

Reconstructing recent divergence: evaluating nonequilibrium population structure in New Zealand chinook salmon

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Abstract

Newly established or perturbed populations are often the focus of conservation concerns but they pose special challenges for population genetics because drift–migration equilibrium is unlikely. To advance our understanding of the evolution of such populations, we investigated structure and gene flow among populations of chinook salmon that formed via natural straying following introduction to New Zealand in the early 1900s. We examined 11 microsatellite loci from samples collected in several sites and years to address two questions: (i) what population differentiation has arisen in the ≈ 30 generations since salmon were introduced to New Zealand, relative to temporal variation within populations; and (ii) what are the approximate effective population sizes and amounts of gene flow in these populations? These questions are routinely addressed in studies of indigenous populations, but less often in the case of new populations and rarely with consideration of equilibrium assumptions. We show that despite the recent introduction, continued gene flow and high temporal variability among samples, detectable population structure has arisen among the New Zealand populations, consistent with their colonization pattern and isolation by geographical distance. Furthermore, we use simple individual-based simulations and estimates of effective population sizes to estimate the effective gene flow among drainages under likely nonequilibrium conditions. Similar methodology may be broadly applicable to other studies of population structure and phenotypic evolution under similar nonequilibrium, high gene flow conditions.

Keywords: contemporary evolution, effective population size, gene flow, genetic equilibrium, temporal variation, transplant

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Introduction

Drift–migration equilibrium and restricted gene flow are common themes in evolutionary theory and empirical analyses. Population structure and the strength of selection among geographically disjunct subpopulations are most easily studied when gene flow is negligible but many species exist as genetically linked subpopulations. Moreover, equilibrium conditions between drift and migration are unlikely over short time scales in many perturbed or new populations (an increasingly large proportion of

biodiversity). Empirical and theoretical population genetic research that does not assume equilibrium and restricted gene flow is required to understand the biology of such populations, which are often of pressing concern (e.g. endangered species or exotic invasive species).

Although most molecular ecologists would acknowledge that many populations probably do not conform to assumed equilibrium conditions, equilibrium approximations are still commonly employed. Gene flow (i.e. $N_e m$), for example, is often estimated using Wright's (1943) F_{ST} approximation despite recognition that the method is potentially highly biased under nonequilibrium conditions and other violations of an infinite island model (Whitlock & McCauley 1999). One factor slowing development of

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population genetic approaches suitable for nonequilibrium cases, such as during the initial development of population divergence, may be that few real-world study systems have been approached explicitly as case studies of nonequilibrium genetics. Introduced populations can provide model systems to study the nonequilibrium patterns that likely characterize the early stages of divergence. Introductions often afford an *a priori* knowledge of population history and phylogeny that can aid in interpreting genetic patterns, and may thus lead to insights useful for interpreting patterns found in populations with unknown histories. For example, if populations show similar patterns of divergence to those known to develop *de novo* over very short time frames, their population structure may reflect recent evolution more than historical contexts; a potentially important insight in planning conservation and management measures.

In this study we consider the evolution of population structure *de novo*. Not all studies of the population genetics of introduced populations are equally suited to this goal. Some studies of population structure following introductions have been limited because comparisons involved only a single year of data (e.g. Quinn *et al.* 1996) or confounded sampling of sites and years (e.g. Gharrett & Thomason 1987; Marsden *et al.* 1995). Such sampling plans assume stable allele frequencies, which risks confounding spatial structure with temporal variation and sampling error. In addition, some introduction studies have involved numerous (or unknown) introductions from multiple sources to various sites within the introduced range (Baker

& Moeed 1987; Krueger & May 1991; Burger *et al.* 2000) or into systems containing existing populations (Hendry *et al.* 1996). Assortment of ancestral lineages by habitat (e.g. Burger *et al.* 2000), introductions of different lineages to different sites (e.g. Baker & Moeed 1987) or varying admixtures of lineages at different sites, are likely to inflate the apparent divergence rate when studied years after the introduction. What is required for the above goal is multiple years of genetic data from populations that all derive from a common source.

The introduction of chinook salmon (*Oncorhynchus tshawytscha*) to New Zealand has provided an opportunity to study the development of population differentiation at a relatively early stage. All chinook salmon in New Zealand derive from introductions to the Waitaki River system, primarily the Hakataramea River, between 1901 and 1907 (McDowall 1994; Fig. 1). Introduced chinook were imported as embryos from the Sacramento River system, California, in particular a Battle Creek population that returned to freshwater in late summer/early fall. By 1907 anadromous fish, resulting from releases of the imported salmon, were observed spawning in the Hakataramea River. Within 5–10 years other spawning populations formed by natural straying and colonization in the other major river drainages along the east coast of the South Island (McDowall 1994), where self-sustaining populations exist today (≈ 5000 –20 000 mature individuals return yearly to the major river drainages; West & Goode 1987). Salmon did not naturally disperse to rivers south of the Waitaki system (notably the Clutha River), but were introduced

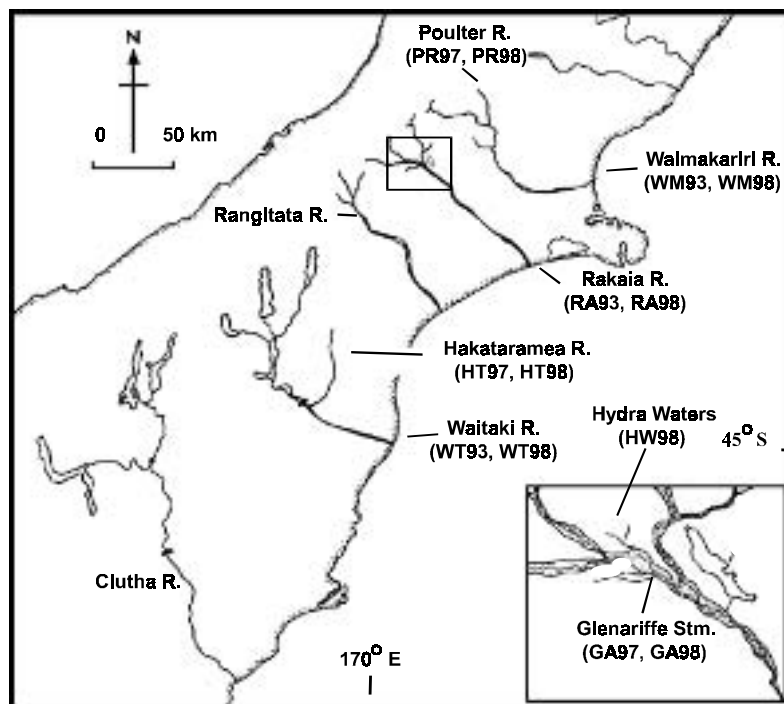


Fig. 1 Map of the central South Island of New Zealand showing major salmon bearing rivers and study sites. Sample abbreviations used in following tables and figures are indicated in parentheses, with the last two digits indicating the sampling year. Not shown are the 1997 and 1998 samples from Battle Creek, CA (BC97 and BC98) and the 1996 sample from the Washougal River, WA (WA96).

there from salmon returning to the Waitaki system. Hence, all chinook salmon populations in New Zealand derive from a common ancestral source introduced to the Waitaki drainage, and most were formed by natural straying and colonization processes.

Several lines of evidence indicated to us that detectable population structuring has arisen in New Zealand. Multiple studies have revealed divergence in phenotypic traits among New Zealand salmon populations (e.g. Quinn & Unwin 1993; Kinnison *et al.* 1998a,b,c, 2001; Quinn *et al.* 2000; Unwin *et al.* 2000) consistent with adaptation to different drainages. Recoveries of adult New Zealand salmon, tagged as juveniles and released during spring and summer from a hatchery between 1978 and 1984, revealed strong philopatry: 96% of recoveries were in the river of origin and of those 89% were recovered at their release site (Unwin & Quinn 1993). Straying across drainages was biased in a northerly direction and greatest into neighbouring rivers and larger rivers. Overall these results clearly indicate an opportunity for partial genetic isolation. Finally, an initial study by Quinn *et al.* (1996), based on a single year of sampling and 24 polymorphic allozyme loci and mitochondrial DNA (mtDNA), detected variation among New Zealand drainages, albeit on an approximate scale with interannual variation seen within populations in California.

Here we report on a more extensive study of population structure in New Zealand chinook salmon based on 11 microsatellite loci. Our primary objectives were: (i) to evaluate spatial and temporal patterns of population structure in these recently derived populations, and (ii) to estimate effective population sizes and migration rates that may help interpret phenotypic divergence patterns (reviewed in Quinn *et al.* 2001). We put forth the New Zealand populations as a case study in the nonequilibrium genetics of initial population divergence and have thus adopted approaches that do not assume drift-migration equilibrium for all of our analyses (barring occasional comparisons with equilibrium approximations).

Materials and methods

Samples and processing

New Zealand tissue samples were obtained from three sources, the samples of Quinn *et al.* (1996), adults returning to spawning tributaries and fry captured in tributaries and the mainstems of rivers. In our terminology, 'mainstem' refers to a sampling locale within the main channel of a river system, whereas the term 'drainage' is used in reference to the entire river (i.e. mainstem and tributaries) and its associated gene pool. Samples included tissue (muscle or fin tissue) from fry obtained via electrofishing, or from adults (live or fresh dead) collected during weir or

stream survey operations. The samples of Quinn *et al.* (1996) were preserved by freezing; those collected later were preserved in 90% ethanol. We obtained 13 population samples from three major drainages in New Zealand, including samples from the three mainstems in 1993 and 1998 and for a spawning tributary within each system in 1997 and 1998: the Waitaki system including the Hakataramea River, the Rakaia system including Glenariffe Stream, and the Waimakariri system including the Poulter River. We obtained samples from a second spawning tributary in the Rakaia River in 1998, the Hydra Waters, located < 5 km from Glenariffe Stream. Spawning and capture of fry all occur in the same calendar year in New Zealand (Unwin 1986) and thus adult or fry samples referring to the same nominal year correspond to genotypes involved in the same breeding season. Two outgroups were incorporated in the analysis: fall-returning, Battle Creek salmon from the 1997 and 1998 spawning seasons (provided by Jennifer Nielsen, Alaska Biological Sciences Division, U.S. Geological Survey, Anchorage, AK, USA), and 52 chinook salmon from the Washougal River, a tributary of the Columbia River, WA (from Jeffrey Olsen, Gene Conservation Laboratory, Alaska Department of Fish and Game, Anchorage, AK, USA). The identity of sample types (Quinn *et al.* 1996, adult fin clip or fry sample) and their corresponding sample sizes (ranging from 23 to 80 individuals) are listed in Table 1.

DNA samples were extracted from 20 to 50 mg of tissue using protocols based on the Gentra Systems® (Minneapolis, MN, USA) Puregene DNA isolation kit and were rehydrated in TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0). All samples were quantified by spectrophotometry and diluted to 100 ng/μL for amplification using the polymerase chain reaction (PCR). Eleven loci were coamplified for each individual in two 10 μL multiplexed reactions of five and six loci, respectively (Table 2), as described by Olsen *et al.* (2001). PCR amplifications were performed in a Perkin-Elmer 9600 thermocycler, using fluorescently labelled primers. Microsatellite alleles were size fractionated and detected using 6.0% denaturing polyacrylamide gels in an Applied Biosystems Inc. (ABI) 373XL automated DNA sequencer in GeneScan® XL mode. Multilocus genotypes were then scored by size (bases) relative to an internal, fluorescent-labelled, DNA size-standard (Genescan-500® TAMRA) using GENESCAN ANALYSIS® Version 3.1 and GENOTYPER® Version 2.5 software. An individual of known genotype was amplified and run on every gel to ensure consistency of amplification and scoring.

Population structure analysis

Allelic richness was computed, taking into account rarefaction associated with sample size, using the program FSTAT (Goudet 2001). Tests of conformation to Hardy-Weinberg

Table 1 Microsatellite loci, primer concentrations and PCR profiles used for examination of New Zealand chinook population structure

Multiplex / locus	Forward or reverse	Source	Conc. (μ M)	Thermal profile $^{\circ}$ C (min) + $^{\circ}$ C (min) + $^{\circ}$ C (min)
Multiplex 1*				
<i>T-Omy325</i> †	F	O'Connell <i>et al.</i> (1997)	0.07	
	R		0.07	
<i>H-Oneμ8</i> †	F	Scribner <i>et al.</i> (1996)	0.06	5 cycles TD:‡
	R		0.06	94 $^{\circ}$ (1.0) + 63.5 $^{\circ}$ \rightarrow 59.5 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>T-Ots100</i> §	F	Nelson & Beacham (1999)	0.13	7 cycles:
	R		0.13	94 $^{\circ}$ (1.0) + 58.5 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>H-Ocl1</i> †	F	Condrey & Bentzen (1998)	0.06	17 cycles:
	R		0.06	94 $^{\circ}$ (0.42) + 58.5 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>F-Oki23</i> §	F	A. Spidle, unpublished	0.12	1 cycle:
	R		0.12	72 $^{\circ}$ (40)
Multiplex 2*				
<i>F-Ots2</i> †	F	Banks <i>et al.</i> (1999)	0.16	
	R		0.16	
<i>F-Ots101</i>	F	Small <i>et al.</i> (1998)	0.24	5 cycles TD:‡
	R		0.24	94 $^{\circ}$ (1.0) + 54.8 $^{\circ}$ \rightarrow 50.8 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>T-Ots3</i> †	F	Banks <i>et al.</i> (1999)	0.32	7 cycles:
	R		0.32	94 $^{\circ}$ (1.0) + 49.8 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>T-Ots102</i> §	F	Nelson & Beacham (1999)	0.15	18 cycles:
	R		0.15	94 $^{\circ}$ (0.42) + 49.8 $^{\circ}$ (0.5) + 72 $^{\circ}$ (0.25)
<i>H-Oneμ10</i> †	F	Scribner <i>et al.</i> (1996)	0.30	1 cycle:
	R		0.30	72 $^{\circ}$ (30)
<i>H-Ots104</i> §	F	Nelson & Beacham (1999)	0.37	
	R		0.37	

*Prefix of locus indicates fluorescent label: H = Hex, T = Tet, F = Fam. †Dinucleotide repeat. ‡Touch-down reaction with annealing temperature decreasing by 1 $^{\circ}$ C each cycle. §Tetranucleotide repeat (primarily).

equilibrium (HWE) and genotypic linkage disequilibrium were performed using Markov chain methods in GENEPOP Version 3.1d (Raymond & Rousset 1995). Markov chain methods in GENEPOP were also used to test for genic and genotypic divergence in allele frequencies among populations for all loci and population pairs. Multilocus pairwise estimates of F_{ST} were estimated by θ (Weir & Cockerham 1984) computed using GENETIX Version 4.0 (Belkhir 1999).

The choice of correction procedure for multiple contrasts depended on whether individual tests represented component evaluations of an overarching hypothesis (e.g. combined loci inference of population divergence) or whether individual tests were themselves informative of direct interest (e.g. which sets of populations differ). If the inference was whether sufficient multilocus or multigroup tests supported a given overarching hypothesis (e.g. HWE over multiple loci at a given site or over multiple sites for a given locus) we estimated the likelihood (L) of obtaining as many (or more) significant tests as actually obtained by chance using the binomial likelihood function (Chapman *et al.* 1999)

$$L = \sum_{i=r}^n C(1 - \alpha)^{n-i} (\alpha)^i$$

where n is the total number of tests, r is the number of significant tests at a given α (alpha level of statistical significance), and C is the factorial constant $n!/(r!(n-r)!)$. When this likelihood was < 0.05 the null hypothesis of such a result by chance was rejected and the overarching hypothesis was considered supported. Sequential Bonferroni testing (cf. Rice 1989) was employed when inference concerned which individual tests, out of a group of related tests at a common α , were significant.

Estimates of θ were used to construct a neighbour-joining (NJ) tree using the NEIGHBOUR component of PHYLIP Version 3.5c (Felsenstein 1993). Because of our knowledge of the history of the New Zealand populations we were able to contrast empirical population structure inferred from our microsatellite dataset with *a priori* predictions. We predicted that the following hierarchical tree topology would occur within New Zealand: samples from the same sites in different years should form clades, sites within drainages should form clades, and finally drainages of closest geographical proximity should form clades. We tested whether our empirical tree corresponded closer with the predicted tree than expected for a tree of random topology (i.e. no meaningful population or temporal structure). Unrooted trees can be decomposed into a finite

Table 2 Diversity of allelic variation. Source = origin of samples, N = range of sample sizes per sample and locus, A_T = total number of alleles per locus, H_E = expected heterozygosity corrected for sampling bias, H_O = observed heterozygosity. Results of sample by locus and overall locus and sample tests of conformance to Hardy–Weinberg expectations are provided. Site abbreviations are as indicated in Fig. 1

		Battle Creek, CA			Waitaki Drainage, New Zealand				Rakaia Drainage, New Zealand				Waimakariri Drainage, New Zealand				
		WA96	BC97	BC98	WT93	WT98	HT97	HT98	RA93†	RA98	GA97	GA98	HW98	WM93	WM98	PR97	PR98
Source‡		O	A	A	Q	F	A	F	Q	F	A	A	A	Q	F	A	F
N		50–52	57–60	39–40	79–80	47–50	65–68	23	71–78	58–60	22–24	57–61	49–50	66–71	59–60	28–31	66–73
<i>Ocl1</i>	A_T	12	6	7	6	5	6	5	6	6	5	6	6	6	6	5	6
	H_E	0.83	0.77	0.77	0.73	0.63	0.72	0.70	0.75	0.73	0.77	0.71	0.77	0.75	0.75	0.71	0.75
	H_O	0.78	0.74	0.83	0.74	0.62	0.79	0.74	0.76	0.73	0.83	0.82	0.86	0.76	0.82	0.61*	0.74
<i>Oki23</i>	A_T	18	21	19	19	17	14	13	16	16	14	16	15	16	15	13	18
	H_E	0.92	0.95	0.94	0.91	0.92	0.87	0.91	0.87	0.87	0.92	0.91	0.90	0.90	0.88	0.91	0.90
	H_O	0.54*	0.83*	0.85	0.64*	0.74*	0.63*	0.70	0.56*	0.58*	0.71*	0.69*	0.66*	0.68*	0.56*	0.74	0.66*
<i>Omy325</i>	A_T	9	11	9	6	5	8	5	5	5	5	6	5	6	6	4	4
	H_E	0.75	0.54	0.63	0.41	0.51	0.49	0.49	0.46	0.45	0.61	0.50	0.53	0.51	0.55	0.54	0.52
	H_O	0.69	0.50	0.68	0.40	0.62	0.49	0.52	0.54	0.43	0.63	0.52	0.54	0.48	0.48	0.55	0.48*
<i>Oney10</i>	A_T	8	9	9	7	5	7	4	7	7	6	7	6	6	7	6	6
	H_E	0.70	0.48	0.59	0.50	0.47	0.57	0.40	0.59	0.53	0.46	0.55	0.51	0.48	0.53	0.41	0.44
	H_O	0.79	0.48	0.58	0.51	0.60	0.60	0.39	0.63	0.48	0.46	0.59	0.50	0.52	0.53	0.42	0.38
<i>Oney8</i>	A_T	14	12	12	9	9	9	8	9	10	8	8	9	11	8	9	11
	H_E	0.85	0.78	0.87	0.77	0.66	0.69	0.69	0.68	0.62	0.73	0.71	0.59	0.73	0.72	0.74	0.75
	H_O	0.80	0.83	0.85	0.81	0.72	0.61*	0.61	0.74	0.63	0.75	0.65	0.62	0.73	0.65	0.74	0.71
<i>Ots100</i>	A_T	33	37	29	27	24	27	18	21	28	22	25	27	28	29	17	28
	H_E	0.94	0.97	0.95	0.92	0.93	0.93	0.93	0.92	0.94	0.95	0.95	0.93	0.94	0.93	0.91	0.94
	H_O	0.94	0.90	0.95	0.94	0.88*	0.88	0.91	0.92*	0.97	1.00	0.87	0.94	0.93	0.90	0.90	0.99
<i>Ots101</i>	A_T	27	28	25	23	22	21	15	21	21	18	24	20	23	20	17	23
	H_E	0.96	0.96	0.95	0.92	0.93	0.91	0.90	0.91	0.93	0.92	0.94	0.92	0.93	0.92	0.91	0.93
	H_O	0.90*	0.92	0.97	0.87	0.92	0.96	0.87	0.86*	0.91	0.88	0.97	0.90	0.90	0.92	0.84	0.82*
<i>Ots102†</i>	A_T	32	29	25	30	24	28	22	18	21	18	24	21	25	23	17	21
	H_E	0.97	0.94	0.96	0.94	0.93	0.95	0.96	0.92	0.95	0.95	0.95	0.94	0.94	0.95	0.93	0.92
	H_O	0.68*	0.61*	0.63*	0.66*	0.68*	0.65*	0.55*	0.31*	0.58*	0.68*	0.60*	0.61*	0.56*	0.49*	0.50*	0.55*
<i>Ots104</i>	A_T	26	20	20	18	15	18	15	19	17	15	16	15	17	14	14	14
	H_E	0.96	0.94	0.94	0.90	0.91	0.92	0.93	0.93	0.92	0.92	0.90	0.89	0.90	0.91	0.92	0.88
	H_O	0.92*	0.98	0.95	0.90	0.88	0.94	0.96	0.91*	0.88	0.92	0.92	0.84	0.93	0.92	0.94	0.92
<i>Ots2</i>	A_T	12	10	12	11	11	11	8	11	11	8	10	8	8	11	6	9
	H_E	0.83	0.78	0.83	0.72	0.75	0.78	0.66	0.76	0.78	0.76	0.72	0.67	0.77	0.79	0.76	0.75
	H_O	0.85*	0.73	0.80	0.66	0.78	0.78	0.78	0.78*	0.75	0.83	0.67	0.60	0.75*	0.77	0.71	0.71

Table 2 Continued

	Battle Creek, CA			Waitaki Drainage, New Zealand			Rakaia Drainage, New Zealand			Waimakariri Drainage, New Zealand						
	WA96	BC97	BC98	WT93	WT98	HT97	HT98	RA93†	RA98	GA97	GA98	HW98	WM93	WM98	PR97	PR98
O/s3																
A_T	9	10	10	9	9	10	9	8	9	9	9	10	9	9	9	9
H_E	0.86	0.84	0.85	0.86	0.87	0.86	0.87	0.85	0.86	0.88	0.85	0.85	0.82	0.86	0.82	0.82
H_O	0.83	0.83	0.79	0.86	0.90	0.90	0.91	0.88	0.85	0.96	0.80*	0.88	0.76	0.88	0.77	0.88
Across all loci (11)																
A_T	200	195	178	166	147	160	123	142	152	129	152	143	156	150	118	150
H_E	0.87	0.81	0.84	0.78	0.77	0.79	0.77	0.79	0.78	0.81	0.79	0.77	0.79	0.80	0.78	0.78
H_O	0.79	0.76	0.81	0.72	0.75	0.75	0.72	0.72	0.71	0.76	0.74	0.73	0.72	0.72	0.70	0.71
Across HWE loci (9)																
A_T	150	135	134	117	106	118	88	108	115	97	112	107	115	112	88	111
H_E	0.85	0.78	0.82	0.75	0.74	0.76	0.73	0.76	0.75	0.78	0.76	0.74	0.76	0.77	0.75	0.75
H_O	0.83	0.77	0.82	0.74	0.76	0.77	0.74	0.78	0.74	0.81	0.76	0.74	0.75	0.76	0.72	0.74

*Deviation from Hardy-Weinberg expectations ($P \leq 0.05$). †Failed likelihood method test by exceeding number of samples or loci expected to violate tests of Hardy-Weinberg equilibrium by chance. ‡Sources are as follows: A = Mature Adults; F = Fry; Q = Samples collected by Quinn *et al.* (1996), and O = genotypes provided by Jeff Olsen.

number of subunit trees, each describing the affinities of four taxonomic units called quartets [number of quartets = $n(n-1)(n-2)(n-3)/24$, where n = number of taxonomic units]. Tree dissimilarity was measured as the number of differing quartets, d , between two trees (Estabrook *et al.* 1985). Ten thousand random topology trees, with the same number of labelled taxonomic groups as the predicted and empirical trees, were created using the program COMPONENT Version 2.00a (Page 1993) and d was estimated for comparison of each with the predicted tree. Dissimilarity of the empirical and predicted trees was then compared with the distribution of d for the random trees.

The hypothesis that population structure follows an isolation-by-distance (IBD; Wright 1943) pattern was further examined using a Mantel test (15 000 permutations implemented in GENEPOP Version 3.1) of the nonparametric significance of the correlation between $F_{ST}(1 - F_{ST})^{-1}$ and distance, using θ as an estimator of F_{ST} , as appropriate for the case of populations along primarily linear habitats (Rousset 1997). Interannual and among-site variation in F -statistic analogs were examined by hierarchical analysis of molecular variance (AMOVA, see Michalakis & Excoffier 1996 for general formulation) implemented in ARLEQUIN Version 2.0 (Schneider *et al.* 2000). Three AMOVA tests were performed, with the significance of structure provided by nonparametric permutation. These included analyses of years within mainstems, years within tributaries, and samples (1997 and 1998 tributaries and 1998 mainstems) within major New Zealand spawning systems, all within the total genetic diversity for the samples in the analysis.

Temporal variation in allele frequencies between fry samples collected in 1993 and 1998 were used to estimate the effective number of breeders per generation (N_b) for the Waitaki and Waimakariri drainages using the methodology of Waples (1990b; equation 6) with the parameter b estimated using a BASIC script described by Tajima (1992: $b = 3.12$ or 3.22 for New Zealand pops, respectively, for 5 years). Rakaia mainstem samples were excluded because the sample collected in 1993 showed evidence of potentially nonrandom sampling (see below). Alleles with mean frequencies, across samples at a site, of < 0.02 were combined to create one or several pseudo-alleles with frequencies > 0.02 (R. Waples, personal communication) to maximize precision and to minimize bias associated with using alleles with very low frequencies (cf. Waples 1990b). In total, there were 95 effective alleles for the Waimakariri samples and 93 for the Waitaki samples. Mean generation length, used to estimate the generational effective population size (N_e) from N_b as described by Waples (1990a), was estimated at 3.34 years for the Waitaki population (from Quinn & Unwin 1993) and 3.14 years (the New Zealand average) for the Waimakariri population. Confidence bounds were estimated according to Waples (1989).

Under a range of conditions, including the absence of drift–migration equilibrium (or a small number of sub-populations), the effective number of migrants, $N_e m$, is not related to F_{ST} by Wright's simple approximation (Whitlock & McCauley 1999). However, as a statistic of population divergence arising since colonization, F_{ST} is still dependent on effective migration rates (i.e. $N_e m$) and patterns. We used simulations to determine the relationship among $N_e m$ and F_{ST} under divergence from initial random distribution of genotypes among populations. We then in turn estimated what combination of N_e and m would result in values of θ approximating those observed under non-equilibrium conditions in New Zealand. This approach took advantage of our knowledge of the history of these populations (e.g. generations since introduction).

Simulations were run using the program EASYPOP Version 1.7.2 (Balloux 1999) assuming random mating over 27 generations for five populations (the number of major spawning rivers on the east coast of the South Island: the Clutha, Waitaki, Rangitata, Rakaia and Waimakariri rivers) in both a one-dimensional stepping-stone array and an island array. These migration patterns were chosen as two extremes (migrants only exchanged with neighbouring populations vs. migrants exchanged equally among all populations) that bracket the domain of the more realistic, but more complex, IBD pattern (most migrants with neighbouring populations but some with more distant populations). Runs simulated divergence (measured as the multilocus estimator of $F_{ST} : G_{ST}$) at nine independent loci (number ultimately used in our analysis of New Zealand structure), each with 17 allelic states (approximately the mean number of alleles per locus for the loci actually used in population structure analyses). To estimate how far the populations were from drift–migration equilibrium we simulated 1000 generations under both models, employing our inferred N_e and m , and determined if values of F_{ST} at 27 generations approached asymptotic values.

A final nonequilibrium method of detecting population structure and dispersal among drainages involved the use of a multilocus genotype (re)assignment test with leave-one-out classification. We used a Bayesian approach implemented in the GENECLASS Version 1.0 software of Piry & Cornuet (1999) that was inspired by the methods of Rannala & Mountain (1997). Classification success was examined for tributaries and mainstems, separately in each sampling year. Success of classification for a given sample was compared with classification under random assignment using χ^2 tests.

Results

All loci were polymorphic in all samples (Table 2). The number of alleles per locus across all sites, including the North American populations, ranged from 11 for *Oneu10*

to 64 for *Ots100*, for a total of 285 with a mean of 25.9 alleles per locus. However, the Washougal River outgroup (hereafter WA) contributed a large number of unique alleles (46 over all loci; 16.1% of the total allelic richness). Taking into account sample sizes, New Zealand salmon possessed the lowest allelic richness at all loci (average number of alleles per locus evaluated at $n = 50$; NZ = 14; CA = 17.3; WA = 18.1). The WA population had the highest mean expected heterozygosity (0.86), followed by Battle Creek (0.82), and the average for the New Zealand populations was the lowest (0.78). Paired tests over all-loci confirmed that Battle Creek fish had significantly higher expected heterozygosity than New Zealand fish (mean difference = 0.04, $P = 0.002$) as well as significantly higher allelic richness (corrected to $n = 50$: mean difference = 3.3 alleles; $P < 0.001$). However, within New Zealand there was no indication that allelic richness (evaluated at $n = 216$) differed significantly among drainages (paired tests over loci: $P = 0.147$).

Two loci deviated significantly from HWE expectations, *Oki23* and *Ots102* (Table 2). Both showed evidence of heterozygote deficiencies ($P < 0.05$) for at least 13 of 16 population samples ($P < 0.001$ by LM), and they were excluded from further analyses. The Rakaia mainstem sample from 1993 also deviated strongly from HWE at *Ots100*, *Ots101* and *Ots104* ($P < 0.001$ in all cases). Examination of linkage disequilibrium for the Rakaia (1993) sample also indicated significant correlations among genotypes across loci (26 of 36 possible loci pairings). In combination these results strongly suggested nonrandom sampling or admixture (Wahlund effect), hence this sample was excluded from further analyses (with the exception of pairwise comparisons shown in tables).

Counts of significant ($P < 0.05$) pairwise genic and genotypic tests of frequency differences by locus (Table 3) indicated numerous cases in which samples differed by more loci than expected by chance. By the LM method the likelihood of two or more tests out of nine (loci) occurring by chance is 7.0% and the likelihood of three or more is only 0.8%. Thus $\alpha = 0.05$ lies between two and three significant loci per sample comparison. There was strong evidence that gene and genotype frequencies differed considerably between the New Zealand samples and the Battle Creek samples (Table 3). Likewise, there were many cases in which sample frequencies differed significantly among sites within a sampling season in New Zealand (Table 3). Similarly, comparisons of θ among samples (sites and years) suggested greater divergence between the Battle Creek and New Zealand mean pairwise ($\theta = 0.016$) than were found within New Zealand (tributaries, mean $\theta = 0.008$; mainstems, mean $\theta = 0.011$), but still far more tests were significant for divergence within New Zealand, at both $P < 0.05$ and $P < 0.001$, than expected by chance ($P < 0.001$ by LM). No tributaries differed significantly from their respective mainstems in 1998 when samples were collected in both locations.

Table 3 Pairwise values of θ (above the diagonal) and counts of significant ($P < 0.05$) genotypic and genic tests of allele frequency differences (below diagonal: genotypic/genic) between samples for nine microsatellite loci. By LM method, likelihood of two or more significant tests is 0.07 and likelihood of three or more significant tests is 0.008 (in bold). Tests of interannual variation are shown in boxes. Abbreviations are as indicated in Fig. 1

	New Zealand Tributary									New Zealand Mainstem									North America			
	HT97	HT98	GA97	GA98	HW98	PR97	PR98	WT93	WT98	RA93	RA98	WM93	WM98	BC97	BC98	WA96						
HT97‡																						
HT98‡	2/0																					
GA97§	2/2	1/1																				
GA98§	3/2	3/3	1/1																			
HW98§	3/3	2/2	0/0	1/1																		
PR97¶	2/2	3/3	0/0	2/1	3/2																	
PR98¶	7/7	6/5	2/3	4/5	5/5	3/2																
WT93‡	2/2	0/1	3/4	6/6	4/4	4/5	7/9															
WT98‡	3/3	1/1	4/4	4/4	5/5	3/3	8/8	3/4														
RA93§	7/7	6/6	3/3	5/5	7/7	7/6	8/8	8/8	9/9													
RA98§	2/2	2/2	0/0	0/0	0/0	0/1	5/6	5/6	6/7	5/5												
WM93¶	4/4	4/4	1/1	1/2	3/3	1/1	3/3	7/7	8/8	7/7	2/2											
WM98¶	4/3	2/2	0/0	2/2	1/1	2/2	5/5	4/4	6/7	7/7	0/0	3/3										
BC97	4/4	4/3	4/4	5/5	4/4	4/4	7/7	8/7	7/7	8/8	6/6	6/6	4/4									
BC98	6/5	5/2	2/2	7/8	7/6	5/4	9/9	9/9	7/7	9/9	8/7	7/7	6/6	0/0								
WA96	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	9/9	0.031*						

* $P < 0.001$, † $0.001 < P < 0.05$. ‡Waitaki drainage. §Rakaia drainage. ¶Waimakariri drainage.

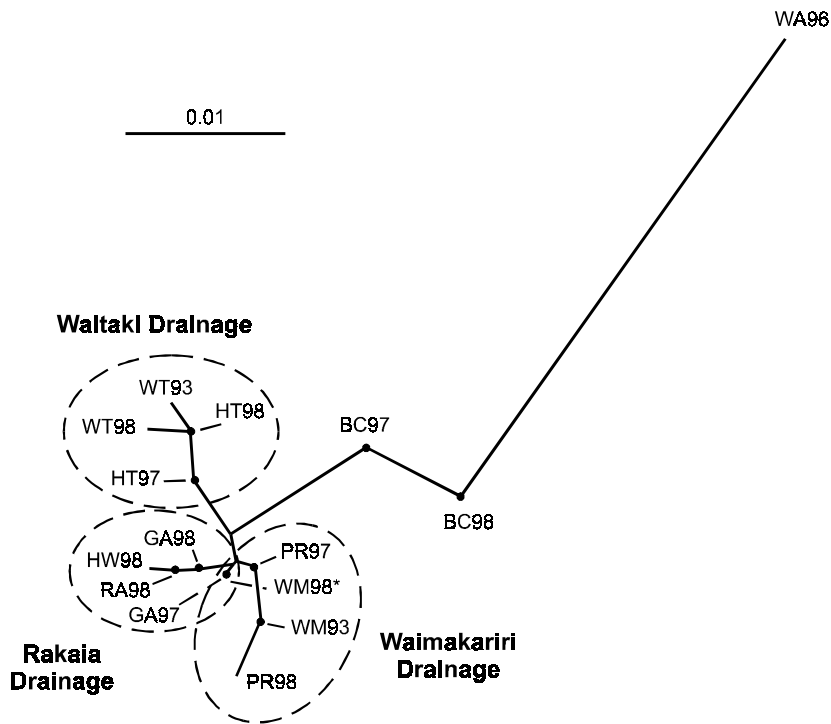


Fig. 2 Neighbour-joining tree of θ -values for all samples (except Rakaia 1993). Dashed lines mark lineages associated with New Zealand drainages. Abbreviations are as indicated in Fig. 1. *The Waimakariri 1998 sample (WM98) is misaligned with the Rakaia drainage samples.

Frequencies differed more often between 1993 and 1998 samples (3–5 loci tests per sample comparison), than between samples at a given site in 1997 and 1998 (0–3 loci tests). Still, in two of three populations (Hakataramea and Poulter) θ differed between the 1997 and 1998 samples. All New Zealand sites displayed significant variation between 1993 and 1998 mainstem samples (even with Bonferroni correction). Interannual θ averaged 0.003 for tributaries and 0.004 for mainstem samples (excluding Rakaia samples). Interannual θ was not significant for the Battle Creek population (Table 3). Based on variation in allele frequencies between 1993 and 1998 in New Zealand, estimates of N_b were 205 for Waimakariri fish (loci = 9, alleles = 95) and 179 for Waitaki fish (loci = 9, alleles = 93). Given age structures of the populations these values correspond with generational N_e values of 643 fish (95% CI = 298–2416) for Waimakariri salmon and 598 fish (95% CI = 286–1871) for Waitaki salmon.

A NJ tree constructed using θ corresponded closely with expectations based on geography and history (Fig. 2). All New Zealand samples appeared as a single lineage distinct from the North American samples, but aligned much more closely with Battle Creek fish than with the WA samples. The New Zealand samples fell into three groups (lineages) that, with only one exception, corresponded exactly to the three major New Zealand drainages. The Waimakariri 1998 sample appeared at the base of the Rakaia branch, close to the node separating the closely related Rakaia and Waimakariri groups. Samples from the Waitaki system (including samples from the Waitaki and Hakataramea

river) were consistently divergent from the other two river systems. Overall, sample structure in New Zealand was much closer to *a priori* predictions than expected by chance, as indicated by the fact that dissimilarity (d number of differing quartets) between the predicted topology and our empirical topology was much smaller than d for well over 95% of random topologies (Fig. 3).

IBD was evident for transformed pairwise θ s [transform: $\theta(1 - \theta)^{-1}$] among mainstem samples ($r = 0.68$, $P = 0.067$; Fig. 4a) and among tributary samples ($r = 0.62$, $P = 0.003$, Fig. 4b). The Hydra Waters sample differed from the Glenariffe samples by an amount equivalent to that of interannual variation at most sites. We inferred that neutral genetic divergence at this small spatial scale was insignificant. This sample was not included in AMOVA for time and tributary effects to avoid confounding intradrainage tributary variation with among drainage structure inferred from tributary samples.

AMOVA (Table 4) indicated that most variation was contained among individuals within populations ($\approx 99\%$), consistent with the high variability of the microsatellite loci used. There was significant structure among samples within and among drainages ($P < 0.001$ in both cases). Variation among years at a given site, $\theta_{Y(L)}$, was significant for both mainstem and tributary samples ($P < 0.02$). After accounting for the magnitude of temporal variation at sampling sites, AMOVA indicated that remaining variation among mainstem sites was not significant ($P = 0.33$), whereas variation among tributary sites was still suggested ($P = 0.06$).

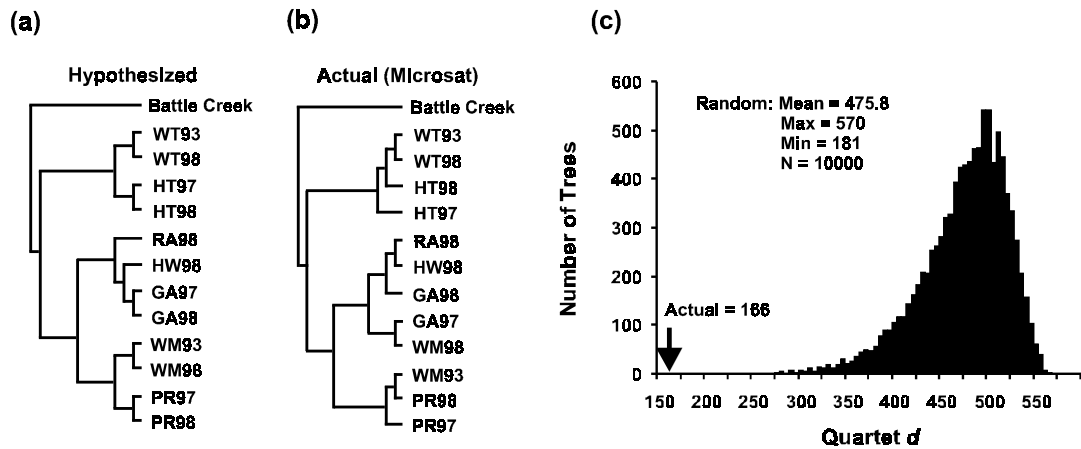


Fig. 3 Quantitative comparison of congruence between phenetic tree inferred from neighbour-joining analysis (see Fig. 2) and hypothesized tree based on history and presumed gene-flow pattern among New Zealand populations. Distances along branches are ignored. (a) Hypothesized tree, (b) tree inferred from results of neighbouring-joining analysis, (c) values of d (number of differing quartets) between 10 000 random (labelled) trees and hypothesis tree compared with value of d between actual tree and hypothesis tree.

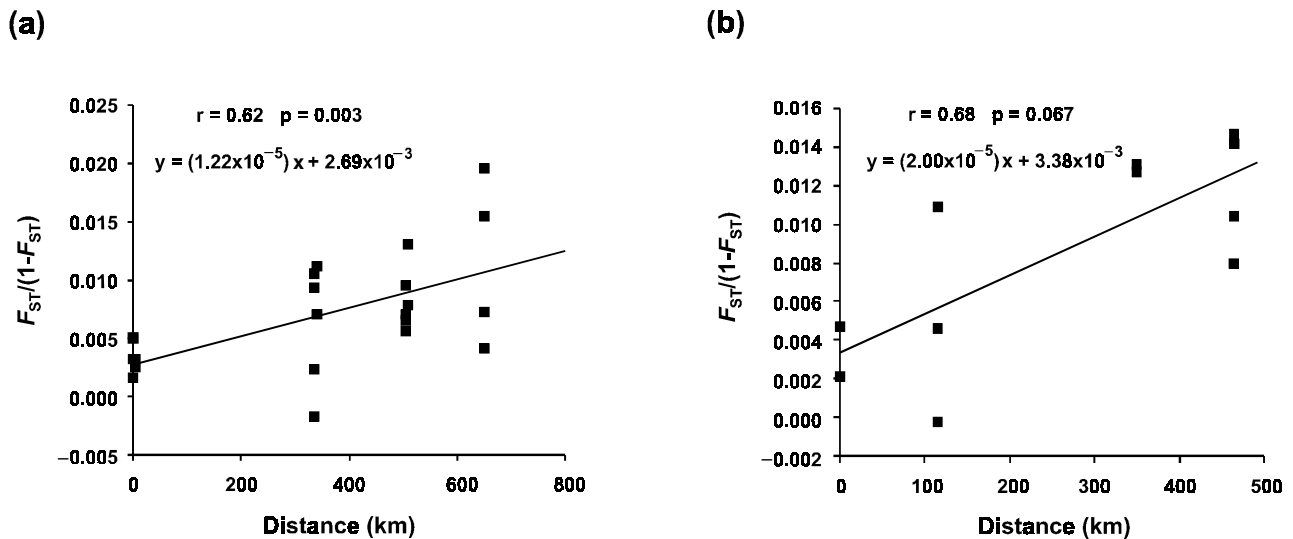


Fig. 4 Isolation, inferred from $F_{ST}/(1 - F_{ST})$, for (a) tributary samples along shortest combined river and marine distances (1997 vs. 1998 data yield 0 distances), (b) mainstem samples along shortest marine distances (1993 vs. 1998 data yield 0 distances). Geographic distance between Glenariffe and Hydra Waters sites in (a) is only 5 km and thus the points representing their divergence are among those values near 0 km.

Simulations of F_{ST} values for a 5 population array over 27 generations indicated that expected microsatellite divergence under the stepping-stone and island models were very similar except at high very gene flow (Fig. 5). Comparison of simulated F_{ST} values with θ -values obtained among New Zealand drainages ($\theta \approx 0.01$) suggested that the degree of divergence among drainage-based populations would be consistent with $N_e < 1000$, regardless of migration rate (Fig. 5). Effective population sizes much less than 300 fish would result in F_{ST} values much larger than detected in New Zealand within realistic bounds for migration (e.g. $m < 0.1$). For an estimated N_e of 600 fish per drainage, fitting with estimates based on the temporal

method, simulations suggest that the corresponding m would be ≈ 0.023 to obtain a mean F_{ST} of 0.01, and thus $N_e m \approx 14$ immigrants per drainage per generation (Fig. 5).

Extended simulations (1000 generations) using $N_e = 600$ and $m = 0.023$ indicated that asymptotic F_{ST} would not be expected until ≈ 50 –100 generations under the island model and not until over 200 generations under the stepping-stone model (Fig. 6). Although equilibrium F_{ST} under the island model would be comparable with that currently observed among New Zealand populations, the IBD pattern of New Zealand populations suggests that a pure island scenario is unlikely. The closer gene flow was to a stepping-stone pattern (i.e. most migrants exchanged with

Table 4 Analysis of molecular variance (AMOVA) describing contributions of time and sample location (tributary or mainstem) to population structure

Grouping strategy	Source of variation	σ^2	% of total	θ	$\theta_{L(T)}$	$\theta_{Y(L)}$
Time within mainstems	Total	3.42	100.00			
	Within samples	3.38	98.83			
	Between samples	0.04	1.17	0.012**		
	Between locations	0.03	0.83		0.008	
	Between years within locations	0.01	0.33			0.003**
Time within tributaries	Total	3.40	100.00			
	Within samples	3.37	99.08			
	Between samples	0.03	0.92	0.009**		
	Between locations	0.02	0.57		0.006*	
	Between years within locations	0.01	0.34			0.003**
				θ	$\theta_{D(T)}$	$\theta_{S(D)}$
Samples within drainages	Total	3.39	100.00			
	Within samples	3.37	99.22			
	Between samples	0.03	0.78	0.008**		
	Between drainages	0.02	0.54		0.005**	
	Between samples within drainages	0.01	0.24			0.002**

T, total; L, location; Y, year; S, sample; D, drainage. ** $P \leq 0.01$, * $P \leq 0.05$.

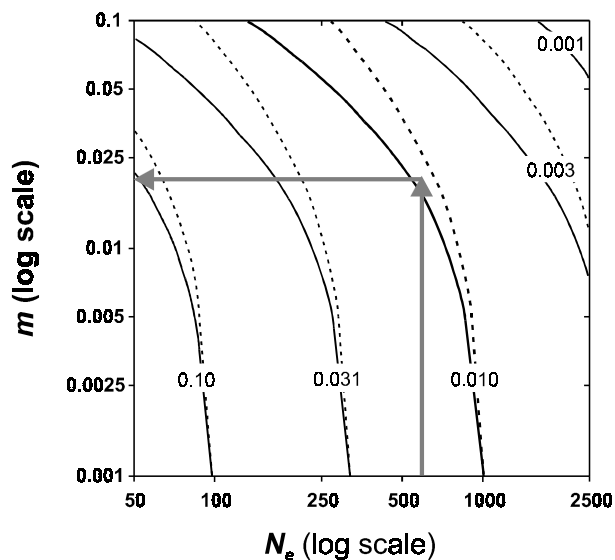


Fig. 5 Isoclines of F_{ST} (estimated by G_{ST}) relative to different combinations of effective population size (N_e) and m for an array of five populations diverging for 27 generations under drift with island (solid) or one-dimensional stepping-stone (dashed) patterns of migration. Simulations were performed using EASYPOP Version 1.7.2 (Balloux 2001) and used nine loci with 17 alleles each. Note that axes and isocline spacing are presented on logarithmic scales. Grey arrows show translation of N_e of 600 into m of 0.023 ($N_e m \approx 14$) along isoclines of $F_{ST} = 0.010$ (approximate mean value observed in New Zealand).

neighbouring populations) the more likely that populations have yet to reach drift–migration equilibrium at the time of analysis. Wright's F_{ST} method yielded a higher estimate of $N_e m$, about 25 fish per drainage per generation

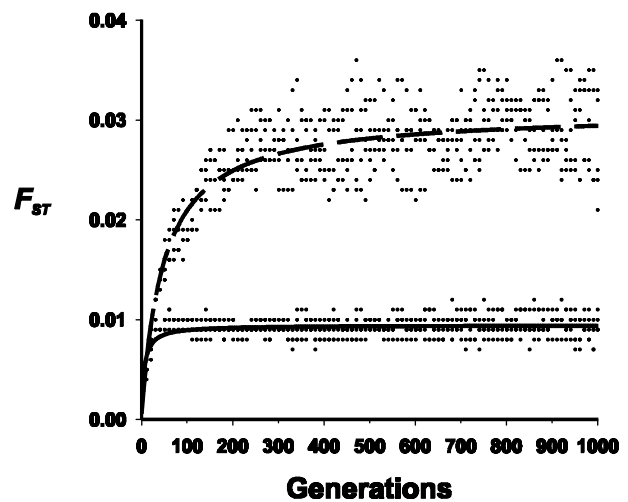


Fig. 6 Simulated long-term divergence, expressed as multilocus F_{ST} (i.e. G_{ST}), among five populations in island (solid) or stepping-stone (dashed) arrays with $N_e = 600$ per population, $m = 0.023$ ($N_e m = 14$) and nine loci with 17 alleles each. Real New Zealand populations are at ≈ 27 generations at the time of writing and likely conform to an isolation-by-distance pattern resulting in a divergence trajectory bracketed between the island and stepping-stone trajectories shown here.

for all populations considered together (based on $F_{ST} = 0.01$) and 22.1–56.7 individuals per generation for F_{ST} estimates between pairs of populations. In contrast the private alleles method estimated fewer migrants (5.8–11.1) per generation.

Multilocus genotype assignment classified individuals to their respective system significantly better than expected

by chance ($P < 0.001$) in all cases. The mean percentage of individuals of a given population correctly classified to their respective population was 77.6% for the two mainstem samples tested in 1993 (Waitaki and Waimakariri fish; 50% expected by chance). The mean percentage correct classification was 52% for the three mainstem samples in 1998 (Waitaki, Rakaia and Waimakariri), 40.1% for the three tributary samples in 1997 (Hakataramea, Rakaia and Poulter) and 57.6% for the same three tributary samples in 1998 (relative to 33.3% expected by chance averaged over all three tests). Misclassification trends suggested a tendency in 1998 for incorrectly assigned Waitaki/Hakataramea fish to be classified as Rakaia/Glenariffe fish and for misclassified Rakaia/Glenariffe and Waimakariri/Poulter fish to be classified as each other, respectively, consistent with IBD.

Discussion

Our results suggest that New Zealand chinook salmon have developed detectable population structure among drainages over a time scale of ≈ 27 generations. This structure evolved following rapid colonization of multiple river systems from sources introduced to a single drainage. The structure follows an isolation-by-distance pattern despite significant interannual variation within locations. Furthermore, although confidence bounds are large and simulations suggest that these populations are probably not in drift-migration equilibrium, we estimated N_e at ≈ 600 individuals per major drainage and N_{em} at ≈ 14 immigrants per drainage per generation by simulating divergence from a common gene pool under non-equilibrium conditions.

Divergence among New Zealand drainages (average pairwise $\theta \approx 0.01$) is subtle compared with values noted over similar geographical scales within the natural range of the species in North America. In California and the Sacramento River system chinook salmon F_{ST} analogues have been estimated as high as 0.078 (Bartley & Gall 1990) for allozymes and 0.084 for microsatellite loci (Myers *et al.* 1998). However, these studies included populations from outside the Sacramento–San Joaquin system and seasonal races that contributed substantially to genetic variation within the Sacramento–San Joaquin system. Examination of five Sacramento–San Joaquin populations using 47 polymorphic allozyme loci, including four fall-run populations and one winter-run population, estimated F_{ST} at 0.016 (Gall *et al.* 1991), much closer to the values we found among fall-run New Zealand populations (Table 2). Our overall estimates of θ for New Zealand drainages (Table 4) were comparable to, if somewhat larger than the F_{ST} estimate of 0.005 among New Zealand drainages documented by Quinn *et al.* (1996) using 24 polymorphic protein loci.

The pattern of population structure within New Zealand conformed to our expectations. The New Zealand popula-

tions aligned more closely with Battle Creek fish than with WA salmon, as expected based on their ancestral affiliation and with previous allozyme work (Quinn *et al.* 1996). The New Zealand salmon also showed lower levels of heterozygosity and allelic richness, consistent with an overall founder effect upon introduction of the species to New Zealand as suggested by Quinn *et al.* (1996). Despite significant interannual variation at sites within New Zealand, a spatial signal was apparent. Among 12 samples from different sites and years within drainages, all but one grouped on branches of a NJ tree according to the river system where they were collected (Figs 2 and 3). Although samples from the same site (e.g. tributary or mainstem samples) in different years did not always cluster together as originally hypothesized, the microsatellite-based tree conformed much closer to our a priori expectations than would be expected by chance (i.e. a random topology) (Fig. 5). The relative geographical proximity of the Rakaia and Waimakariri drainages was also mirrored by close placement of the corresponding branches on the NJ tree. This result is consistent with IBD, and with studies of tagged releases in which straying was found to be a function of proximity and river discharge (Unwin & Quinn 1993). Although we did not analyse samples from the Rangitata drainage, which lies between the Waitaki and Rakaia drainages, it seems almost assured that Rangitata salmon would be intermediate in allelic distribution and divergence to that found in contrasting the Waitaki and Rakaia populations. We would similarly anticipate that the Clutha population, the most Southern salmon drainage would be most similar to the Waitaki salmon and diverge most from the Rakaia and Waimakariri salmon (Fig. 1).

Temporal genetic variation is potentially as important as spatial variation in describing population structure, particularly of contemporary populations, but has comprised much less of the efforts of molecular ecologists (examples for fishes: McClenaghan *et al.* 1985; Lacson & Morizot 1991; Jordan *et al.* 1992; Nielsen *et al.* 1999). In addition to providing information about real changes in genetic variation over time, temporal samples can help assure that apparent spatial divergence is not an artefact of sampling error when structure is subtle, although it is important how such inference is made. When the magnitude of temporal sample variation within sites is very low, compared with the magnitude of among site variation, population structuring is likely real (as opposed to a statistical artefact) and persistent. However, our results demonstrate that the presence of significant temporal variation within sites does not conversely imply a lack of persistent spatial structure (and partial genetic isolation). Although among-drainage divergence in New Zealand is only of the order of that seen between years at Battle Creek (Table 4, Fig. 2, Quinn *et al.* 1996) and AMOVA indicated that temporal variation is large enough

that it could explain a significant component of spatial variation, our NJ analysis clearly indicated that a temporally and spatially cohesive structure was associated with drainages (years within drainages still tended to group with each other). Population structure in New Zealand is thus likely real and persistent. A structure in flux (i.e. in which genetic constitution of individual populations changes rapidly) is still a structure, and significant temporal variation within sites can reflect the drift processes expected to hasten among site divergence.

Significant temporal variation in allele frequencies in New Zealand is likely a result of the effective number of breeders and sampling error. Indeed, temporal variation in allele frequencies is the basis for the 'temporal method' of estimating effective population size (Pollack 1983; Waples 1989). This indirect method assumes random sampling and that gene flow does not significantly impact gene frequencies over the time frame examined (Nei & Tajima 1981; Waples 1990b). The influence of effective gene flow on gene frequencies, and thus on estimates of N_e , over the approximately two generations we examined would likely be negligible compared with other sources of error, and few if any studies of N_e are assured of truly random sampling. Additional evidence indicates that we have not overestimated N_e . Each of the river systems we sampled contains ≈ 5000 – $20\,000$ spawning salmon annually (cf. West & Goode 1987). Taking only half of the low end census value as the minimum population size and using a modest estimate of N_b/N of 0.10 (low for chinook salmon: R. Waples, personal observation the effective number of breeders in a given season would still be 250 fish, corresponding to a generational N_e of ≈ 800 fish (given a mean generation time of 3.14 years). Furthermore, local N_e much smaller than 300 fish would result in much larger F_{ST} values than actually observed, unless migration rates were substantially higher (e.g. $m > 0.1$).

Comparison of estimates of $N_e m$ based on Wright's (1943) method with our estimates under simulations suggested that Wright's method likely overestimated $N_e m$ by several fold under the conditions found in New Zealand. Following a perturbation or colonization event it may take tens to hundreds of generations for populations to recover equilibrium (Crow & Aoki 1984; Waples 1998), which may bias equilibrium estimates of $N_e m$ (Slatkin 1985; Waples 1998). At the time scale and likely gene flow rates of New Zealand salmon, the island and stepping-stone models provided similar predictions for divergence at a given N_e (at higher m and longer time scales they differ considerably; Fig. 5). However, extended simulations based on $N_e = 600$ and $m = 0.023$ (likely parameters for New Zealand salmon) suggested that equilibrium may not be reached until ≈ 200 generations for the stepping-stone model, whereas for the island model it may be reached at ≈ 50 – 100 generations (Fig. 6).

Our simulations confirm that equilibrium methods will tend to be inaccurate under nonequilibrium conditions for a finite number of subpopulations. F_{ST} (or its multilocus estimator G_{ST} in this case) is not constant across the range of combinations of N_e and m that multiply to give the same value of $N_e m$, rather divergence tends to be less at larger values of N_e even as m decreases proportionately (Fig. 5). This violates the basic premise of Wright's infinite-island model, and other equilibrium models, that relate a given F_{ST} to a single number of effective migrants regardless of population size. Slatkin & Barton (1989) and Cockerham & Weir (1993) have additionally suggested that θ may overestimate $N_e m$ for the F_{ST} method at high m .

In contrast, estimates based on the private alleles method were closer to, but lower than, the estimate from simulations (14 individuals per generation) under nonequilibrium conditions. Slatkin (1985) estimated that significant bias of $N_e m$ from private alleles estimates following colonization may persist for times less than N_e generations. Given the generation length and potential effective size of New Zealand populations, bias in estimates from this method might be expected for up to 1800 years.

New or perturbed populations, such as those in New Zealand, will seldom satisfy the assumptions of commonly used indirect methods for estimating $N_e m$. We therefore believe a simulation approach, such as the one employed in this study, is preferable and more widely applicable under such conditions. Further refinements might be appreciated by incorporation of founder effects and changing population sizes and migration rates over time. However, we did not detect evidence of substantial founder effects beyond the initial introduction of salmon to New Zealand (allelic richness did not differ among New Zealand drainages), and the populations appear to have grown to their full sizes very rapidly.

Recent developments in the use of assignment tests based on multilocus genotypes promise to offer powerful tools for identifying the origins of migrants and rates of dispersal among some populations without direct assumptions of equilibrium (see Davies *et al.* 1999 for review). However, although our ability to classify individuals to their drainage was significantly better than expected by chance, the assignment test proved a very poor estimator of among site dispersal. If a misclassified individual actually represented among-site dispersal, then immigrants would have represented in the order of 30–50% of each population per year, far greater than our indirect generational estimate of gene flow of $\approx 2.3\%$. It is highly unlikely that even selection would account for such a large difference between dispersal and gene flow. Rather, misclassification most likely reflected subtlety of allele frequency differences among drainages and the limited sample sizes used in building classification rules. In the case of systems in which population structure is a recent advent and gene frequency

differences subtle, assignment tests may have limited power, within practical bounds of sample size and numbers of available loci, to detect straying (much less actual gene flow).

The estimated effective number of migrants ($N_e m$) into each of the New Zealand populations could be considered large from the perspective of maintenance of genetic differentiation in selectively neutral traits (Slatkin 1987; Mills & Allendorf 1996). It would be reasonable to expect fairly high gene flow among the New Zealand populations given the rate with which they were colonized and the straying that takes place (Unwin & Quinn 1993). Such high gene flow implies that observed divergence among New Zealand populations in quantitative traits such as migration and breeding timing (Quinn *et al.* 2000), ovarian investment (Kinnison *et al.* 2001) and juvenile growth rates (Unwin *et al.* 2000) is unlikely to have arisen because of random processes. However, population divergence is nonetheless apparent in allelic frequencies in New Zealand and the expectations of quantitative trait divergence under drift and migration are insufficiently understood to assure that phenotypic divergence is purely the result of selection. These expectations may themselves be most readily addressed in future individual-based simulations of quantitative trait divergence under neutrality.

Extensive gene flow may slow the evolution of adaptive differences among populations (Slatkin 1987) but once adaptive differences begin to accrue they can reduce the relative fitness of immigrants and contribute to the development of isolating mechanisms (Rice & Hostert 1993; Hendry *et al.* 2000; Quinn *et al.* 2000). This process has the potential to be self-reinforcing, and, thus, gene flow may have changed over time. Adaptive divergence may contribute to fitness costs for current strays in New Zealand. Glenariffe Stream salmon had 1.7 and 2.9 times higher survival at sea than Hakataramea River fish when released in two different years from Glenariffe (i.e. local for the Glenariffe fish), but the two populations performed similarly when released from a site that was home to neither (Quinn *et al.* 2001). Given evidence of selection against strays, our estimate of $N_e m$ would thus represent a value integrating higher early gene flow and may overestimate current levels.

Evidence of genetic structure under nonequilibrium conditions can reflect remnants of founder effects or occasional bottlenecks that are gradually eroded by gene flow, particularly over shorter geographical scales (resulting in IBD). Such a scenario has been hypothesized for temporal and spatial genetic variation in a damselfish (*Stegastes partitus*, Lacson & Morizot 1991) and theoretically examined by Austerlitz *et al.* (1997). However, as stated above, there is no genetic or historic indication that founder effects or bottlenecks have been significant in the history of population structure within New Zealand beyond the initial

introduction of the species from North America. Nonetheless, releases of hatchery fish in New Zealand over the last 20 years (particularly Rakaia salmon into the Waimakariri system) may have eroded or impeded some population structuring regardless of how it developed.

Conclusions

Our results provide insights into population divergence over short time scales in neutral allelic variation. Examination of microsatellite variation suggested that, despite considerable interannual variation, detectable population structure developed among anadromous salmon in different river drainages from a common founding group within 27 generations. This structure resulted from genetic drift within populations following natural colonization, and persists in the face of gene flow from straying and some recent translocations by humans. The isolation of these populations has apparently been sufficient to permit the evolution of adaptive variation, which may have reinforced isolation by enhancing the reproductive success of local salmon over strays. Furthermore, although the New Zealand populations likely deviate from drift–migration equilibrium in allelic variation, we estimated $N_e m$ using simulation methods that are less reliant on equilibrium assumptions. We hope that our results will encourage others to continue to develop and incorporate individually tailored simulation approaches as a means of addressing nonequilibrium conditions posed by new or perturbed populations. Future conservation and management are likely to be better served by the flexibility afforded by implementation and refinement of such simulations than they would be by restricted adherence to current deterministic approximations.

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This study is part of an international programme investigating contemporary evolution and genetics of New Zealand salmon, with implications for evolutionary biology, conservation and aquaculture. It was conducted as part of doctoral and postdoctoral studies of Michael Kinnison at the University of Washington and Dartmouth College, respectively. Michael Kinnison has recently joined the faculty of the University of Maine and intends to continue studies of contemporary evolution and fish ecology. Paul Bentzen specializes in molecular ecology of aquatic species. Martin Unwin studies the ecology and management of New Zealand fishes and Thomas Quinn investigates the behaviour, ecology and evolution of salmonids.
