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Proc. R. Soc. B published online 11 July 2012
doi: 10.1098/rspb.2012.1158

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FirstCite® e-publishing

Proc. R. Soc. B
doi:10.1098/rspb.2012.1158
Published online

Genetic change for earlier migration timing in a pink salmon population

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To predict how climate change will influence populations, it is necessary to understand the mechanisms, particularly microevolution and phenotypic plasticity, that allow populations to persist in novel environmental conditions. Although evidence for climate-induced phenotypic change in populations is widespread, evidence documenting that these phenotypic changes are due to microevolution is exceedingly rare. In this study, we use 32 years of genetic data (17 complete generations) to determine whether there has been a genetic change towards earlier migration timing in a population of pink salmon that shows phenotypic change; average migration time occurs nearly two weeks earlier than it did 40 years ago. Experimental genetic data support the hypothesis that there has been directional selection for earlier migration timing, resulting in a substantial decrease in the late-migrating phenotype (from more than 30% to less than 10% of the total abundance). From 1983 to 2011, there was a significant decrease—over threefold—in the frequency of a genetic marker for late-migration timing, but there were minimal changes in allele frequencies at other neutral loci. These results demonstrate that there has been rapid microevolution for earlier migration timing in this population. Circadian rhythm genes, however, did not show any evidence for selective changes from 1993 to 2009.

Keywords: microevolution; genetic change; salmon; circadian rhythms; climate change; migration timing

1. INTRODUCTION

It is becoming increasingly apparent that adaptive microevolution can occur rapidly in wild populations [1–4]. Nonetheless, there is a paucity of empirical evidence for rapid adaptive microevolution (i.e. genetic change) in response to climate warming, largely because it is unclear whether many climate-induced phenotypic changes have a genetic basis or are due to phenotypic plasticity [5]. In other words, observed phenotypic changes may be due to the same genotypic distribution producing a new phenotypic distribution (plasticity). Appropriate methods, including genetic data or quantitative genetic designs, will help clarify the influences of plastic and genetic adaptations to climate change [6–9], and will help predict and quantify the impacts of global change on ecosystems and biodiversity. This information is critically important given the proliferation of evidence suggesting that life-history traits are changing in many populations as a response to global climate change [10,11].

Generally, migration events are timed to coincide with environmental conditions that maximize individual fitness, and many species will have to change their migration timing to match new environmental conditions produced by climate change [12,13]. Changes in migration timing for Pacific salmon populations may be particularly necessary [14,15], because salmonid phenological events—the timing of seasonal life-history events—are often highly adapted to local thermal conditions in freshwater rivers, streams and lakes, and also the ocean [16–18]. Phenological traits are generally heritable in salmonid populations (median $h^2 = 0.51$ [19]), and it is hypothesized that microevolutionary changes in migration timing may be one mechanism that would allow salmon populations to persist under climate warming [14,20,21]. A general trend towards earlier migration timing observed in many salmonid species and populations [22–26] supports this hypothesis, but molecular genetic evidence for microevolution towards altered migration timing is non-existent.

In this study, we use phenotypic data on migration timing, archived genetic samples and data from a marker locus, the allele frequencies of which were experimentally altered more than 30 years ago, to determine whether change in migration timing in a population of pink salmon has a genetic basis (i.e. microevolution). Although rare, experimental genetic data in salmon populations can provide a tool by which genetic changes can be tracked in natural populations [27]. Specifically, we observed that both even- and odd-year adult pink salmon that spawn in a warming Alaskan stream (figure 1) are migrating into freshwater earlier and are migrating over a shorter period of time (figures 2 and 3 [15,26]). Owing to a strictly semelparous, 2-year life cycle, pink salmon have the potential for rapid rates of adaptive evolution relative to other salmon species (in terms of number of years). The combination of high trait heritability, short generation time and observed phenotypic change provides a suitable context to study evolutionary change over a contemporary and relatively short time-frame.

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2012.1158 or via http://rspb.royalsocietypublishing.org.

Received 18 May 2012
Accepted 15 June 2012

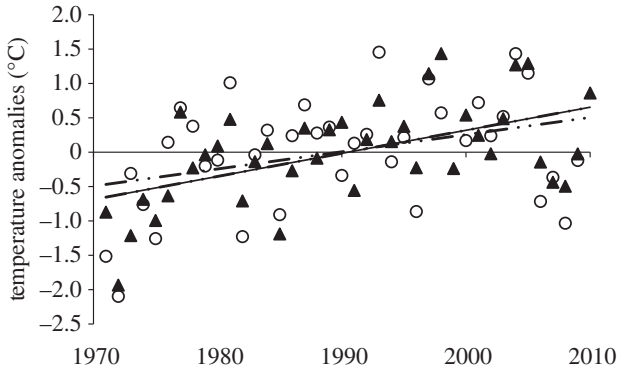

Figure 1. Yearly mean temperature anomalies for stream temperature in Auke Creek (black triangle, solid line), and ambient temperature at Auke Bay (white circle, dashed line), Alaska.

In many vertebrate species, maturation schedule and migration timing are influenced by endogenous circadian or circannual rhythms that are driven by photoperiod [28–30]. Photoperiod also appears to be a primary cue that initiates adult maturation and migration timing in Pacific salmon [18,31]. Recently, researchers have identified crucial molecular components of the circadian rhythm cycle in salmon, including genes in *Clock* (a transcription factor) and *Cryptochrome* (an inhibitor) [32,33]. *OtsClock1b* has been used to detect Chinook salmon population structure that was not evident from neutral microsatellite locus data [32]. Latitudinal clines in *OtsClock1b* allele frequencies exceed neutral expectations for Chinook salmon (*Oncorhynchus tshawytscha*), chum salmon (*Oncorhynchus keta*), and pink salmon (*Oncorhynchus gorbuscha*), and indicate that local adaptation may be responsible for patterns of clock gene frequencies across geographical space [34,35]. Additionally, *OtsClock1b* and *Cryptochrome2b* map to genomic regions that explain variation in growth and development in juvenile coho salmon (*Oncorhynchus kisutch* [33]). To test our hypothesis that there has been genetic change for earlier migration timing, we used more than 30 years of temporal genetic data (17 complete generations) from the odd-year population and predicted that there would be a significant decrease over time in a neutral genetic marker manipulated to alter allele frequencies in the late-migrating portion of the population as well as evidence of directional selection at circadian rhythm genetic loci.

2. METHODS

(a) *Study site*

Auke Creek is a small lake-outlet stream near Juneau, Alaska. There have been complete daily counts (census) of all adult pink salmon migrating into Auke Creek since 1971. Some experimental hatchery activity occurred in the 1970s; since that time, however, there has been little hatchery activity. Historically, the distribution of migration timing of the Auke Creek pink salmon population was moderately bimodal and had relatively distinct early- and late-migrating population components that were separated by approximately 20 days (figure 3 [36,37]). This bimodality in the migration distribution was associated with distinct phenotypic differences. Towards the end of August, returning adults tended to be very 'dark' and in advanced states of maturity; beginning in September, ocean fresh 'bright' individuals would arrive signifying the beginning of the late migration [38].

(b) *Experimental genetic marker for late-migration timing*

Experimental manipulations in 1979 introduced a putatively neutral genetic marker into the late-migrating portion of the odd-year Auke Creek pink salmon population. A neutral marker was used so that it would be possible to genetically track late-migrating individuals without influencing their fitness. Selective breeding was used to alter the frequency of two neutral alleles so that late-migrating fish were genetically differentiated from earlier-migrating fish. Specifically, individuals that migrated into Auke Creek after 15 September were used in the genetic marking experiment (i.e. the latest-migrating individuals). A large effective population size ($N_e \sim 400$) was used, and there was no evidence for natural selection at this locus after the 1979 marking event or genetic heterogeneity between the pre- and post-experimental populations at other allozyme loci (for details on the experimental design, see earlier studies [27,38]). Within the late-migrating portion of this population, the frequency of the **70* allele at the *MDH B1,2** allozyme locus was substantially increased from 0.056 in 1979 to 0.256 in 1983. Additionally, the frequency of the **130* allele was decreased in the latest migrating individuals from 0.046 in 1979 to 0.023 in 1983 (the third allele, **100*, changed by necessity due to these manipulations). From 1981 to 1989, the allele frequencies at this locus did not substantially change (i.e. stayed at pre-experimental levels) in the early-migrating portion of the population (**70* = 0.04–0.05; **130* = 0.04–0.05; figure 4), and experimental allele frequencies in late-migrating fish remained stable and differentiated from early-migrating fish (**70* = 0.20–0.30; **130* = 0.01–0.03). Therefore, these alleles genetically marked late-migrating individuals and allow us to infer whether changes in the migration timing distribution are due to changes in the genotype for migration timing. Hence, selection towards earlier migration timing should change the frequency of these alleles towards the frequencies of the early-run fish, thus confirming our prediction. The experimental manipulations led to allele frequency differences between early- and late-migrating fish that were substantially larger for the **70* allele than for the **130* allele. We frequently refer only to the **70* allele with the notation LMMA for 'late-migration marker allele'. When we refer to the entire locus, we use LMML for 'late-migration marker locus'.

(c) *Genetic data*

To obtain allele frequency data for the LMML and a control locus not associated with the late portion of the population, approximately 5–30 (generally 10) fish were sampled each day from fish migrating into Auke Creek, Alaska in 1983, 1985, 1987, 1989, 1991, 1993, 2001 and 2011 (but see additional details later). All fish were sampled for skeletal muscle tissue as they passed through the weir, except for samples in 1993. In 1993, samples were collected from newly dead carcasses on each day that fish mortalities (i.e. post-spawning) were observed. Starch gel protein electrophoresis was used to resolve allozyme banding patterns [27]. Data were obtained from fish in 1983 ($n = 645$), 1985 ($n = 587$), 1987 ($n = 459$), 1989 ($n = 524$), 1991 ($n = 507$), 1993 ($n = 550$), 2001 ($n = 490$) and 2011

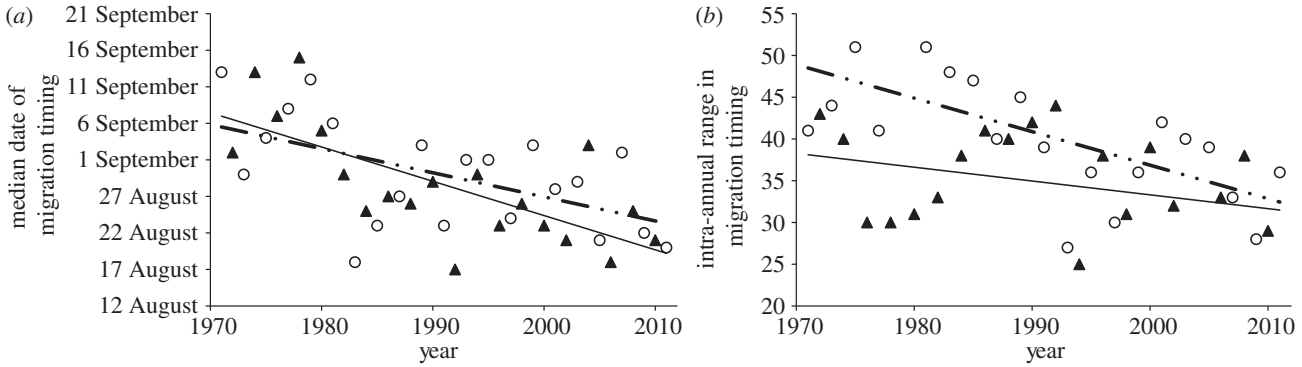

Figure 2. Change in migration timing of pink salmon in Auke Creek, Alaska. (*a*) Median date of migration timing versus year for odd- (white circle, dashed line) and even-year (black triangle, solid line) pink salmon populations. (*b*) Phenotypic variance in migration timing versus year for odd- and even-year pink salmon (symbols as mentioned earlier). Phenotypic variance was measured as the number of days over which the central 95% of the migration timing distribution entered Auke Creek.

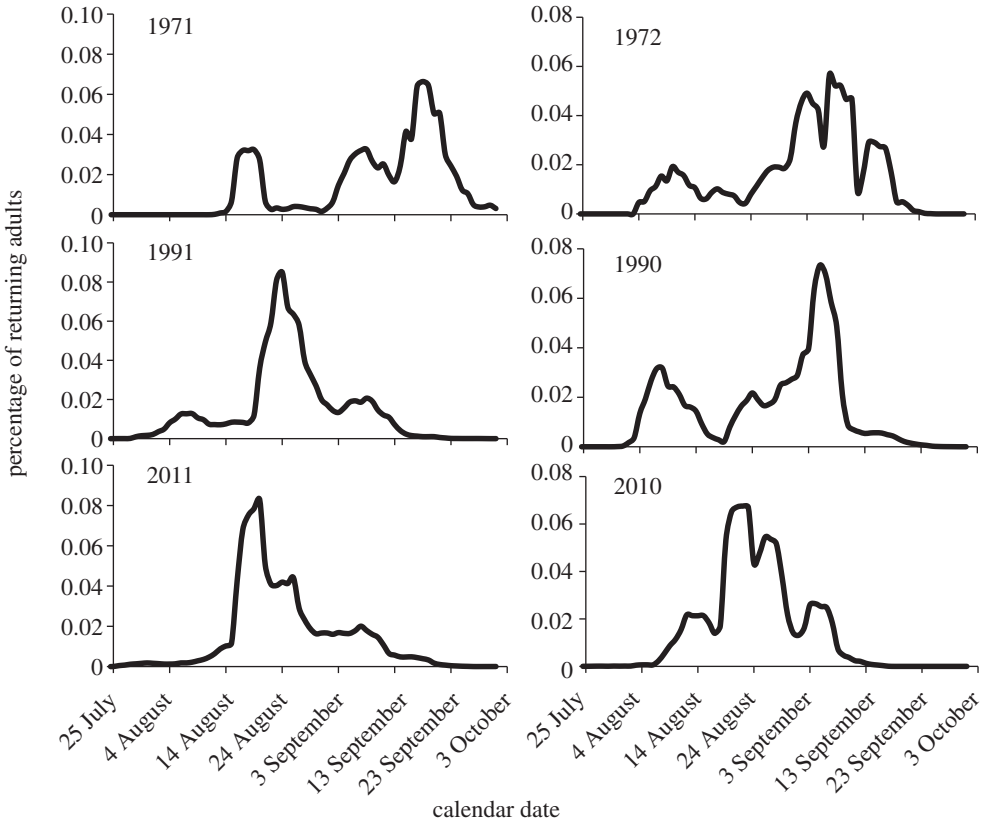

Figure 3. Pink salmon migration timing distributions from 3 years representing the beginning, middle and end of our time series. The data series are 5-day running averages of the total percentage of migrating adults on each day. The odd-year population is on the left and even-year is on the right.

($n = 606$). Allele frequencies were obtained for the allozyme locus *G3PDH-1** in 1979 ($n = 179$), 1981 ($n = 203$), 1983 ($n = 726$) and 2011 ($n = 551$). *G3PDH-1** is not associated with migration timing and was used as a comparison (selectively neutral control [39]) with the LMML allele frequencies.

Microsatellite data were obtained from approximately 10 individuals sampled every other day during the migration in 1993, 2001 and 2009. Approximately 160–190 individuals were genotyped at each locus in each year (electronic supplementary material, table S1). DNA was extracted from all samples with the protocol described [40], and was amplified at 23 putatively neutral microsatellite loci and three candidate loci that are part of the circadian rhythm gene complex (electronic supplementary material, table S1). PCR amplification used optimized, locus-specific temperature profiles and a Qiagen multiplex PCR kit (Qiagen, Valencia, CA). PCR products were visualized on a LI-COR 4300 DNA Analyzer. Allele sizes were estimated with SAGA Generation 2 software (LI-COR, Lincoln, NE). All data are available from Dryad (doi:10.5061/dryad.m3c53).

(d) *Data analyses*

Multiple approaches were used to describe temporal changes in intra-annual variation in the allele frequencies at the LMML over time. For each year, simple graphical comparisons of 5-day running allele frequency averages of the LMMA were used to track changes in genetic differentiation over time. A binomial *t*-test was used to test for significant

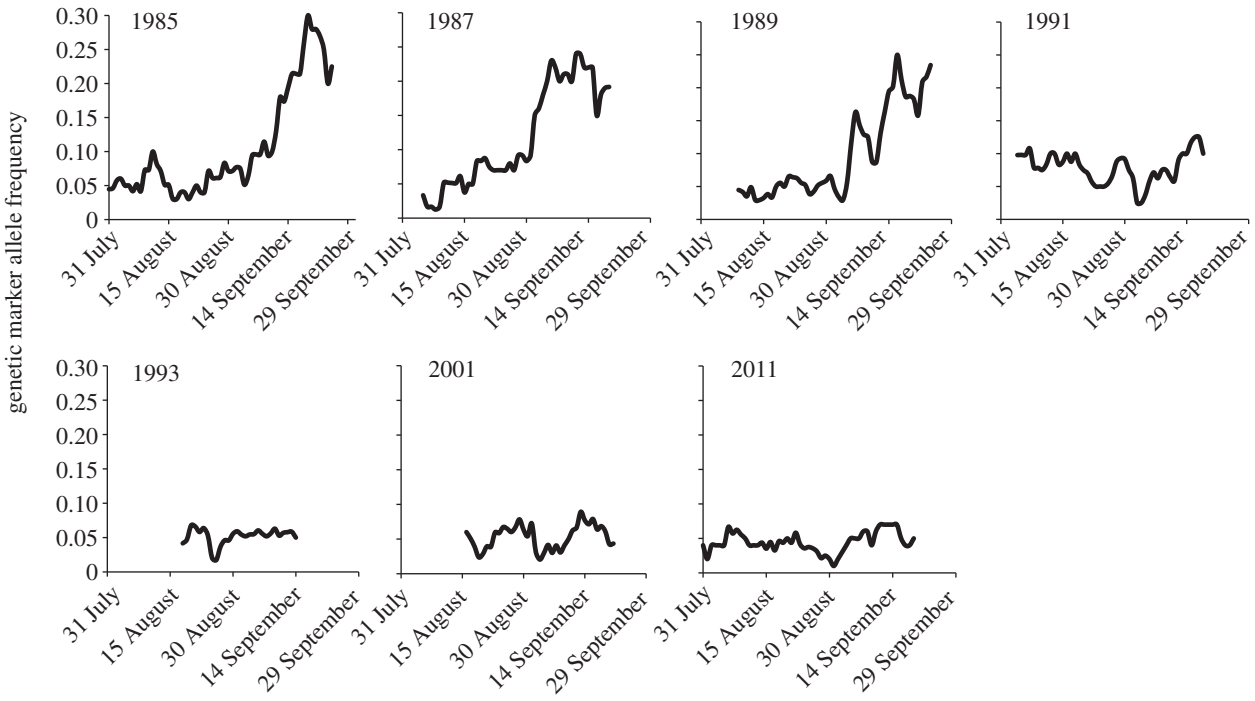

Figure 4. Five-day running averages of the frequency of the late-migration marker allele (LMMA). 1983 is not included because samples were only taken on 3 days for that year.

genetic differences at the LMMA between early- and late-migrating individuals. We used data from the calendar dates that included the first and last 100 fish sampled in each year.

Gene flow between early- and late-migrating fish could erode the genetic structure introduced by the marking effort. Thus, we estimated the overall frequency of the LMMA, because gene flow alone should not change the overall frequency of the allele, whereas demographic changes that reduced the late run would decrease the frequency of this allele. Obtaining an unbiased estimate of the overall frequency and associated uncertainty at the LMMA across the entire migration timing distribution is complicated, because of strong genetic differentiation between early- and late-migrating fish, unequal abundance during different portions of the migration timing distribution and unequal sample representation across the migration timing distribution.

A parametric bootstrap approach that included the genetic and daily census data was used to resolve these issues. In each year, the migration timing distribution was systematically separated into 5-day 'subsamples' starting with the first date that genetic samples were collected. We calculated maximum-likelihood allele frequency estimates (f) for each period (i; $i = 1, 2, 3 \ldots j$) and then drew random parametric bootstrap samples from a binomial distribution, $x_i \sim \mathrm{Bin}(f_i, n_i)$, where n_i are the number of alleles sampled ($2 \times$ number of individuals sampled) in each period. For each year, an overall allele frequency estimate was obtained with $F = (1/(2 \times \Sigma_i^j N_i))\ \Sigma_i^j \alpha_i$, where $\alpha_i = x_i/n_i \times 2 \times N_i$ and N_i is the census number of adult fish migrating into Auke Creek during the same 5-day period. One thousand bootstrap replicates were performed and 95% confidence intervals were calculated by excluding the most extreme 0.025 per cent of the smallest and the largest values. For 1993, we used census data from 8 days prior to the date of the genetic samples (obtained from carcasses), because this approximates the duration of freshwater life for Auke Creek pink salmon [41]. In 1983, samples were taken on only 3 days roughly corresponding to the beginning, middle and end of the migration. To estimate the overall allele frequency for this year, we equally allocated maximum-likelihood allele frequency estimates between the sampled dates for each period. We used weighted allele frequencies at *G3PDH-1** in 1979, 1981 and 1983 from data of McGregor *et al.* [42], and used the approach described earlier to estimate frequencies for 2011. We detected two alleles at *G3PDH-1** and report estimates for the less abundant allele. We did not replicate these analyses at *MDH**130 because the manipulative change in frequency was small (approx. 0.02) and the allele frequencies are very close to 0 (the boundary), resulting in very little power to detect small changes.

Importantly, inter-population gene flow could also influence the frequency of the LMMA, but estimates of contemporary gene flow between pink salmon in Auke Creek and other nearby locations are low (proportion of migrants each generation $m = 0.0015$ [27]). Estimates of direct immigration/straying are also low ($m = 0.02$–0.036; [43,44]). The average frequency of the LMMA in other populations ranges from 0.059 in the most proximate populations (approx. 1–6 km distance) to 0.057 when including populations up to 30 km away [42].

A bootstrap simulation based on allele frequencies at the LMML was used to estimate the total number of fish that belong to the early and late segments of the Auke Creek pink salmon population (essentially a mixed stock analysis). Expectation maximization algorithms [45] were used to allocate fish to the early or late portion of the population by comparing daily running averages of estimated allele frequencies to allele frequencies from a baseline population (in our case this was 1983, because that was the first return of post-experimental fish that randomly mated in the wild [46]). Specifically, the simulation uses running 5-day allele

frequency averages (e.g. days 3–7) to estimate the composition, in terms of origin (early versus late-migrating fish), of the middle date (day 5). The census number of fish migrating into Auke Creek on that day is multiplied by the estimated contribution of early- and late-migrating individuals to yield the estimated daily return of early- and late-migrating fish. This same procedure is performed for each day of the migration to estimate the total contribution of early- and late-migrating fish to the total abundance. Statistical replication is performed through non-parametric bootstrapping of the empirical data. This method makes use of the clear genetic difference between the marked and unmarked portions of the population in 1983 to estimate total population contribution of each phenotype in each subsequent year. The simulation also estimates the median date of migration timing for the early- and late-migrating portions of the population. For all allozyme analyses, data from the LMML were treated as if the locus were diploid [38].

The population genetic parameter F_{temporal} was used to describe and compare change in allele frequencies at the nuclear loci. This parameter measures differences in allele frequencies between two samples [47] and is a powerful method to detect genetic changes in populations [9,48]. Changes in allele frequencies at candidate loci that exceed changes at neutral microsatellite loci are evidence of directional selection at this or closely linked quantitative trait loci. To test for directional selection at candidate loci, we used genetic outlier tests [49]. LOSITAN [50] and Bayescan [51] were used to generate estimates of F_{temporal} and compare these estimates between putatively neutral and candidate microsatellite loci. These are frequentist and Bayesian approaches, respectively. Essentially, these methods attempt to differentiate signals for natural selection from those of genetic drift. Other methods to detect selection at genetic loci exist, but LOSITAN and Bayescan have the lowest type I and II error rates [52]. The recommended settings for LOSITAN were used for all analyses, including an additional 20 000 simulations. In Bayescan, we used 100 000 iterations of burn-in, 20 pilot runs, a thinning rate of 15 iterations, and retained 8000 iterations of the Markov chain Monte Carlo (MCMC) chain to ensure convergence of the posterior distributions with minimal MCMC chain autocorrelation.

3. RESULTS

Throughout the 1980s, the frequency of the LMMA in the latest-migrating fish differed from those of the early-migrating fish ($p < 0.001$; figure 4). Specifically, the allele frequency in samples of fish collected before 1 September was approximately 0.04–0.05 and was 0.21–0.26 in samples collected from the latest-migrating fish. Beginning in 1991, this pattern completely disappeared and the LMMA frequency did not differ significantly between early- and late-migrating fish ($p = 0.69$). A lack of genetic differentiation at the LMMA after 1989 was confirmed in 1993 ($p = 0.91$), 2001 ($p = 0.27$) and 2011 ($p = 0.85$). There was a strong decrease—approximately threefold—in the total frequency of the LMMA across the entire migration timing distribution from 0.131 (s.e. = 0.016) in 1983 to 0.043 (s.e. = 0.008) in 2011 (figure 5). The frequency of the LMMA was relatively stable during the 1980s, but decreased rapidly and significantly ($p < 0.05$) between 1989 and 1993. The frequency of this allele has been relatively constant since 1993.

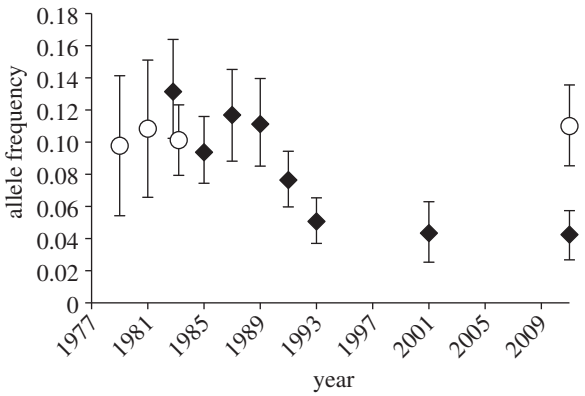

Figure 5. Frequency of the late-migration marker allele (LMMA; black diamond) and the alternate allele at control locus *G3PDH-1 *200* (white circle) across years. Error bars are the 95% CIs for each estimate.

This rapid change in the LMMA contrasts with the stable frequencies of the *200* allele at the control locus *G3PDH-1*, which changed very little (figure 5); its frequency was 0.098 (s.e. = 0.022) in 1979, 0.108 (s.e. = 0.011) in 1981, 0.101 (s.e. = 0.011) in 1983 and 0.109 (s.e. = 0.013) in 2011, which suggests that genetic drift had minimal effects on *G3PDH-1**, and hence the entire population, during this time period. These results support the hypothesis of directional selection for earlier migration timing. The altered allozyme frequencies at the marker locus in the late-migrating portion of the population changed substantially during the study period, but such changes were not observed at another locus.

Results from the bootstrap simulation demonstrate that during the 1980s the late-migrating genetically marked component of the population accounted for 27 to 39 per cent of the total abundance (table 1). This proportion decreased rapidly after 1989 and was approximately 5 per cent (s.e. = 2.4%) in 2011. Because of the loss of intra-annual genetic differentiation by time at the LMML after 1989, the simulation was unable to differentiate the early- and late-migrating population, as demonstrated by the overlap in the median dates of migration timing (table 1). Therefore, estimates from 1991 to 2011 should be used with caution. However, the primary pattern is clear and consistent with the other results; the late-run portion of the population used to be an important component of the total population abundance, but is no longer. Nevertheless, overall abundance has not changed [26].

Conversely, genetic outlier analyses did not provide any evidence for selection at the candidate loci associated with circadian rhythms. Results from LOSITAN indicate that none of the 26 microsatellite loci used in this study appear to be under directional or balancing selection (electronic supplementary material, figure S1*a*). Locus-specific estimates of F_{temporal} were low (0–0.007) across all loci, suggesting that the combined effect of genetic drift and selection has been weak at these loci. Nonetheless, it is noteworthy that the candidate locus *Cryptochrome2b* had the highest F_{temporal} value of all loci (0.007). This locus had the lowest expected heterozygosity (0.044), and therefore was located in the region of the plot in which selection is the most difficult to detect

Table 1. Estimates of the abundance (N) of the early and late (genetically marked) portions of the population. M is the estimated median date of migration timing of the early- and late-migrating portions of the population based on the number of days after 1 July. PLR is the proportion of the overall population composed of late-migrating fish.

year	run	N	s.e. (N)	M	s.e. (M)	PLR	s.e. (PLR)
1985	early	17619.11	972.38	52.52	0.09		
	late	6494.89	972.38	68.67	2.51	0.27	0.04
1987	early	4812.46	375.12	51.77	0.50		
	late	3052.54	375.12	61.86	0.17	0.39	0.05
1989	early	3403.25	223.64	56.92	1.25		
	late	1596.75	223.64	69.70	1.87	0.32	0.04
1991	early	5668.26	226.46	53.35	0.09		
	late	937.74	226.46	53.35	1.78	0.14	0.03
1993	early	1545.25	53.37	67.64	0.27		
	late	137.75	53.37	67.20	4.48	0.08	0.03
2001	early	6959.42	217.64	59.46	0.17		
	late	569.58	217.64	58.03	1.15	0.08	0.03
2011	early	25634.18	650.21	51.04	0.59		
	late	1347.82	650.21	53.92	6.99	0.05	0.02

(i.e. has the widest 95% confidence regions). Similarly, results from Bayescan suggest that directional selection is not acting at any of these loci; but there may be mild balancing selection at *Ots101* (electronic supplementary material, figure S1*b*).

The small values of F_{temporal} and relatively large N_e ($N_e = 271$ [53]) at these microsatellite loci indicate little genetic drift in this population and support the idea that the radical changes in the LMML over the course of the study were due to selection against the late-migrating portion of the population. For example, F_{temporal} was 0.025 at the LMML from 1989 to 1993 [47], a value that is over three times greater than that observed at any of the microsatellite loci (though the time periods are not overlapping). We used LMMA frequencies from other populations in an island–continent model to estimate the migration rate (m—the proportion of the population that are immigrants [54]) that would be necessary to achieve the observed changes in the LMMA in the Auke Creek population from 1989 to 1991. Depending on the scale of the analysis (including only nearby locations versus more distant populations) or whether the analysis was restricted to late-migrating fish, m would need to be 0.69–0.85 to satisfy the observed genetic changes. For populations around Auke Creek, these values are 19–24 times higher than the largest demographic estimate of m (i.e. dispersal [42]), and 460–566 times larger than genetic estimates of m [27]. As such, migration is not a likely explanation for the observed genetic changes in the LMML.

4. DISCUSSION

In order to understand whether phenological shifts in a population of pink salmon were due to microevolution, we used genetic data collected from 1979 to 2011 and observed evidence for genetic change associated with shifts towards earlier migration timing. Data from the LMML demonstrated that there was a significant decrease—at least threefold—in the late-migrating portion of the odd-year Auke Creek pink salmon population. This provides evidence of a rapid microevolutionary change in this population that has proved exceptionally elusive in other studies [5,9]. The trend towards earlier migration timing in this population does not appear to be anomalous, because it is replicated in the even-year population that uses the same freshwater habitat, and in other salmonid species and life histories (figures 2 and 3) [23–26]. Importantly, another recent study that used a modelling approach determined that microevolution for earlier migration timing has occurred in a population of sockeye salmon in the Columbia River [20]. Together, these results provide compelling evidence that recent climate change has influenced the evolutionary dynamics of salmonid populations and their adaptation via migration timing to their respective habitats.

The LMML indicated that a major selection event occurred between 1989 and 1993. Although we do not know the specific selective pressures that led to earlier migration timing in this population, stream temperatures during peak migration timing in 1989 were the second highest on record, and we observed substantial genetic changes at the LMML in the progeny from this spawning generation. Migrating pink salmon appear to avoid high stream temperatures; given the trend in migration timing, changes in the genetic marker and increasing stream temperatures in Auke Creek [26], it appears that earlier-migrating fish may have higher fitness in warmer years. Adaptations-by-time [55] for different thermal regimes and biotic interactions are well documented for this population, and there is evidence that early-migrating adult fish are adapted to warmer conditions at multiple life stages and life-history events (e.g. juvenile developmental rates and migration timing, and adult migration timing, lifespan and breeding date) [37,41,56,57]. These patterns of local adaptation result in a strong temporal structuring of the population [37]. Another possible explanation is that warm stream temperatures may have caused reproductive overlap (and hence gene flow) between early- and late-migrating fish, and the resulting evolutionary changes are due to outbreeding depression.

Stream temperatures during peak migration approach upper lethal limits in some years [18], which could potentially act as a constraint to further microevolution. However, there are no temporal trends in migration timing for the first 5 per cent ($p = 0.854$) and first 25 per cent

($p = 0.102$) of the migration timing distribution. This observation and data at the LMML suggest that there has been a truncation of the migration timing distribution and strong selection against the latest migrating fish, resulting in the near elimination of this phenotype. The rapid genetic changes imply that climate-induced selection on life-history traits may not result in gradual evolutionary shifts. Rather, selection events may be extreme and episodic, and have severely different consequences for different phenotypes (i.e. near elimination of the late-migrating phenotype). Interestingly, the median phenotype appears to have undergone a continuous shift towards earlier timing (as opposed to rapid truncation in one generation), indicating that plasticity must also be responsible for the observed change in migration timing.

Temporal structuring owing to migration timing complicates whether this represents the evolution of a single population, or selection against one population and a demographic response (increase in abundance) in another. Like other salmon populations, pink salmon in Auke Creek are probably best described as a single population exhibiting intra-population genetic structure owing to heritable differences in migration timing [55]. This is corroborated by weak, but statistically significant genetic differentiation between early- and late-migrating fish at neutral loci (i.e. there is gene flow between reproductively isolated components of the migration timing distribution) [42,53].

Interestingly, the progeny of early-migrating adult pink salmon historically (1970s–1980s) had lower early marine survival than progeny of later-migrating fish [40,58]. To better understand these phenotypic and evolutionary changes, it would be valuable to determine whether this pattern is still present, or if these populations are stable because of increased fitness (compensation) at some other population vital rate (e.g. reproductive success in freshwater). Additionally, it is unclear whether these changes could lead to future trophic mismatches between juvenile pink salmon and the availability of marine resources [15]. Despite the fact that Auke Creek has undergone significant warming and there have been substantial phenological shifts, both odd- and even-year pink salmon populations are stable [26], and population abundance in 2011 was the second highest on record. Given that changes in migration timing can influence population dynamics, it seems plausible that the observed changes in migration timing have allowed these populations to remain resilient to environmental change [59].

Selection was not detectable in the circadian rhythm genes we used in this study. It is possible that these loci do not directly influence migration timing [60]. Along the same lines, migration timing is probably a complex quantitative trait influenced by many genes. If selection did not act on our candidate loci for migration timing, then it must have acted on other loci that influence migration timing. However, archived genetic samples were available dating back only to 1993 and it is possible that selection occurred at circadian rhythm genes before this date. This is hinted at by the LMML data, which showed a decrease in the late-migrating phenotype/genotype between 1989 and 1993. Alternatively, sampling more selectively neutral markers would increase our power to detect subtle differences in genetic change (e.g. changes at *Cryptochrome2b*).

Genetic variation for migration timing is an important aspect of biocomplexity in Pacific salmon populations that decreases population stochasticity [61]. Along with the shift in the distribution of migration timing and loss of the late-migrating component of the population, there is no longer distinct bimodality in the distribution of migration timing in the even- or odd-year populations. We no longer observe the clear phenotypic distinction between early- and late-migrating individuals that was once present in the system [27,37]. Apparently, the very-late-migrating phenotype has been greatly reduced or potentially lost. Although microevolution may have allowed this population to successfully track environmental change, it may have come at the cost of a decrease of within-population biocomplexity—the loss of the late run [61]. This is not a surprising result; by definition, directional selection will decrease genetic variation. However, it does highlight the importance of maintaining sufficient genetic and phenotypic variation within populations in order for them to have the ability to respond to environmental change. In this particular population, genetic and phenotypic variation have allowed for evolutionary changes in an important life-history trait, the result of which is that this population is persisting through rapid temperature warming. These findings are an important empirical advancement towards understanding the process of climate-induced microevolution in wild populations.

We thank J. Joyce, J. Echave, S. Taylor, NOAA and previous members of the Gharrett laboratory for helping to collect much of this data, and Mark Lindberg and Milo Adkison for comments on earlier drafts of this manuscript. An Alaska EPSCoR Graduate Research Fellowship and Institute of Arctic Biology Summer Research Fellowship supported R.P.K. This research was prepared by R.P.K. and D.A.T. under North Pacific Research Board project 1110 and award 45965 from the National Oceanic and Atmospheric Administration, US Department of Commerce, administered by the Alaska Department of Fish and Game. Data obtained prior to 1991 were obtained with support from the Alaska Sea Grant Programme. The statements, findings, conclusions and recommendations are those of the author(s) and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration, the US Department of Commerce or the Alaska Department of Fish and Game.

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