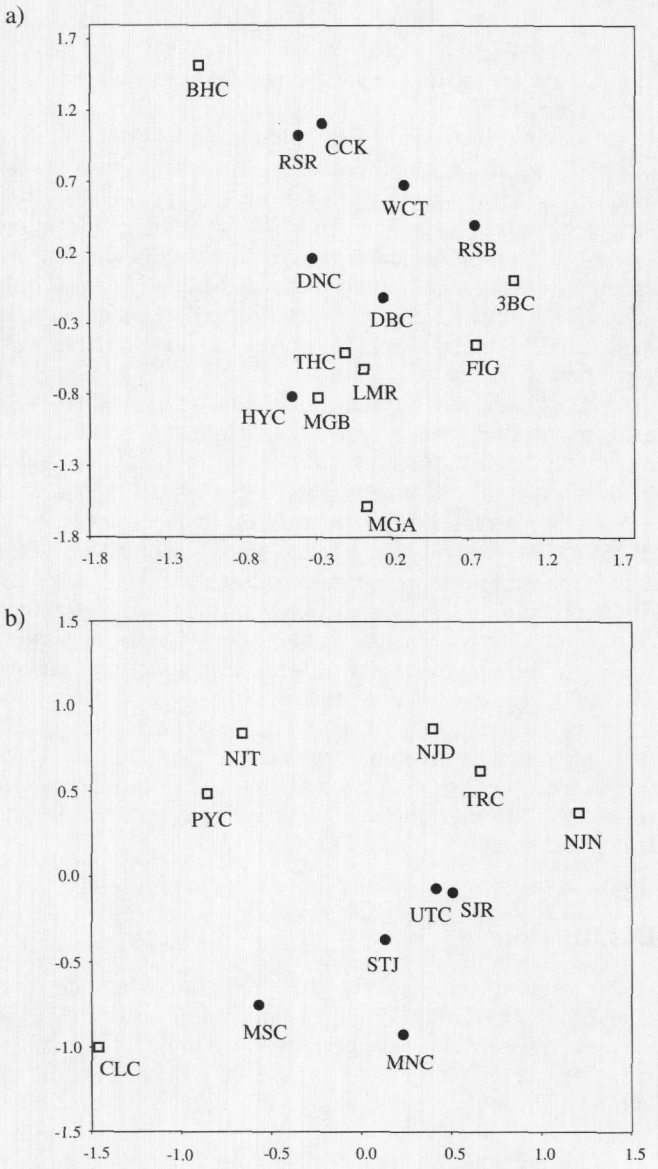
Overall jackknifed estimates of F_{ST} among populations within each of the Mulgrave-Russell (F_{ST} = 0.19 ± 0.10 P < 0.0001) and Johnstone drainages (F_{ST} = 0.28 ± 0.06, P < 0.0001) showed that there were significant levels of population subdivision. Exact tests of pairwise population differentiation averaged over loci revealed many significant results. After applying the sequential Bonferroni adjustment, 45 of 91 Mulgrave-Russell comparisons were significant and in the Johnstone, 50 of 55. Perusal of gene frequencies among populations of the two drainages further exemplifies the high degree of population differentiation. For instance, in the Mulgrave-Russell drainage, there was a high frequency of the *ADH*3* allele in populations in Behana Creek (BHA, BHB96, and BHB97) relative to other Mulgrave River populations (Appendix 1). Among Johnstone River sites, the frequency of the *PEPB*2* allele at site CLC (North Johnstone) was 1.0, while at NJN, little more than 8 km stream distance away, the frequency was 0.24 (Appendix 2). For these same two sites, the frequency of the *GPI-1*3* allele was 1.0 at CLC and 0.16 at NJN. At the *PEPB** locus, site MNC was fixed for a different allele than that found at CLC, sites that are within the same drainage (Appendix 2). At MSC (South Johnstone), the frequency of the *PEPB*3* allele was 0.57, while at STJ 5 km downstream, it was 0.84 (Appendix 2). Some sites also showed very low levels of heterozygosity at all loci (e.g., MNC) and tended to be upstream or tributary sites (Appendix 2), with the mean heterozygosity lower upstream than downstream for three of the four subcatchments (Table 1).

MDS ordination of Rogers' (1972) genetic distance revealed no clear separation by subcatchments and the presence of "outlier" sites which were divergent from their nearby counterparts (Fig. 2). For example, in the Mulgrave-Russell drainage, the position of site BHC in ordination space was widely divergent from other Mulgrave River populations (Fig. 2*a*), due mainly to the high frequency of the *ADH*3* allele (see Appendix 1). In the Johnstone drainage, site CLC, which had large gene frequency differences at the *ADH** and *PEPB** loci, was an outlier site (Fig. 2*b*).

Table 1. Mean heterozygosity (H) and standard error (in parentheses) for downstream and upstream sites in each subcatchment.

	Downstream	Upstream
Russell	0.0584 (0.0201)	0.0993 (0.0087)
Mulgrave	0.1003 (0.0115)	0.0339 (0.0076)
North Johnstone	0.2569 (0.0256)	0.1441 (0.0690)
South Johnstone	0.1420 (0.0247)	0.0903 (0.0492)

Fig. 2. MDS plots of Rogers' (1972) genetic distance for six allozyme loci of populations of *P. signifer* in the (*a*) Mulgrave-Russell (□, Mulgrave; ●, Russell; stress = 0.11) and (*b*) Johnstone drainages (□, North Johnstone; ●, South Johnstone; stress = 0.10). Site abbreviations as in Fig. 1.



Hierarchical analysis of variation

When the genetic variation was partitioned hierarchically, more variation among the Mulgrave-Russell sites was explained by the differences among sites within a subcatchment than between the subcatchments (15.1% compared with –2.1%) (Table 2). Indeed, the between-subcatchment component of variation was not significant (P = 0.91). Removing the outlier site (BHC) from the analysis resulted in only a small increase in the variation attributed between subcatchments within a drainage (data not shown).

The pattern among the Johnstone River sites was similar to that among the Mulgrave-Russell River sites. Genetic variation among sites within subcatchments (22.5%) was greater than between the subcatchments within the drainage (6.5%) (Table 2). Again, the between-subcatchment component was not significant (P = 0.08). When site CLC was removed from the analysis, there was only a small increase in the variation attributed between subcatchments relative to the drainage (data not shown).

Isolation by distance

There was a significant relationship between $\log(\hat{M})$ and

Table 2. Analysis of molecular variance for populations of *P. signifer* in the Mulgrave-Russell and Johnstone drainages.

Source	% variation	*F* statistic	*P*
Mulgrave-Russell drainage			
Between subcatchments	–2.1	–0.021	0.91 ± 0.0023
Among sites within subcatchments	15.1	0.148	<0.0001
Among all sites	13.0	0.130	<0.0001
Johnstone drainage			
Between subcatchments	6.2	0.062	0.087 ± 0.003
Among sites within subcatchments	22.5	0.239	<0.0001
Among all sites	28.6	0.286	<0.0001

log(stream distance) among all sites of the Mulgrave-Russell drainage (Fig. 3*a*) and among all sites of the Johnstone drainage (Fig. 3*b*). However, the coefficients of determination were low (0.07 and 0.19, respectively). The regression slopes describing the relationship between log($\hat{M}$) and log(stream distance) for all populations of the Mulgrave-Russell were approximately –0.5 and for the Johnstone, –0.65.

The log of stream distance was subsequently plotted against log($\hat{M}$) for populations of the Mulgrave and Russell subcatchments separately. Correlations were insignificant between sites within each subcatchment (Figs. 3*c* and 3*e*). Inspection of the scatterplots among Mulgrave sites only (Fig. 3*c*) identified comparisons between populations that had pairwise values of log($\hat{M}$) near zero or less. These were identified as comparisons involving site BHC (reflected in the MDS ordination, Fig. 2*a*), which when removed from the analysis resulted in a significant relationship between distance and genetic differentiation among populations within the Mulgrave subcatchment ($P = 0.048$, $r^2 = 0.20$) (Fig. 3*c*).

Similar patterns in the isolation by distance analyses to the Mulgrave-Russell were evident among populations of the Johnstone River. Stream distance did not explain any of the differentiation among populations of the North or South Johnstone subcatchments when considered separately (Figs. 3*d* and 3*f*). Inspection of the North Johnstone scatterplot (Fig. 3*d*) revealed comparisons between populations that had pairwise log($\hat{M}$) values of less than –0.5. These were identified as involving site CLC (reflected in the MDS ordination, Fig. 2*b*), which when removed from the analysis revealed a strong correlation between log(distance) and log($\hat{M}$) ($P = 0.002$, $r^2 = 0.78$) (Fig. 3*d*).

Discussion

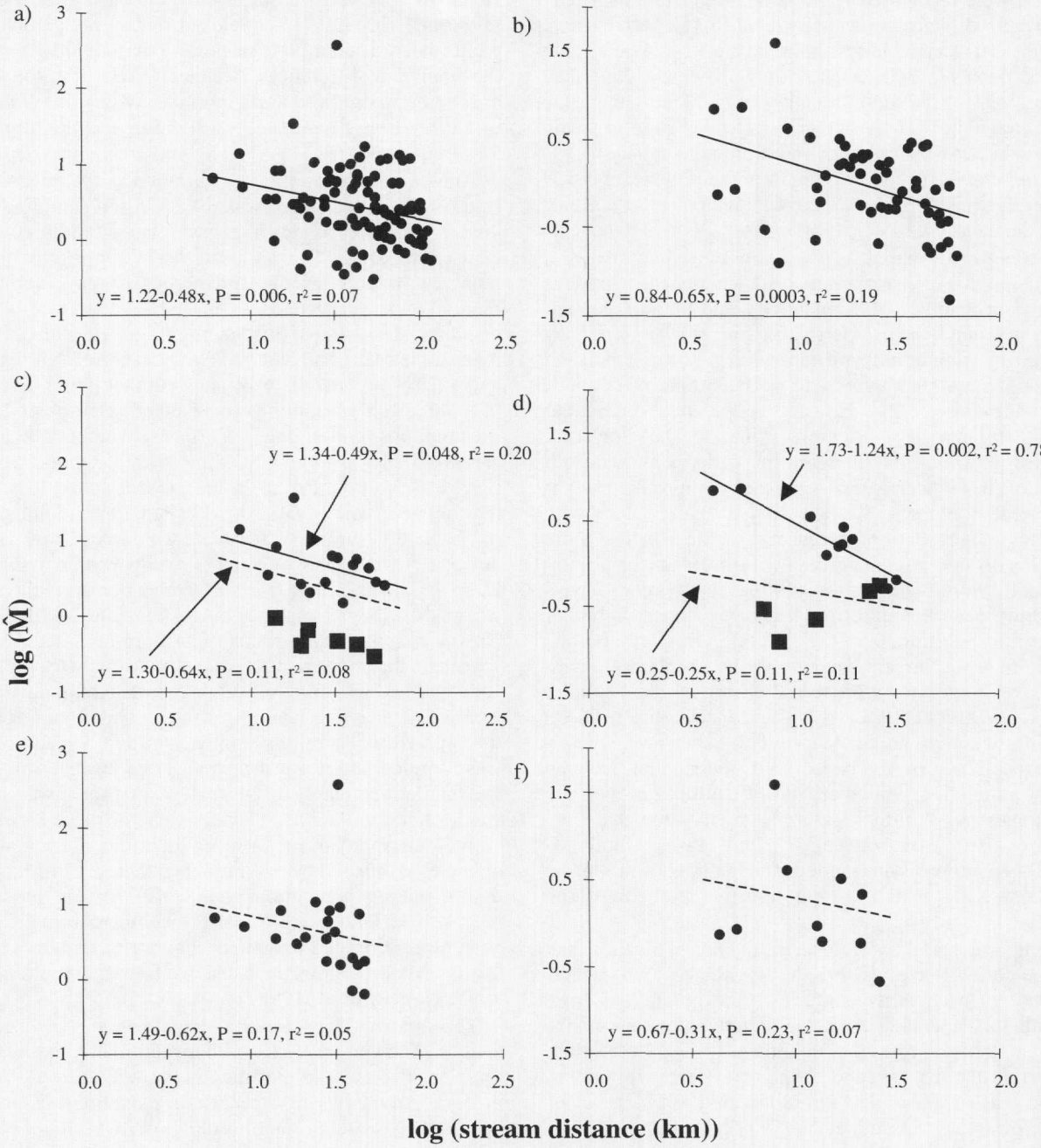
The present study makes the first thorough attempt at quantifying the within-drainage population structure of an Australian freshwater fish. In both of the drainages sampled, there were significant F_{ST} values, which indicated that populations were not panmictic within drainages. Studies in other geographic areas have also shown significant levels of population differentiation within drainages (e.g., Trinidadian guppies, Shaw et al. 1994; Florida freshwater fish, Baer 1998). Shaw et al. (1994) suggested that river order, isolation by distance, and barriers were important contributors to within-drainage gene frequency heterogeneity. A possible bias toward downstream over upstream gene flow may also be important (Shaw et al. 1994; Hernandez-Martich and Smith 1997). Below, we explore some of these issues in relation to the population structure of *P. signifer* within drainages.

Populations of *P. signifer* within drainages appear to have higher levels of connectivity than populations that inhabit separate drainages (42% of genetic variation was among drainages in a broad geographic study, McGlashan 2000), which is consistent with the SHM of Meffe and Vrijenhoek (1988). Thus, the isolation imposed by the drainage boundary appears to partition the distribution of genetic diversity observed. However, the SHM predicts that further partitioning of genetic diversity should also be dependent on divisions within drainages, which was not the case for *P. signifer*. Therefore, other factors must be important in determining the distribution of genetic diversity. The estuarine confluences that separate the Mulgrave from the Russell and the North Johnstone from the South Johnstone do not appear to be important determinants of population structure in this species. If they were, the partitioning of genetic variation among sites within systems should have been greater between subcatchments than among populations within subcatchments, which was not the case.

An alternative model for stream-dwelling species is isolation by distance (IBD). In this model, equilibrium between genetic drift and gene flow may be reached in species where the lifetime dispersal distance is less than the range, and a relationship between distance and genetic differentiation should be apparent (Wright 1943). In *P. signifer*, there was a significant association between genetic differentiation and distance, suggesting that an IBD model may be appropriate. The slopes of the relationships approximated –0.5, which Slatkin (1993) demonstrated is the theoretical expectation from the two-dimensional stepping stone model. This implies that for populations of *P. signifer*, the dendritic nature of streams may approximate a two-dimensional habitat more than a one-dimensional habitat. In other words, connectivity between populations is not necessarily decreased between populations separated by nodes in the stream hierarchy, compared with populations inhabiting the same linear stretch of stream.

The IBD model is, however, not entirely satisfactory for explaining population structure in *P. signifer* within drainages. Although significant, the relationship between geographic and genetic distance was weak; distance explained less than 20% of the genetic differentiation among populations inhabiting the Mulgrave-Russell and Johnstone drainages (Fig. 3). In addition, the relationship between genetic differentiation and geographic distance should be monotonic and the scattergram should have little spread in

Fig. 3. Scatterplots of log(stream distance) versus log($\hat{M}$) for populations of *P. signifer* in the (*a*) Mulgrave-Russell and (*b*) Johnstone drainages and subcatchments, (*c*) Mulgrave, (*d*) North Johnstone, (*e*) Russell, and (*f*) South Johnstone. The broken lines in Figs. 3*c*–3*f* indicate the line of best fit when all pairwise comparisons were considered. The solid line in Fig. 3*c* represents the line of best fit when comparisons involving site BHC (■) were removed from the comparison. The solid line in Fig. 3*d* represents the line of best fit when comparisons involving site CLC (■) were removed from the comparison.



values (i.e., high r^2) (Hutchison and Templeton 1999), which was not the case in the present study. Moreover, theoretical predictions suggest that nearer populations should approach equilibrium before more distant populations (see Slatkin 1993). When populations within subcatchments only were considered, there was little evidence for isolation by distance (noting that the power of the analysis was reduced through lower site numbers). Also, nearer sites often had a large spread of values in genetic differentiation – stream distance comparisons. It may be that there are simply high levels of recurrent gene flow between nearer sites (Hutchison and Templeton 1999). However, the large frequency differences between geographically close sites (e.g., CLC and NJN) may suggest that high levels of gene flow occur only between some nearby sites.

A better model than either the SHM or the IBD model of *P. signifer* population structure within drainages may require the consideration of metapopulation processes such as extinction/

colonisation events and other demographic parameters. Slatkin (1977) and Whitlock and McCauley (1990) suggested that the genetic consequences of founding events can be complex and depend on the number of founders and the characteristics of the founding population(s). In the current study, distinctive populations were recognised from MDS ordination of Rogers' (1972) genetic distance. For instance, site CLC was fixed at two loci that were variable just downstream (e.g., *PGI-1** and *PEPB** loci). Sites in Behana Creek in the Mulgrave River had high frequencies of the *ADH*3* allele that had a low frequency in other nearby sites. These outlier populations were in small, upstream habitats, which may be more prone to stochastic extinction/colonisation events. When these sites were removed from their respective analyses, the predictive power of distance increased dramatically, especially when site CLC was removed.

A more powerful model of gene flow may thus involve a mixed model that considers the distance between sites as well as the probability of connectivity and size of populations that inhabited various parts of a stream system. It may be that nearby populations are more likely to be connected than more distant populations (e.g., isolation by distance plots without outlier sites). However, populations in smaller headwater streams may be more prone to the stochastic effects of drift which may increase the variance in gene frequencies of headwater sites compared with other downstream populations (e.g., isolation by distance plots with outlier sites). If gene flow is reduced to the smaller, more isolated headwater streams, they will take longer to approach equilibrium than populations that have greater levels of gene flow because equilibrium approaches faster at higher migration rates (Whitlock 1992). Although this may be offset by the fact that larger populations (i.e., probably downstream) take longer to approach equilibrium than smaller populations (Whitlock 1992), the weak relationship between genetic differentiation and distance among sites in *P. signifer* may be exacerbated by the inclusion of the smaller, isolated headwater populations which are out of equilibrium with the other populations. Whitlock (1992) demonstrated that estimates of F_{ST} can vary between 0.1 and 0.5 over approximately 20 generations with random fluctuations of migration rate. Values of F_{ST} in the present study were encompassed by this range.

The temporal stability of gene frequencies of sites that were sampled in 1996 and 1997 (approximately two generations) does not necessarily conform to the predictions from metapopulation models because the effective population size of the populations compared is unknown. If they were small, the temporal stability in gene frequencies lends support to the notion that sites sampled are subdivided with low levels of gene flow (e.g., Spruell et al. 1999). However, if they were large, the inertia of larger populations may not necessarily be contrary to the metapopulation concept. The lack of independent data on population size and the small number of generations between sampling periods preclude firm conclusions in this regard. It may be useful to sample the same populations in the future to document temporal changes in gene frequencies.

It is also important to consider other factors that may be contributing to variation in allele frequencies among populations of *P. signifer* in the Mulgrave-Russell and Johnstone drainages. Genetic drift and gene flow are expected to affect all loci equally, whereas the effects of selection are locus specific (e.g., Slatkin 1987). The high F_{ST} estimate among populations in the Mulgrave-Russell drainage was largely generated by frequency differences at the *ADH** locus, in which the frequency of the *ADH*3* allele was high in the Behana Creek and Russell River populations. If selection is favouring the *ADH*3* allele in these populations, there would need to be a high selection coefficient to account for the frequency differences. Major differences in the selection regime between these areas are not obvious; however, selection cannot be discounted as a force in shaping allozyme allele frequencies.

Another possible source of variation in the observed allele frequencies is that there was a bias in the sampling of individuals from only one or a few related families. Recently, Hansen et al. (1997) showed that sampling only limited numbers of families of brown trout which exhibited nonrandom distributions led to biased estimates of allele frequencies. They found that many juveniles within the same reach of stream had an otherwise rare mtDNA haplotype, and microsatellite analysis showed that there were likely only three full-sib families represented (Hansen et al. 1997). Assumptions underlying the Hardy–Weinberg equilibrium are that there is random mating within a large population (Richardson et al. 1986). In the present study, it is possible that some of the small tributary streams are inhabited by only a small number of families, which may have led to the deviations from Hardy–Weinberg proportions at some sites. Little is known about *P. signifer* abundance in the sampled streams or about their spawning behaviour and life history. However, some local groups of fish are dominated by a single male that aggressively defends an area (Semple 1986; Howe 1987), which may lead to assortative associations at a localised scale. The sampling strategy was designed to avoid the problem of sampling families, in that a reach of stream was sampled and not just a small area; however, in some of the smaller upstream sites, it may be that there was a bias toward a few families. By chance, some of these families may be monomorphic at one or more loci, thus biasing allele frequencies compared with samples gathered from larger, randomly mating populations. Studies of stream insects have revealed deviations from Hardy–Weinberg proportions that have been attributed to a small number of females contributing to egg deposition in a reach of stream (Schmidt et al. 1995; Hughes et al. 1998).

The fact that three of the six allozyme loci used in the analysis showed no variation between many sites (i.e., were fixed for the same allele) may have weakened the power to detect a strong isolation by distance relationship. It may be necessary to develop microsatellite loci with higher levels of variability to resolve this question further.

Finally, we must account for the history of drainage evolution whereby drainage-specific perturbations may affect the levels and patterns of genetic differentiation. The geomorphological history of the two drainages appears to be quite different: there is evidence that the Mulgrave and Russell rivers were mobile through much of the Pleistocene, with the mouth switching between Trinity Inlet at the north and the present mouth at Mutchero Inlet in the south. In contrast, a large basaltic lava plume flowed down the "ancestral" Johnstone River about 2 million years ago (De Keyser

1964; De Keyser and Lucas 1968), which must have greatly affected the course of this river. Given these processes, the patterns of differentiation among populations of the Mulgrave-Russell and Johnstone drainages were remarkably similar. It may be that enough time has elapsed since the perturbations for populations within each drainage to approach equilibrium. Analysis of mtDNA variation may further elucidate the importance of historical events to the extent and distribution of genetic diversity among populations of the Mulgrave-Russell and Johnstone drainages.

It is evident that populations of *P. signifer* within the Mulgrave-Russell and Johnstone drainages are not panmictic. Distance was revealed to be an important, but not exclusive, component of the variation at six allozyme loci. It may be that a more complex model, which includes populations that are in equilibrium and nonequilibrium states with other populations, may better describe the patterns of variation. It may also be that the structuring of streams, which divides populations in terms of size and probability of connectedness, may result in a complex metapopulation of interconnected demes. Future work may profit from modelling how the hierarchical structure of streams and the resultant effect on connectedness and population size can influence population structure and gene flow of obligate stream species. In addition, although allozymes provide an easy nuclear marker for analysing large numbers of samples relatively quickly, analysis of mtDNA with its smaller effective population size may be more sensitive to small population size and barriers to dispersal.

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References

Allen, G.R. 1989. Freshwater fishes of Australia. T.F.H. Publications Inc., Neptune City, N.J.

Baer, C.F. 1998. Population structure in a south-eastern US freshwater fish, *Heterandria formosa*. II. Gene flow and biogeography within the St. Johns River drainage. Heredity, **81**: 404–411.

Blaber, S.J.M. 1980. Fish of the Trinity Inlet system of north Queensland with notes on the ecology of fish faunas of tropical Indo-Pacific estuaries. Aust. J. Mar. Freshwater Res. **31**: 137–146.

De Keyser, F. 1964. Innisfail, Qld. 1 : 250 000 Geological Series Sheet SE/55–6. Bureau of Mineral Resources, Geology and Geophysics, Canberra, Australia.

De Keyser, F., and Lucas, K.G. 1968. Geology of the Hodgkinson and Laura Basins, north Queensland. Bur. Min. Resources, Geol. Geophys. Bull., Canberra, Australia.

Guo, S.W., and Thompson, E.A. 1992. Testing differentiation in diploid populations. Biometrics, **48**: 361–372.

Hadfield, A.J., Ivanstoff, V., and Johnston, P.G. 1979. Clinal variation in electropohoretic and morphological characters between two nominal species of the genus *Pseudomugil* (Pisces: Atheriniformes: Pseudomugilidae). Aust. J. Mar. Freshwater Res. **30**: 375–386.

Hansen, M.M., Nielson, E.E., and Mensberg, K.-L.D. 1997. The problem of sampling families rather than populations: relatedness among individuals in samples of juvenile brown trout *Salmo trutta* L. Mol. Ecol. **6**: 469–474.

Hernandez-Martich, J.D., and Smith, M.H. 1997. Downstream gene flow and genetic structure of *Gambusia holbrooki* (eastern mosquitofish) populations. Heredity, **79**: 295–301.

Howe, E. 1987. Breeding behaviour, egg surface morphology and embryonic development in four species of the genus *Pseudomugil* (Pisces: Melanotaeniidae). Aust. J. Mar. Freshwater Res. **38**: 885–895.

Hughes, J.M., Bunn, S.E., Hurwood, D.A., and Cleary, C. 1998. Dispersal and recruitment of *Tasiagma ciliata* (Trichoptera: Tasimiidae) in rainforest streams, south-eastern Australia. Freshwater Biol. **39**: 117–127.

Hutchison, D.W., and Templeton, A.R. 1999. Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the influence of genetic variability. Evolution, **53**: 1898–1914.

Lu, G., Li, S., and Bernatchez, L. 1997. Mitochondrial DNA diversity, population structure, and conservation genetics of four carps within the Yangtze River, China. Can. J. Fish. Aquat. Sci. **54**: 47–58.

McGlashan, D.J. 2000. Consequences of dispersal, stream structure and earth history on patterns of allozyme and mitochondrial DNA variation of three species of Australian freshwater fish. Ph.D. thesis, Griffith University, Brisbane, Australia.

McGlashan, D.J., and Hughes, J.M. 2000. Reconciling patterns of genetic variation with stream structure, earth history and biology in the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae). Mol. Ecol. **9**: 1737–1752.

Meffe, G.K., and Vrijenhoek, R.C. 1988. Conservation genetics in the management of desert fishes. Conserv. Biol. **2**: 157–169.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. U.S.A. **70**: 3321–3323.

Raymond, M., and Rousset, F. 1995. GENEPOP (version 1.2). Population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249.

Richardson, B.J., Baverstock, P.R., and Adams, M. 1986. Allozyme electrophoresis: a handbook for animal systematics and population studies. Academic Press, Sydney, Australia.

Rogers, J.S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. University of Texas publication 7213. pp. 145–153.

Saeed, B., Ivantsoff, W., and Allen, G.R. 1989. Taxonomic review of the family Pseudomugilidae (Order Atheriniformes). Aust. J. Mar. Freshwater Res. **40**: 719–787.

Schmidt, S.K., Hughes, J.M., and Bunn, S.E. 1995. Gene flow among conspecific populations of *Baetis* sp. (Ephemoptera): adult flight and larval drift. J. North Am. Benthol. Soc. **14**: 147–157.

Schneider, S., Kueffer, J., Roessli, D., and Excoffier, L. 1997. Arlequin: exploratory molecular genetics software. Version 1.1. University of Geneva, Geneva, Switzerland.

Semple, G.P. 1986. *Pseudomugil signifer* — maintenance, reproduction and early development of the Pacific blue-eye. Fishes Sahul, **3**: 121–125.

Semple, G.P. 1991. Reproductive behaviour and early development of the honey blue-eye *Pseudomugil mellis* Allen and Ivanstoff

1982 (Pisces: Pseudomugilidae), from the north-east coast division, south-eastern Queensland, Australia. Aust. J. Mar. Freshwater Res. **42**: 277–286.

Shaw, P.W., Carvalho, G.R., Magurran, A.E., and Seghers, B.H. 1994. Factors affecting the distribution of genetic variability in the guppy, *Poecilia reticulata*. J. Fish Biol. **45**: 875–888.

Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent local extinctions. Theor. Popul. Biol. **12**: 253–262.

Slatkin, M. 1987. Gene flow and the geographic structure of populations. Science (Washington, D.C.), **236**: 787–792.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution, **47**: 264–279.

Appendix 1. Allele frequencies, sample sizes (n), and observed heterozygosity (H_O) for populations of *P. signifer* from the Mulgrave-

	Mulgrave sites									
Locus	MGA	LMB	LMA	BHB 96	BHB 97	3BC	BHA	FIG	THC	MGB
*GPI-1**										
n	16	18	37	43	44	36	42	44	43	42
2	0.219	0.111	0.054	0.093	0.148	0.069	0.060	0.068	0.035	0.012
3	0.781	0.889	0.932	0.907	0.852	0.931	0.940	0.932	0.965	0.988
5	—	—	0.014	—	—	—	—	—	—	—
H_O	0.313	0.222	0.135	0.140	0.205	0.139	0.119	0.136	0.070	0.024
*ADH**										
n	15	20	34	43	44	37	42	43	41	42
1	—	—	—	0.012	—	—	—	—	—	—
3	—	0.025	0.074	0.581	0.750	0.162	0.595	0.093	0.073	0.012
4	—	—	—	—	—	—	—	—	—	—
5	1.000	0.975	0.912	0.407	0.250	0.838	0.405	0.895	0.927	0.988
6	—	—	0.015	—	—	—	—	0.012	—	—
H_O	0.000	0.050	0.118	0.419	0.364	0.324	0.333	0.116	0.146	0.024
*PEPB**										
n	16	18	37	43	44	37	42	44	44	42
1	—	—	—	—	0.011	0.014	—	0.023	—	—
2	0.969	1.000	1.000	1.000	0.943	0.905	0.964	0.841	1.000	1.000
3	0.031	—	—	—	0.045	0.081	0.024	0.136	—	—
4	—	—	—	—	—	—	0.012	—	—	—
H_O	0.063	0.000	0.000	0.000	0.114	0.135	0.048	0.273	0.000	0.000
*PGM**										
n	15	18	37	43	44	37	40	44	43	41
2	—	—	—	—	0.011	0.068	—	—	—	0.012
4	1.000	1.000	1.000	1.000	0.989	0.932	1.000	1.000	1.000	0.988
5	—	—	—	—	—	—	—	—	—	—
H_O	0.000	0.000	0.000	0.000	0.023	0.135	0.000	0.000	0.000	0.024
*PGDH**										
n	16	19	36	43	44	37	42	44	44	41
2	0.969	1.000	1.000	0.988	1.000	0.986	0.976	1.000	1.000	1.000
3	0.031	—	—	0.012	—	0.014	0.024	—	—	—
H_O	0.063	0.000	0.000	0.023	0.000	0.027	0.000	0.000	0.000	0.000
*GPI-2**										
n	16	20	34	43	44	37	42	44	43	42
4	—	—	—	—	—	0.027	—	—	—	—
5	1.000	1.000	1.000	1.000	1.000	0.973	0.988	1.000	1.000	1.000
7	—	—	—	—	—	—	0.012	—	—	—
H_O	0.000	0.000	0.000	0.000	0.000	0.054	0.024	0.000	0.000	0.000

Note: Site abbreviations as in Fig. 1.

Spruell, P., Rieman, B.E., Knudsen, K.L., Utter, F., and Allendorf, F.W. 1999. Genetic population structure within streams: microsatellite analysis of bulltrout populations. Ecol. Freshwater Fish, **8**: 114–121.

Ward, R.D., Woodwark, M., and Skibinski, O.F. 1994. A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. J. Fish Biol. **44**: 213–232.

Whitlock, M.C. 1992. Temporal fluctuations in demographic parameters and the genetic variance among populations. Evolution, **46**: 608–615.

Whitlock, M.C., and McCauley, D.E. 1990. Some population genetic consequences of colony formation and extinction: genetic correlations within founding groups. Evolution, **44**: 1717–1724.

Wright, S. 1943. Isolation by distance. Genetics, **114**: 114–138.

Russell drainage.

Russell sites							
HYC96	HYC97	DBC	RSB	DNC	RSR	CCK	WCT
43	44	43	43	44	41	36	44
—	—	0.047	0.035	—	0.037	0.056	0.091
1.000	1.000	0.953	0.965	1.000	0.963	0.944	0.909
—	—	—	—	—	—	—	—
0.000	0.000	0.093	0.070	0.000	0.073	0.056	0.136
42	44	44	43	44	40	36	43
—	—	—	—	—	—	—	—
0.017	0.036	0.148	0.221	0.239	0.425	0.417	0.267
—	—	—	—	—	—	—	0.023
0.983	0.964	0.852	0.779	0.761	0.575	0.583	0.709
—	—	—	—	—	—	—	—
0.071	0.000	0.205	0.349	0.386	0.350	0.500	0.349
44	44	44	43	44	41	34	44
—	—	—	—	—	—	—	0.011
1.000	0.989	0.977	0.837	1.000	0.963	0.956	0.955
—	0.011	0.023	0.151	—	0.037	0.044	0.034
—	—	—	0.012	—	—	—	—
0.000	0.023	0.045	0.186	0.000	0.073	0.088	0.045
44	44	44	43	44	40	38	44
—	—	—	—	—	—	—	0.045
1.000	1.000	0.977	1.000	1.000	1.000	1.000	0.955
—	—	0.023	—	—	—	—	—
0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.091
44	44	44	43	44	41	37	44
0.966	1.000	1.000	1.000	1.000	1.000	1.000	1.000
0.034	—	—	—	—	—	—	—
0.068	0.000	0.000	0.000	0.000	0.000	0.000	0.000
44	44	44	43	44	41	37	44
—	—	—	—	—	—	0.014	—
1.000	1.000	1.000	1.000	1.000	1.000	0.986	1.000
—	—	—	—	—	—	—	—
0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.000

Appendix 2. Allele frequencies, sample sizes (n), and observed heterozygosity (H_O) for populations of *P. signifer* from the Johnstone drainage.

	North Johnstone sites						South Johnstone sites					
Locus	PYC	NJT	CLC	NJN	TRC	NJD	SJR96	SJR97	MNC	UTC	STJ	MSC
*GPI-1**												
n	46	19	41	44	48	43	41	44	44	44	44	44
2	0.022	0.132	—	0.023	—	—	0.098	0.023	—	0.011	0.011	—
3	0.891	0.711	1.000	0.159	0.406	0.430	0.610	0.636	1.000	0.670	0.886	1.000
4	—	—	—	—	0.010	—	—	—	—	—	—	—
5	0.087	0.158	—	0.818	0.583	0.570	0.293	0.341	—	0.318	0.102	—
H_O	0.174	0.368	0.000	0.273	0.500	0.535	0.439	0.523	0.000	0.500	0.227	0.000
*ADH**												
n	46	19	41	44	48	44	41	44	44	44	44	44
1	0.011	—	—	—	—	—	—	—	—	0.011	—	—
3	0.272	0.342	0.012	0.102	0.104	0.159	0.098	0.068	—	0.091	0.068	—
4	—	—	0.244	0.011	—	0.080	—	—	—	—	—	—
5	0.685	0.658	0.744	0.886	0.885	0.761	0.902	0.932	1.000	0.898	0.932	1.000
6	0.033	—	—	—	0.010	—	—	—	—	—	—	—
H_O	0.239	0.474	0.171	0.182	0.188	0.295	0.195	0.136	0.000	0.182	0.136	0.000
*PEPB**												
n	48	19	38	44	47	44	42	44	44	44	44	43
1	0.021	—	—	—	0.021	0.011	0.012	0.023	—	0.011	—	—
2	0.260	0.316	1.000	0.239	0.128	0.284	0.083	0.068	—	0.102	0.136	0.570
3	0.594	0.421	—	0.761	0.606	0.670	0.833	0.807	1.000	0.830	0.841	0.430
4	0.115	0.237	—	—	0.245	0.034	0.071	0.091	—	0.057	0.023	—
6	0.010	0.026	—	—	—	—	—	0.011	—	—	—	—
H_O	0.583	0.632	0.000	0.341	0.447	0.523	0.262	0.318	0.000	0.295	0.318	0.535
*PGM**												
n	47	19	41	44	48	44	42	44	44	44	44	44
1	0.021	—	—	—	—	—	—	—	0.011	—	—	—
2	0.191	0.158	—	0.011	0.052	0.057	—	0.034	—	0.011	0.011	—
4	0.787	0.842	1.000	0.989	0.948	0.943	1.000	0.966	0.989	0.989	0.989	1.000
H_O	0.340	0.316	0.000	0.023	0.104	0.114	0.000	0.068	0.023	0.023	0.023	0.000
*PGDH**												
n	46	17	41	44	48	44	40	44	44	44	44	44
2	0.902	1.000	1.000	1.000	0.990	0.932	1.000	1.000	1.000	1.000	1.000	0.989
3	0.098	—	—	—	0.010	0.068	—	—	—	—	—	0.011
H_O	0.196	0.000	0.000	0.000	0.021	0.136	0.000	0.000	0.000	0.000	0.000	0.023
*GPI-2**												
n	48	19	41	44	48	44	42	44	44	44	44	43
4	0.010	—	—	—	—	—	—	—	—	—	—	—
5	0.979	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
7	0.010	—	—	—	—	—	—	—	—	—	—	—
H_O	0.042	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Site abbreviations as in Fig. 1.