Testing unknown Gadopsis samples against a broad reference dataset for genetic SNP markers.

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Summary

The unknown Gadopsis samples are clearly from the region encompassing the Gellibrand and Aire rivers.

Introduction

The genus Gadopsis consists of two recognised species, G. marmoratus, the River Blackfish and G. bispinosus, the Two-spined Blackfish. More recent work (Hammer et al. 2014; Unmack et al. 2017) demonstrate that each of the recognised species contain cryptic diversity that almost certainly represent different species including five within G. marmoratus (SEV, SBA, SWV, NMD, NGW) and two within G. bispinosus (BE and BG). Prior to European settlement G. marmoratus would have been widespread and abundant in most aquatic habitats except for the upper most high elevation reaches of streams. Today they are very patchily distributed and are absent from much of their former range.

The current study is designed to test the geographic origins of 16 Gadopsis samples of unknown origin. These samples were combined with a broad dataset spanning samples from most rivers across the range of the genus to provide a robust test of their origins.

Materials and Methods

Taxonomic sampling

I took the existing previously analysed DNA samples for Gadopsis that covered the entire range of each species. These samples included most of the rivers across the entire distribution of the genus (Hammer et al. 2014). This provides the ideal baseline to use when trying to identify unknown samples to determine their provenance. A few additional samples were included from Unmack et al. (2017) to fill in space in the sequencing plates which helped to bump up sample sizes and slightly improve the geographic coverage.

A total of 188 individuals were submitted for SNP sequencing with Diversity Arrays Technology. Fish were included from 74 populations plus 16 unknown individuals (Table 1). This included a thorough sampling of major drainages in central southern Victoria to allow a good test of the source of the unknown fish as this was the region they were suspected of being from.

SNP genotyping and data filtering

DNA was extracted by Diversity Arrays Technologies (DArT Pty Ltd, Canberra, Australia) sequenced for a SNP dataset using DArTseq™, a variation of the double-digest RAD technique which combines next generation sequencing, complexity reduction using restriction enzymes, and implicit fragment size selection, as described by Kilian et al. (2012). All details of the sequencing methods used follow Georges et al. (2018).

SNP Filtering

The SNP data and associated metadata were read into a genlight object (adegenet, Jombart, 2008) to facilitate processing with package dartR 2.2.3 (Gruber, Unmack, Berry, & Georges,

2018). Three datasets were generated, one for the “Full” dataset, the second for “SBA” (Southern Bass) individuals, and a third for “Gellibrand” containing individuals from the Gellibrand, Aire and unknown samples. For the latter two datasets any monomorphic loci were removed as the first step. Reproducibility (based on DArT’s repeated sequencing of ~33% of individuals to check they obtain the same answer twice) was filtered at 0.99. For missing data I filtered by locus and then by individuals. For each respective dataset loci were filtered at 0.7 (<30% missing data), 0.8 and 0.99. For the Full dataset it is best to allow more missing data as less related populations tend to have more missing data due to having more mutations in the enzyme cutting sites that accumulate over time. In addition tree based analyses are less impacted by missing data. For the third dataset I filtered stringently as this was PCA based analysis which benefits from having less missing data. Filtering for missing data per individual was at 0.6, 0.7 and 0.9. This resulted in elimination of 7, 1 and 0 individuals for each respective dataset. There is always some compromise by retaining as many individuals as possible and not having excess missing data.

SNP Analyses

I used a mix of tree and ordination based analyses to examine the relationship between individuals. For the Full and SBA datasets I generated phylogenetic trees using Maximum likelihood (ML) applied to concatenated sequences. ML analysis were conducted using RAxML 8.2.12 (Stamatakis 2014) on the CIPRES cluster (Miller et al. 2010) using the model GTRCAT and searching for the best-scoring ML tree using the model GTRGAMMA in a single program run, with bootstrapping set to finish based on the autoMRE majority rule criterion. The tree was imported to Mega 7.0 (Kumar et al. 2016), formatted and mid point rooted.

For the SBA and Gellibrand datasets I visualized genetic similarity among individuals and populations using ordination (Principal Coordinates Analysis or PCoA, Gower, 1966) as implemented in the gl.pcoa and gl.pcoa.plot functions of dartR.

In addition, for the SBA dataset I examined patterns of fixed differences, private alleles and heterozygosity using the gl.fixed.diff and gl.report.heterozygosity functions in dartR.

Results

After filtering a total of 34,216, 8,488 and 1,577 polymorphic SNP loci were scored for the Full, SBA and Gellibrand datasets respectively.

Maximum Likelihood (ML) was run on the Full and SBA datasets with RAxML producing trees with respective likelihood scores of -319448.982609 and -67179.830838 and the rapid bootstrap search finished at 150 and 400 replicates (Figures 1 & 2). As is often the case with SNP datasets most deeper nodes for the Full dataset had strong support (100). The resulting

tree was quite similar to previous phylogenetic work (Hammer et al. 2014, Unmack et al. 2017) with the exception of the relationship between the southern Victorian clades, with SWV being sister to the SBA and SEV clades in my results. This analysis clearly places the unknown samples within the SBA clade with a close relationship to fish from the Gellibrand and Aire rivers. Bootstrap support for the SBA dataset was lower, but most nodes had moderate to strong support. Most fish were placed into groups that closely matched geography, reflecting the strong ability of SNP data to discriminate populations at finer geographic scales. The phylogenetic results from SBA confirm the results from the Full dataset, that the unknown fish group closely with fish from the Gellibrand and Aire rivers.

Table 1. Locality data, species and sample size examined for Gadopsis. Samples are organised first based on species, then by the informal taxonomy (Species2), then by geography.

Location Basin Species1 Species2 N Back Ck Cann marmoratus SEV 4 Delegate R Snowy marmoratus SEV 3 Haunted Stream Tambo marmoratus SEV 3 Thompson R Thompson marmoratus SEV 2 upper LaTrobe R LaTrobe marmoratus SEV 4 Turtons Ck Tarwin marmoratus SEV 4 Greig Ck Tarra marmoratus SBA 4 Deep Ck Franklin marmoratus SBA 4 Tin Mine Ck Franklin marmoratus SBA 1 Blackfish Ck Wilsons Prom marmoratus SBA 2 Minnieburn Ck Lang Lang marmoratus SBA 2 Diamond Ck Bunyip marmoratus SBA 2 Tarago R Bunyip marmoratus SBA 4 Donnellys Ck Yarra marmoratus SBA 2 Running Ck Yarra marmoratus SBA 1 Lerderderg R Werribee marmoratus SBA 3 Barwon R Barwon marmoratus SBA 2 Kuruc-A-Ruc Ck Woady Yaloak marmoratus SBA 2 Gellibrand R Gellibrand marmoratus SBA 3 Loves Ck Gellibrand marmoratus SBA 3 Ford R Aire marmoratus SBA 2 Styx R Derwent marmoratus SBA 2 Wye R Wye marmoratus SBA 2 Ansons R Ansons marmoratus SBA 1 Boobyalla R Boobyalla marmoratus SBA 1 Great Forester R Great Forester marmoratus SBA 1 Minnow R Mersey marmoratus SBA 2 Leven R Leven marmoratus SBA 2 Black R Black marmoratus SBA 2 Relapse Ck Arthur marmoratus SBA 2 unknown unknown marmoratus SBA 16 Brucknells Ck Hopkins marmoratus SWV 4 Mt Emu Ck Hopkins marmoratus SWV 2 Darlots Ck Darlots marmoratus SWV 2 Bridgewater Lakes Glenelg marmoratus NGW 2 Stokes R Glenelg marmoratus NGW 2 Wannon R Glenelg marmoratus NGW 3 Upper Glenelg Glenelg marmoratus NGW 1 Muddy Ck Glenelg marmoratus NGW 2 Mosquito Ck SE SA marmoratus NGW 2 Ewens Ponds SE SA marmoratus NGW 2 Henry Ck SE SA marmoratus NGW 1 Fyans Ck divers Wimmera marmoratus NGW 1 McKenzie R Wimmera marmoratus NGW 4 Mount Cole Ck Wimmera marmoratus NGW 1

Location Basin Species1 Species2 N

Nowhere Ck Wimmera marmoratus NGW 2

Browns Ck Condamine marmoratus NMD 2

Molong Ck Gwydir marmoratus NMD 2

McDonald R Naomi marmoratus NMD 2

Shawns Ck Castlereagh marmoratus NMD 2

Catherines Ck Lachlan marmoratus NMD 2

Stony Ck Murrumbidgee marmoratus NMD 2

Coppabella Ck Upper Murray marmoratus NMD 1

Kiewa R Kiewa marmoratus NMD 1

King R Ovens marmoratus NMD 1

Scrubby R Ovens marmoratus NMD 3

Seven Ck Goulburn marmoratus NMD 2

Birch Ck Loddon marmoratus NMD 2

Avoca R Avoca marmoratus NMD 2

Angas R Lower Murray marmoratus NMD 2

Marne R Lower Murray marmoratus NMD 4

Nangkita Ck Lower Murray marmoratus NMD 4

Rodwell Ck Lower Murray marmoratus NMD 4

Tookayerta Ck Lower Murray marmoratus NMD 1

Cotter Murrumbidgee bispinosus BE 2

Cotter R ACD Murrumbidgee bispinosus BE 2

Cotter R VC Murrumbidgee bispinosus BE 5

Goobarragandra R Murrumbidgee bispinosus BE 3

Cudgewa Ck Upper Murray bispinosus BE 2

Ovens R Ovens bispinosus BE 4

Stony Ck Ovens bispinosus BE 2

Hollands Ck Broken bispinosus BE 4

Criss Cross Ck Goulburn bispinosus BG 2

Goulburn R Goulburn bispinosus BG 1

Taggerty R Goulburn bispinosus BG 2

PCA results on SBA are similar to the phylogenetic results, with populations grouping geographically (Figure 3). It also highlights the unknown fish have a close relationship to fish from the Gellibrand and Aire rivers. The Gellibrand PCA results are shown from completeness sake (Figure 4). At this scale SNPs tend to be very good at separating individual populations if gene flow is low. It shows that the unknown fish are not from identical sites to the ones my samples came from.

Average observed heterozygosity by population varied from 0.005 to 0.062 (Table 2).

GMunkn.2 unknown.2

Figure 1. RAxML tree for

GMunkn.10 unknown.10

GMunkn.12 unknown.12

Gadopsis based on 34,216

GMunkn.15 unknown.15

GMunkn.7 unknown.7

GMunkn.9 unknown.9

SNPs. Clade names are

GMunkn.11 unknown.11

GMunkn.5 unknown.5

shown in bold.

GMunkn.14 unknown.14

Gellibrand,

69

GMunkn.3 unknown.3

GMunkn.6 unknown.6

100

Aire and

GMunkn.8 unknown.8

GMunkn.16 unknown.16

94

GMunkn.13 unknown.13

unknowns

GMunkn.4 unknown.4

GMunkn.1 unknown.1

GM02109.4 Loves Ck Gellibrand

GM27 Loves Ck Gellibrand

99

GMGell.3 Gellibrand R Gellibrand

74

GM73 Gellibrand R Gellibrand

GMGell.4 Gellibrand R Gellibrand

59

100

GM26 Loves Ck Gellibrand

GM23 Ford R Aire

89

GM02110.1 Ford R Aire

100

GM68 Relapse Ck Arthur

GM69 Relapse Ck Arthur

100

84

100

GM10 Barwon R Barwon

100

GM11 Barwon R Barwon

GM94 Kuruc-A-Ruc Ck Woady Yaloak

GM95 Kuruc-A-Ruc Ck Woady Yaloak

100

100

GM86 Donnellys Ck Yarra

99

GM85 Donnellys Ck Yarra

GM88 Lerderderg R Werribee

75

96

GMLerd.1 Lerderderg R Werribee

100

GM87 Lerderderg R Werribee

82

100

GM66 Great Forester R Great Forester

59

GM67 Boobyalla R Boobyalla

GM71 Black R Black

GM70 Black R Black

100

100

GM64 Leven R Leven

100

GM63 Leven R Leven

100

GM60 Wye R Wye

99

GM59 Wye R Wye

98

82

GM65 Ansons R Ansons

98

GM114 Styx R Derwent

81

GM113 Styx R Derwent

GM61 Minnow R Mersey

100

GM62 Minnow R Mersey

99

74

GM13 Deep Ck Franklin

SBA

GM0295.3 Deep Ck Franklin

100

GM0295.4 Deep Ck Franklin

100

GM12 Deep Ck Franklin

100

GM21 Tin Mine Ck Franlkin

100

GM7 Blackfish Ck Wilsons Prom

93

GM6 Blackfish Ck Wilsons Prom

GM0297.4 Greig Ck Tarra

95

GM0297.3 Greig Ck Tarra

100

GM15 Greig Ck Tarra

98

GM14 Greig Ck Tarra

87

100

GM25 Minnieburn Ck Lang Lang

GM24 Minnieburn Ck Lang Lang

GM34 Diamond Ck Bunyip

100

GM35 Diamond Ck Bunyip

90

100

GM8 Tarago R Bunyip

GM0282.3 Tarago R Bunyip

GM0282.4 Tarago R Bunyip

GM9 Tarago R Bunyip

100

GM105 Back Ck Cann

100

GMBack.4 Back Ck Cann

GM104 Back Ck Cann

GMBack.3 Back Ck Cann

SEV

GM2 Delegate R Snowy

10010093

GM0263.3 Delegate R Snowy

GM0263.4 Delegate R Snowy

GMThom.1 Thompson R Thompson

GMThom.2 Thompson R Thompson

100

100

100

GM4 Haunted Tambo

GM5 Haunted Tambo

GM0266.4 Haunted Stream Tambo

100

100

GM18 Turtons Ck Tarwin

95

94

GM0278.3 Turtons Ck Tarwin

100

GM19 Turtons Ck Tarwin

GM0278.4 Turtons Ck Tarwin

GM16 LaTrobe R LaTrobe

100

GM17 LaTrobe R LaTrobe

100

GM0281.3 upper LaTrobe R LaTrobe

SWV

78

GM0281.4 upper LaTrobe R LaTrobe

100

GM75 Darlots Ck Darlots

GMDarl.4 Darlots Ck Darlots

100

GM22 Mt Emu Ck Hopkins

100

GM31 Mount Emu Ck Hopkins

Figure 1 continued

88

GM0309.3 Brucknells Ck Hopkins

SWV

100

GM30 Brucknells Ck Hopkins

GM32 Brucknells Ck Hopkins

100

GM33 Brucknells Ck Hopkins

87

76

GM99 Mosquito Ck SE SA

100

GM98 Mosquito Ck SE SA

74

GM58 Henry Ck SE SA

GM39 Stokes R Glenelg

59

GM38 Stokes R Glenelg

99

71

GM76 Muddy Ck Glenelg

GM77 Muddy Ck Glenelg

100

100

GM78 Upper Glenelg Glenelg

100

GM53 Ewens Ponds SE SA

GM52 Ewens Ponds SE SA

100

GMA09122.2 Bridgewater Lakes Glenelg

100

NGW

GMA09122.1 Bridgewater Lakes Glenelg

100

GM20 Wannon R Glenelg

GM29 Wannon R Glenelg

100

100

GM48 McDonald R Naomi

100

GM37 Wannon R Glenelg

71

GM36 Fyans Ck divers Glenelg

99

GM89 Nowhere Ck Wimmera

GM90 Nowhere Ck Wimmera

100

GM42 Mount Cole Ck Wimmera

80

GM54 McKenzie R Wimmera

GM55 McKenzie R Wimmera

GM79 McKenzie R Wimmera

81

GM80 McKenzie R Wimmera

100

100

GM56 Catherines Ck Lachlan

GM57 Catherines Ck Lachlan

100

GM82 Angas R Lower Murray

95

GM81 Angas R Lower Murray

GM111 Rodwell Ck Lower Murray

97

GM112 Rodwell Ck Lower Murray

100

GM97 Stony Ck Murrumbidgee

71

NMD

GM96 Stony Ck Murrumbidgee

100

100

GM101 Nangkita Ck Lower Murray

GM100 Nangkita Ck Lower Murray

100

GM119 Nangkita Ck Lower Murray

87

GM106 Tookayerta Ck Lower Murray

64

Gb11 Scrubby R Ovens

Gb12 Scrubby R Ovens

100

97

Gb10 Scrubby R Ovens

57

GM44 King R Ovens

100

GM91 Kiewa R Kiewa

GM43 Coppabella Ck Upper Murray

99

GM107 Marne R Lower Murray

100

GM109 Marne R Lower Murray

GM108 Marne R Lower Murray

73

100

GM92 Avoca R Avoca

GM93 Avoca R Avoca

95

GM116 Birch Ck Loddon

64

GM117 Birch Ck Loddon

GM40 Seven Ck Goulburn

GM41 Seven Ck Goulburn

100

100

GM84 Molong Ck Gwydir

100

88

GM83 Molong Ck Gwydir

GM49 McDonald R Naomi

100

GM47 Shawns Ck Castlereigh

100

GM46 Shawns Ck Castlereigh

GM51 Browns Ck Condamine

BG

54

GM50 Browns Ck Condamine

100

59

Gb1 Taggerty R Goulburn

85

Gb3 Taggerty R Goulburn

100

GBGoul.2 Goulburn R Goulburn

GBCris.1 Criss Cross Ck Goulburn

GBCris.2 Criss Cross Ck Goulburn

100

100

GBHoll.4 Hollands Ck Broken

100

GBHoll.3 Hollands Ck Broken

GBHoll.2 Hollands Ck Broken

100

GBHoll.1 Hollands Ck Broken

93

100

Gb16 Ovens R Ovens

100

Gb15 Ovens R Ovens

100

Gb14 Ovens R Ovens

88

Gb8 Cudgewa Ck Upper Murray

BE

GM1907.2 Goobarragandra R Murrumbidgee

100

100

GM1907.3 Goobarragandra R Murrumbidgee

GM1907.1 Goobarragandra R Murrumbidgee

GM20AA.1 Cotter R ACD Murrumbidgee

100

GM18AA.5 Cotter R VC Murrumbidgee

100

GM18AA.2 Cotter R VC Murrumbidgee

GM18AA.4 Cotter R VC Murrumbidgee

GM18AA.1 Cotter R VC Murrumbidgee

Gadgbi10 Stony Ck Ovens

Gb5 Cotter Murrumbidgee

GM20AA.2 Cotter R ACD Murrumbidgee

Gadgbi9 Stony Ck Ovens

Gb6 Cotter Murrumbidgee

GM18AA.3 Cotter R VC Murrumbidgee

59

0.02

Figure 2. RAxML tree for the SBA Gadopsis clade based on 8,488 SNPs.

GMunkn.7 unknown.7 unknown GMunkn.9 unknown.9 unknown GMunkn.11 unknown.11 unknown GMunkn.12 unknown.12 unknown GMunkn.15 unknown.15 unknown GMunkn.10 unknown.10 unknown GMunkn.5 unknown.5 unknown GMunkn.2 unknown.2 unknown GMunkn.3 unknown.3 unknown GMunkn.6 unknown.6 unknown

Gellibrand,

78

GMunkn.16 unknown.16 unknown

GMunkn.8 unknown.8 unknown

100 GMunkn.13 unknown.13 unknown

99 GMunkn.14 unknown.14 unknown

Aire and unknowns

GMunkn.4 unknown.4 unknown

54

GMunkn.1 unknown.1 unknown

GMGell.3 Gellibrand R Gellibrand

GMGell.4 Gellibrand R Gellibrand

GM02110.1 Ford R Aire

GM23 Ford R Aire

100

GM73 Gellibrand R Gellibrand

100

GM02109.4 Loves Ck Gellibrand

GM27 Loves Ck Gellibrand

99

GM26 Loves Ck Gellibrand

79 100

GM94 Kuruc-A-Ruc Ck Woady Yaloak

100

GM95 Kuruc-A-Ruc Ck Woady Yaloak

Port

GM10 Barwon R Barwon GM11 Barwon R Barwon

100

100

GM86 Donnellys Ck Yarra

99

93

GM85 Donnellys Ck Yarra

Phillip / Barwon

GM88 Lerderderg R Werribee

100

GM87 Lerderderg R Werribee

10084

GMLerd.1 Lerderderg R Werribee

far NW Tasmania

GM68 Relapse Ck Arthur

GM69 Relapse Ck Arthur

100

100

GM62 Minnow R Mersey

100

GM61 Minnow R Mersey

GM113 Styx R Derwent

79

GM114 Styx R Derwent

99

most of northern

GM65 Ansons R Ansons

57

GM60 Wye R Wye

99

Tasmania

GM59 Wye R Wye

100

GM63 Leven R Leven

GM64 Leven R Leven

100

100

GM70 Black R Black

GM71 Black R Black

100

GM67 Boobyalla R Boobyalla

GM66 Great Forester R Great Forester

100

100

GM9 Tarago R Bunyip

71

GM0282.4 Tarago R Bunyip

53

GM8 Tarago R Bunyip

98

Western Port

GM34 Diamond Ck Bunyip GM0282.3 Tarago R Bunyip 100

GM35 Diamond Ck Bunyip GM24 Minnieburn Ck Lang Lang

GM25 Minnieburn Ck Lang Lang

100

73

GM0295.3 Deep Ck Franklin 52

GM13 Deep Ck Franklin

100

98

GM12 Deep Ck Franklin

100

GM0295.4 Deep Ck Franklin

South

GM21 Tin Mine Ck Franklin

100

GM7 Blackfish Ck Wilsons Prom

68

GM6 Blackfish Ck Wilsons Prom

Gippsland

GM0297.4 Greig Ck Tarra

95

GM0297.3 Greig Ck Tarra

100

GM15 Greig Ck Tarra

100

GM14 Greig Ck Tarra

63

0.02

Figure 3. PCA plot for SBA populations coloured by river.

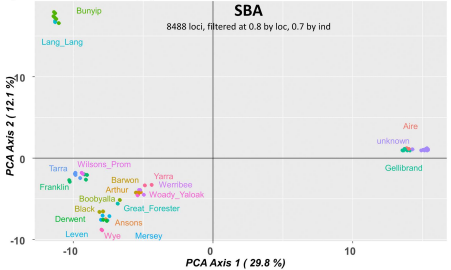


Figure 4. PCA plot for Gellibrand, Aire and unknown populations.

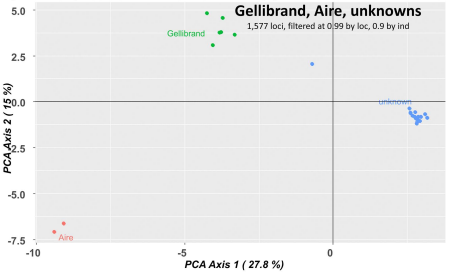


Table 2. Observed heterozygosity by river, sorted from lowest to highest. N indicates the sample size.

pop N Ho

Wye 2 0.005

Aire 2 0.007

Arthur 2 0.009

Woady Yaloak 2 0.014

unknown 16 0.024

Boobyalla 1 0.025

Gellibrand 6 0.027

Lang Lang 2 0.036

Franklin 5 0.037

Barwon 2 0.038

Ansons 1 0.039

Derwent 2 0.043

Bunyip 6 0.044

Werribee 3 0.045

Black 2 0.046

Mersey 2 0.047

Leven 2 0.056

Wilsons Prom 2 0.052

Yarra 2 0.056

Great Forester 1 0.062

Tarra 4 0.064462

Discussion

The SNP results very closely match previous work on this genus (Hammer et al. 2014; Unmack et al. 2017). The unknown samples are all unequivocally part of the SBA clade of Gadopsis marmoratus. We have samples from the majority of the rivers that contain Gadopsis in the SBA region. The SNP data allow populations by river to be very well separated from one another, and nearby rivers tends to group together, matching the geography and biogeographic separation of rivers across southern Victoria. For instance, Victorian populations broadly grouped into the following regions: South Gippsland, Western Port Bay, Port Phillip Bay and the adjacent Barwon River, and the Gellibrand and Aire rivers.

Given the lack of any major unsampled rivers it is quite clear the unknown samples are from the Gellibrand / Aire region. In addition to the evidence from the phylogenetic and PCA analyses, the levels of heterozygosity of the unknown samples match closely with those from the Gellibrand River, thus being consistent with the other results.

It is critical that any fish from the “unknown” population do not get stocked into regions containing the other Gadopsis clades. These are likely to eventually each get described as separate species as the genetic divergences between them are quite large and there is good preliminary morphological evidence for the separation of some clades (e.g., northern vs southern G. marmoratus) from the early unpublished PhD research of Andrew Sanger. It is highly likely that fish from these different clades could hybridise which would potentially impact on their integrity.

I do not see any issues with stocking fish from this unknown population into waterways in the Gellibrand or Aire rivers, although with some slight caution given we only had a very limited sample from the Aire River. Any consideration of stocking beyond these two river systems would require additional careful consideration. There is an increasing push in the conservation biology literature to consider translocations between less genetically related populations to bolster genetic diversity and fitness (Frankham et al. 2019). However, determining what to stock where depends on many factors, such as other populations present within that river system that could be sourced for mixing, availability of fish, and which other populations to use as part of the mix.

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