

Eco-geographic units, population hierarchy, and a two-level conservation strategy with reference to a critically endangered salmonid, Sakhalin taimen *Parahucho perryi*

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Abstract Hierarchical population structure can result from range-wide geographic subdivision under conditions of environmental heterogeneity and weak gene flow. While a lower level of structure can be formed by local populations within eco-geographic regions, an upper level can be characterized by variation between populations from different regions, and thus, be represented by evolutionarily significant units (ESUs) defined by environmental, ecological and genetic variation. Selection of ESUs may depend on the sequence of using these three sources of variation. We propose to determine ESUs by first using non-genetic, ecological and geographical gradients for defining preliminary population groups (*eco-geographic units*, EGUs) and then testing whether the boundaries of these units are genetically coherent and thus represent

ESUs or warrant their further modification. We evaluate this approach using Sakhalin taimen, an East Asian endangered endemic fish. Forty-one samples (473 fish) were drawn from thirty populations across the species range and genotyped at microsatellite DNA markers. We assign the populations into ESUs based on geographic and life history criteria and subsequent application of genetic diversity analyses. The ESUs appeared to be greatly diverged genetically. Within ESUs, local populations are genetically differentiated, have low effective sizes, show signatures of demographic decline and extremely restricted gene flow. Conservation plans aimed to restore or maintain a specific threatened population should take into account such hierarchical structure, and in particular be based on the genetic resources drawn from each population or using ecologically and genetically similar populations from the same ESU as donors for restoration of the population.

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Introduction

Decline in the abundance and range of a threatened species can lead to population fragmentation, increased reproductive isolation, and loss of adaptations due to genetic drift. To develop conservation actions, one needs to identify conservation units, by choosing or prioritizing genetically distinct and reproductively independent local populations for protection. Analyzing definitions in the U.S. Endangered Species Act, Waples (1991) developed a concept of evolutionarily significant units (ESUs) as a population segment important for guiding conservation measures via the establishment of two criteria: (1) it is reproductively isolated from other such units, (2) it represents an important evolutionary component of the species. The two conservation goals for ESUs are: (1) protect the existing pattern of within- and between-population genetic variation that provides environmental adaptation, and (2) protect evolutionary processes that generate genetic diversity and select for adaptive characters (Waples 1991; Moritz 2002; Allendorf et al. 2012; Funk et al. 2012).

Both goals actually refer to adaptive genetic variation. The challenge is to identify conservation units recognizing that it is hard to differentiate populations based on adaptive genotypes as such information is often lacking. Recent genome-wide studies suggest that local adaptations may be based on either parallel (Pearse et al. 2014) or non-parallel (Perrier et al. 2013) genetic changes. Even for quantitative adaptive traits with high heritability, between-population differences caused by local adaptations may not be evident because such traits are usually expressed through the interaction of multiple genes and varying environmental factors (Waples 1991; Gagnaire et al. 2012) and thus would require long-term population-specific mating experiments to understand (Falconer 1960). The molecular basis for local adaptation in salmonids is largely unknown (Fraser et al. 2011). For endangered animal species with complex population structure, such experimental designs are usually logistically impossible, and thus the explicit incorporation of adaptive genetic variation into conservation programs can be impractical to carry out.

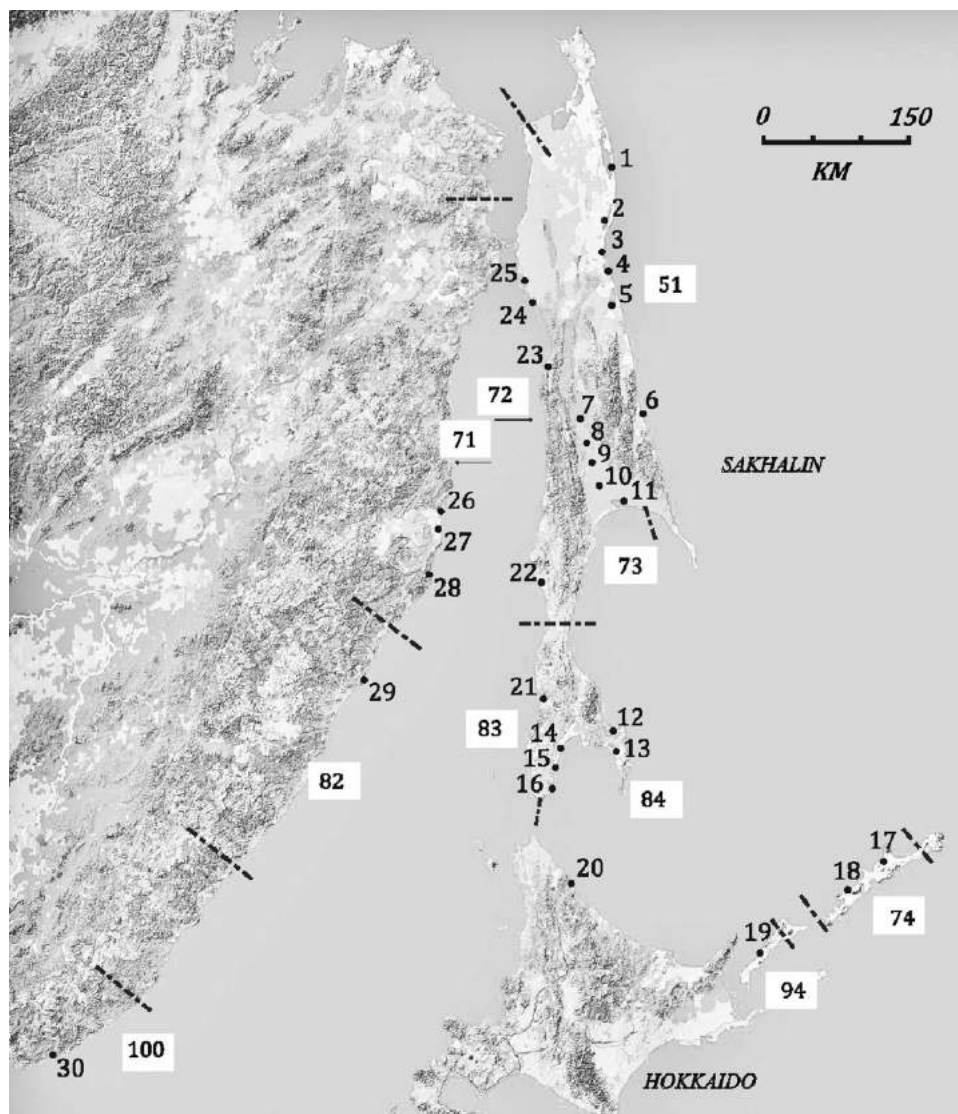
To incorporate adaptive genetic variation into the ESU concept, Moritz (2002) proposed protecting environmental gradients as a surrogate for adaptive phenotypes and their underlying adaptive evolutionary processes. To achieve this, a two-step strategy was suggested: (1) identify geographical areas that represent genetic diversity of the species, and (2) protect contiguous adaptively important environmental gradients within these areas. Another important step in defining ESUs is producing a spatial template that captures the reproductive isolation processes and movement of individuals across habitats—

a main focus of landscape genetics (Manel et al. 2010; Sork and Waits 2010). Combining knowledge of population differentiation based on genetic markers and environmental factors can reveal linkages between population structure and environmental parameters (e.g., Dionne et al. 2008).

Waples et al. (2001) proposed to consider ESUs in terms of three sources (axes) of variation: ecology/environment, life history, and genetics. Within this context, Waples (2006) suggested: (1) determine a genetic tree first, and (2) subdivide the tree using the ecology and life history axes. However, for an endangered species with restricted migration and low population size, genetic trees or other representations of genetic variation (e.g., multi-dimensional scaling) may be misleading due to the combined effect of severe decline in population size, strong genetic drift, artificial reproduction and translocation and small sample sizes. In attempt to circumvent these difficulties, we use the three-axes concept (Waples 2006) with an altered methodology. Namely, we first use the two non-genetic axes for defining population groups across ecological and geographical gradients (herein referred to as *eco-geographic units*, *EGUs*), and only subsequently test whether these unit boundaries are genetically coherent and thus represent ESUs or warrant further division into ESUs.

We elaborate such an approach using Sakhalin taimen *Parahucho perryi* (Brevoort, 1856) as an example. Sakhalin taimen is an endemic iteroparous salmonid species in the Russian Far East and Japan, designated as Critically Endangered by the IUCN (Rand 2006). It is additionally recognized as endangered in national Red Book lists of The Russian Federation and Japan and listed as one of the world's one hundred most threatened species (Baillie J et al. 2012). One of the largest salmonids in the world, *Parahucho* is a riverine top predator that matures at the age of 6–8 years, lives for more than 20 years, and can reach more than one and a half meters in length (Gritsenko 2002; Zolotukhin and Semenchenko 2008; Fukushima et al. 2011). Their current geographic range is restricted to the Russian Far East (Sakhalin, Iturup and Kunashir islands, and Khabarovsk Krai and Primorye) and northern Japan (Hokkaido). Major threats to Sakhalin taimen include illegal fishing and loss of habitat due to the expanding human presence and extensive land use practices. These fish are particularly sensitive to these threats given their large body size, late age of maturation and irregular spawning. Sakhalin taimen occupy a variety of habitats during their life history that shape gene flow and population structure. These habitats, across environmentally different regions of the species range, include headwater and lower reaches of rivers, lakes, brackish lagoons, estuaries and the coastal marine.

Fig. 1 Geographic location of genetic samples from Sakhalin taimen populations. Numbers in white squares represent the environmental/geographic division (geographic provinces) by Martynenko (2007), the adjacent provinces are separated by dashed lines; an additional province is represented by a sample from Hokkaido. Other numbers stand for Sakhalin taimen populations: 1 Piltun Bay, 2 Val River, 3 Dagi River, 4 Tym River, 5 Nabil River, 6 Langeri River, 7 Poronay River and its tributaries (8 Onorka, 9 Brusnichnaya, 10 Elnaya), 11 Nevskoe Lake, 12 Tunaicha Lake, 13 Vavaiskoe Lake, 14 Urjum River, 15 Uljanovka River, 16 Moguchi River, 17 Lebedinoe Lake, 18 Kuibyshevka River, 19 Valentina Lake, 20 Sarafutsu River, 21 Tainoe Lake, 22 Ainskoe Lake, 23 Agnevo River, 24 Viakhtu River and Viakhtu Bay, 25 Tyk Bay, 26 Ulika River, 27 Tumnin River, 28 Koppi River, 29 Samarga River, 30 Kievka River



Materials and methods

Geographic subdivision and population samples across the species range

The Russian Far East territory is extremely diverse environmentally and has been subdivided into geographic provinces according to four environmental characteristics: latitudinal temperature gradients and forest types, longitudinal climatic changes, landscape regions, and marine drainage basins (Martynenko 2007). Projecting this template onto the river watersheds supporting Sakhalin taimen produces ten separate regions (Fig. 1). Hereafter we refer to these as *geographic provinces*.

From 30 populations of Sakhalin taimen, 41 samples were drawn (a few populations were represented by two or three samples taken in different years or seasons), totaling

473 individuals were used for genetic analysis (Fig. 1; Tables 1 and A1). One population, the Tainoe Lake (#21 in Fig. 1), was artificially landlocked in the 1920s by a dam erected on the Tyi River. The samples were collected via catch-and-release. Most tissue samples stem from fin clips fixed immediately in 96 % ethanol, while some stem from archived skin collections (Table A1). Additionally, we took 13 fin clips from Sakhalin taimen in the Khabarovsk city fish market with the aim of identifying potential source populations. Individuals were typed at 19 microsatellite loci (details in Table A2).

Assignment into migratory phenotypes

Sakhalin taimen exhibit a variety of migratory phenotypes, ranging from freshwater residency to anadromy (Zimmerman et al. 2012), although Gritsenko (2002) has

Table 1 Summary of sampling data. Shown are the population names, the sample code (numbered 1–30, Fig. 1) whereby capital letters refer to multiple samples across time, and the number of individuals sampled (for more details see Appendix, Table A1)

Regions (province)	Population	Sample code	Sample size	
Northeastern Sakhalin (51)	Piltun River	1	8	
	Val River	2	14	
	Dagi River	3A	31	
	Dagi Bay	3B	9	
	Tym River	4	11	
	Nabil River	5A	9	
		5B	11	
	Terpenie Bay (73)	Langeri River	6	10
		Poronay River	7	11
		Onorka River	8	12
Brusnichnaya River		9	11	
Elnaya River		10	21	
Nevskoe Lake		11	8	
Southeastern Sakhalin (84)	Tunaicha Lake	12A	10	
		12B	6	
	Vavaiskoe Lake	13A	10	
		13B	18	
		Urjum River	14	3
	Uljanovka River	15A	4	
		15B	7	
	Moguchi River	16	6	
		Lebedinoe Lake	17	7
	Iturup Island (74)	Kuibyshevka River	18	7
Valentina Lake		19	6	
Kunashir Island (94)	Sarafutsu River	20	9	
Hokkaido	Tainoe Lake	21	9	
Southwestern Sakhalin (83)	Ainskoe Lake	22A	30	
Western Sakhalin (72)		22B	20	
		22C	10	
Khabarovsk Krai (71)	Agnevo River	23A	3	
		23B	10	
	Viakhtu River	24A	32	
	Viakhtu Bay	24B	10	
	Tyk Bay	25	10	
	Ulika River	26	15	
		Tumnin River	27A	17
27B			9	
Koppi River	28A	13		
	28B	11		
Primorje (82)	Samarga River	29	9	
	(100)	Kievka River	30	6
Khabarovsk Krai	Khabarovsk fish market	–	13	

characterized the species as *semi-anadromous* assuming that they do not migrate far from the natal river mouth. The strontium-to-calcium ratio in their otoliths can help recognize anadromous versus non-anadromous behaviors (Edo et al. 2005; Honda et al. 2010; Zimmerman et al. 2012), but this method requires sacrificing individual fish.

Given their rare status, there is insufficient data available to properly characterize the full suite of migratory phenotypes expressed in each population. As a proxy, we used another set of catch data collected during the active feeding period (from the end of July to October, 1999 to 2011) from a variety of habitats in watersheds across Sakhalin Island

(see Table A3 for a full description of methods and data). Based on the relative distribution of abundance of each taimen population, we assigned each taimen population of Sakhalin Island to one of the following four dominant migratory phenotypes: *freshwater resident* (FR), *lake* (LK), *lagoon* (LG), and *semi-anadromous* (SA).

Data analyses

Standard population statistics

Allele diversity (expected heterozygosity) and its sampling error were computed as in Nei (1987), unbiased F_{ST} estimator θ_{ST} (Weir and Cockerham 1984) and its bootstrap confidence interval across loci, and exact Fisher tests for Hardy–Weinberg expectations (Zaykin et al. 1995) were calculated with the GDA software (Lewis and Zaykin 2001). The homogeneity among population samples was evaluated by computing a confidence interval (CI) of θ_{ST} by bootstrapping over loci. For multiple comparisons, a sequential Bonferroni correction was applied holding a nominal significance level of 0.05.

Genetic clusters of samples

We used an individual based method of clustering multi-locus genotypes by Pritchard et al. (2000). An optimal number of clusters was inferred using the criteria L(K) and ΔK (Evanno et al. 2005) on simulations of $K = 1 \div 20$ populations with ten iterations *per K* under an admixture model with independent allele frequencies (burn-in and MCMC lengths of 100,000 generations). Given an optimal K , the individuals were assigned into K clusters using a model of informative priors (Hubisz et al. 2009) with burn-in and MCMC lengths of one million generations; different population samples, and multiple samples from the same population as well, were individually labeled. Each higher-level cluster inferred was subdivided into lower-level sub-clusters applying the same methods with $K = 1 \div 10$. Output files were generated with STRUCTURE 2.3.4 (<http://pritchardlab.stanford.edu/structure.html>) and visualized using CLUMPAK (<http://clumpak.tau.ac.il/index.html>).

Spatial structure

Autocorrelation analyses with a fifty kilometer distance class and a Mantel test for evaluation of the correlation between genetic and geographic distance matrices were performed as suggested in Smouse and Peakall (1999) and Peakall and Smouse (2012). Pairwise geographic distances were estimated with the coastal-line distance between the corresponding lake or river mouth.

Gene flow and assignment tests

Recent migration rates between Sakhalin taimen populations were estimated with the method of Wilson and Rannala (2003) using software BayesAss 3.0.2.Windows (<http://sourceforge.net/projects/bayesass/files/BA3/>). Population assignment tests were provided with the approach of Paetkau et al. (2004) using the Bayesian estimate from Rannala and Mountain (1997) in the software GeneClass2 (Piry et al. 2004).

Effective population size

Current effective number of spawners were estimated with linkage disequilibrium (Waples and Do 2010), heterozygote excess (Pudovkin et al. 2010), and molecular coancestry (Nomura 2008) methods using software NeEstimator2 (Do et al. 2014).

Results

Microsatellite variation

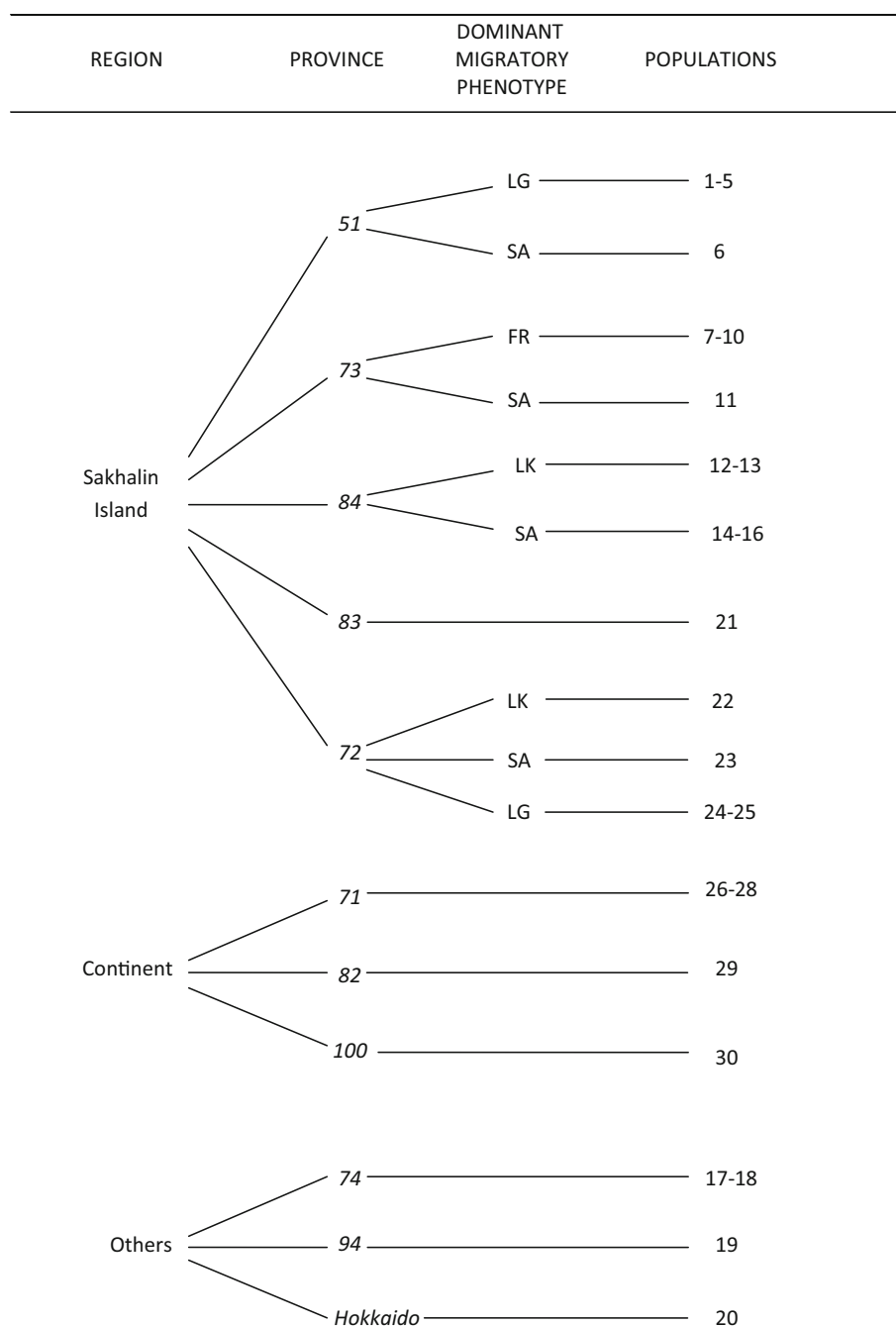
Among 779 tests for Hardy–Weinberg equilibrium, 42 tests (or 5.4 %) were significant with $p < 0.05$ (Table A4), prior to table-wide corrections, but no tests remained significant after applying a Bonferroni correction. Across all samples, 261 alleles were found at 19 loci; the within-population allele diversity averaged across samples was 0.52 (single-locus and single-population estimates are given in Tables A5 and A6). The total F_{ST} -value was 0.13 (pairwise estimates in Table A7). Multiple samples from Nabil, Agnevo and Koppi rivers, and from Vavaiskoe and Ainskoe lakes, did not significantly differ (Table A7), whereas two samples from the Tumnin River were somewhat heterogeneous ($p = 0.012$), but two samples from Tunaicha Lake and two samples from Uljanovka River were highly heterogeneous ($p \ll 0.005$).

Hierarchical population structure

Eco-geographic units (EGUs) of Sakhalin taimen populations

The genetic samples of Sakhalin taimen were distributed over eleven geographic provinces (ten in Russia and one in Hokkaido) (Fig. 1). To characterize Sakhalin taimen populations at a finer scale, a further subdivision into the dominant migratory phenotypes was added to produce eco-geographic units (EGUs). Based on the results of a principal components analysis of the relative distribution of biomass (Table A3), Sakhalin Island populations appear to

Fig. 2 Assignment of populations to EGUs. For Sakhalin Island, EGUs are composed of a combination of the geographic province (Fig. 1) and the migratory phenotype (Fig. B1). For example, 51-LG is an EGU corresponding to the lagoon phenotype within province 51 (the geographic province 83 is represented by an artificially landlocked population only). Outside of Sakhalin Island, EGUs correspond to geographic provinces only as information on migratory phenotypes was not available



be dominated by semi-anadromous and lagoon migratory phenotypes, and more rarely, lake and freshwater resident forms (Fig. B1). In our data, the freshwater resident migratory phenotype is represented by fish from the Poronay River (#s 7–10 in Fig. 1), and lake populations by samples from Vavaiskoe, Tunaicha, and Ainskoe lakes (#s 12, 13, 22), all of which have open access to the sea. Nine EGUs of taimen therefore were identified across Sakhalin Island based on both the geographic province and the dominant migratory phenotype, whereas the other EGUs

(outside of Sakhalin Island) were selected using geographic criteria only due to the lack of life history data (Fig. 2).

STRUCTURE analysis and correspondence to EGUs

The individual-based STRUCTURE analysis revealed five main genetic clusters, each of which corresponded to one or more EGUs (Fig. 3 compared to Fig. 2). Samples from Iturup, Kunashir and Hokkaido islands were distributed among these genetic clusters irrespective of geography, although

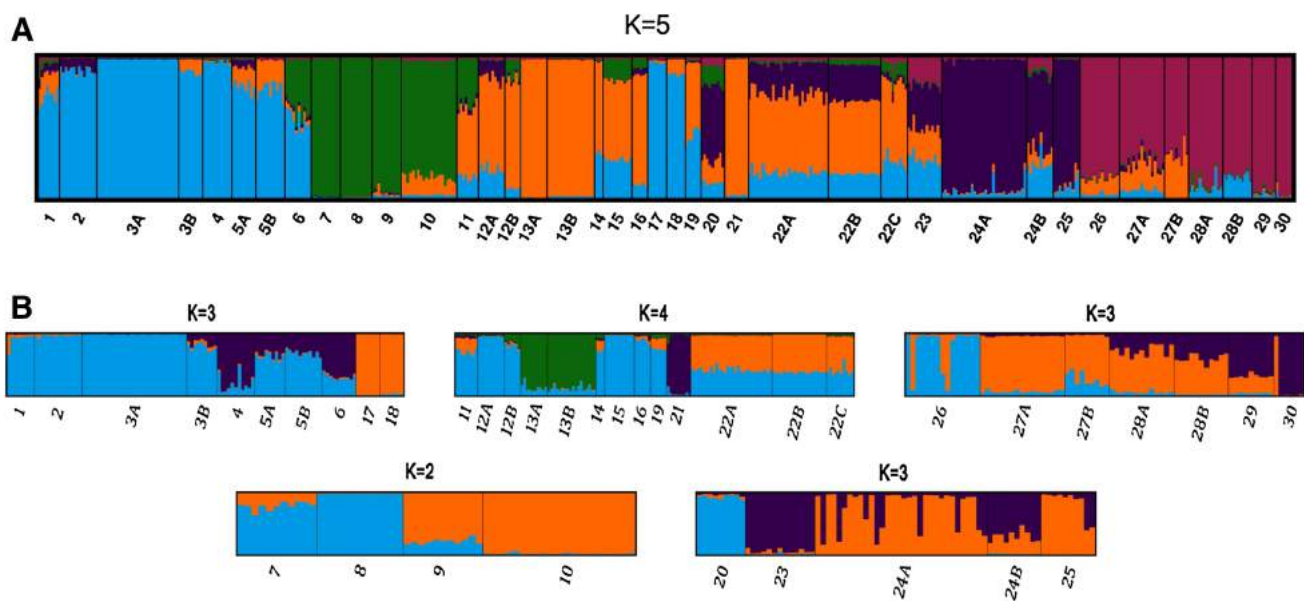


Fig. 3 Population clusters revealed by the STRUCTURE analysis. **a** The initial analysis revealed an optimal number of clusters ($K = 5$) following the likelihood approach by Evanno et al. (2005).

b Subdivisions within clusters followed the same procedure and revealed from $K = 2$ to $K = 4$ sub-clusters for each main cluster identified in the initial analysis. Population codes are in Table 1

very small samples sizes from these regions coupled with a high level of genetic drift due to low population size may have affected the results. Nonetheless, the second level of the hierarchical STRUCTURE analysis revealed 15 sub-clusters that distinguished the latter populations except Valentina (#19 in Figs. 1, 3). This second-level genetic subdivision determined from STRUCTURE demonstrated a finer correspondence between the genetics and ecology, although not without ambiguity. Indeed the majority of genetic sub-clusters corresponded to EGUs as can be seen from Figs. 2 and 3. Population-based approach performed on genetic distances appeared to be less coherent to EGUs than the individual-based one although some clusters were in good concordance with EGUs (data not shown). Therefore, the EGUs are actually ESUs.

The EGUs can be considered to reflect an upper level of genetic differentiation of Sakhalin taimen supported by large F_{ST} -values. Namely, the between-EGU F_{ST} estimate (θ_p) for five Sakhalin Island’s EGUs, each of which contained at least two populations (this is a requirement of the hierarchical GDA-analysis), was 0.079 with a 95 %-confidence interval (CI) of (0.057–0.104), which is more than half the total between-population θ -value, 0.150 (CI 0.126–0.180). Genetic differentiation of Sakhalin Island populations based on geography alone, neglecting migratory phenotype, was considerably less, $F_{ST} = 0.048$ (CI 0.034–0.063), and that based on migratory phenotype alone was 0.032 (CI 0.019–0.047). Therefore, the more broadly defined EGUs, taking into account both geographic discontinuities and migratory phenotype, provide a finer genetic stratification of taimen than those based on geographic or migratory

phenotype criteria alone. This further confirms the importance of the three-axes approach of Waples et al. (2001).

Populations within EGUs represent a second level of hierarchy. Moreover, all pairwise comparisons between taimen populations were statistically highly significant within EGUs indicated by high F_{ST} -values: 0.084 (CI 0.057–0.117) in northeastern Sakhalin (EGU 51-LG); 0.099 (CI 0.062–0.141) in the Poronay River basin (73-FR); 0.074 (CI 0.044–0.110) and 0.074 (CI 0.028–0.122) in southern Sakhalin within the lake (84-LK) and semi-anadromous (84-SA) forms, respectively; 0.037 (CI 0.013–0.064) in northwestern Sakhalin (72-LG), 0.042 (CI 0.027–0.061) in northernmost continental rivers (71), and 0.284 (CI 0.164–0.392) in Iturup Island (74). Therefore, each Sakhalin taimen population looks to be genetically unique. The latter F_{ST} -estimate is extreme, which is probably due to genetic drift in these small isolated island populations; alternatively, the small sample sizes may have inflated F_{ST} -estimates. Therefore more attention should be paid to confidence intervals or standard errors, rather than the point estimates themselves.

Gene flow

Spatial structure analyses revealed that a distance of about 200–300 km, within which the correlation between the geography and genetic distances was still significant, can be viewed as a geographic range within which gene flow could be expected (Fig. 4). It is worth mentioning that the geographic size of each of the EGUs falls into this distance range. Within that range, the geographic-genetic correlation

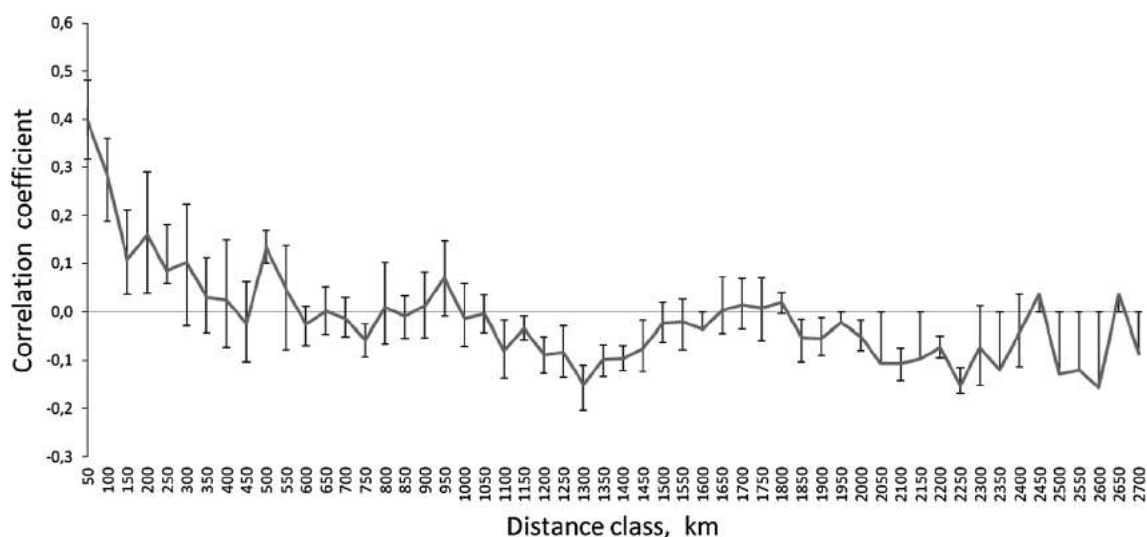


Fig. 4 Spatial structure analysis of Sakhalin taimen populations. Autocorrelations were computed with a 50-km step, vertical bars stand for the 95 % confidence intervals of correlation coefficient estimates. For the fresh-water resident Sakhalin taimen in Poronay River, the distance from the mouth upward to the actual sample site

was added. For estimated distances between mainland coastal sites and islands, it was assumed that fish must pass through the northernmost sample sites at the Tatar Strait, Tyk Bay (sample #25) and Ulika River (#26), (Fig. 1)

works well, except for southern Sakhalin (Fig. B2). Such correlations assume historic gene flow between spatially close populations. Estimates of recent (over the last generations) migration rates (Table A8) revealed higher estimates of short-distance migration rates, namely between populations within EGUs: their average rate was 0.023 with an average standard error of 0.013 across 48 possible pairwise migration paths, whereas the average migration rate between EGUs was smaller, 0.0087, with an average standard error of 0.0081, across 764 possible migration routes between populations. This suggests an isolation-by-distance model because each EGU occupies a relatively small territory. However, these migration patterns appear complex and asymmetrical. Indeed, highly significant apparent migration from the Dagi River population into neighboring Val and Nabil populations and probably into other taimen populations of northeastern Sakhalin was revealed, but not vice versa, as well as migration from Ainskoe into Agnevo and from Koppi into Samarga populations (Table A8). Within-EGU short-distance straying of Sakhalin taimen from natal rivers appears evident from population assignment tests on fish caught in brackish bays. For example, six of nine individuals from the Dagi Bay sample were assigned to the Dagi River population, while three fish were assigned to neighboring Nabil and Tym rivers (the three rivers are from the same EGU); probably, the fish migrated to the bay in search of food. Another example concerns samples from the Viakhtu River and nearby Tyk Bay (same EGU): the majority of individuals sampled in the Tyk Bay were assigned to the Viakhtu River population.

One might evaluate historic gene flow rates assuming Wright's migration-drift equilibrium model whereby the number of effectively reproducing migrants *per* generation is determined by the equation $N_e m = (1 - F_{ST}) / (1 - F_{ST}) 4F_{ST} 4F_{ST}$, where N_e and m are the effective population size and gene flow rate *per* generation, respectively. The average within-EGU F_{ST} -value is 0.069 which results in roughly $N_e m \approx 3.4$ reproducing migrants per generation, or one reproductively successful migrant in three years if the reproductive period lasts ten years. Therefore, even short-distance, within-EGU, historic genetic exchange between Sakhalin taimen populations does not seem frequent, although it may be sufficient to establish a correlation between geographic and genetic distances.

Trends in population size

Contemporary effective population sizes were obtained with three methods that gave quite different estimates (Table A9). Lower bounds of confidence intervals provide more insight than point estimates; we will refer to estimates based on the linkage disequilibrium method as this approach produced the majority of non-infinite values. Most "effectively" abundant were the taimen populations of Dagi River in northeastern Sakhalin, Ainskoe Lake in western Sakhalin, and coastal Koppi and Tumnin rivers. Interestingly, as mentioned above, these very populations were the sources of recent migration. A possible explanation is that migration out was more frequent from large populations; alternatively, migrants are more easily

distinguished in small populations. Many taimen populations appeared to have a small effective number of spawners (Table A9). Among those are populations of southern Sakhalin, a region with high human activity; therefore, the correlation of geographic and genetic distances across southern Sakhalin (Fig. B2) could be obscured as a result of strong genetic drift. Most likely, the reduction in Sakhalin taimen abundance began some time ago as suggested by negative historical trends in population sizes revealed with three tests (Fig. B3).

Discussion

Because of the relatively high degree of concordance with boundaries of EGUs and genetic variation in *P. perryi*, we conclude that EGUs can be considered ESUs, and we refer to them hereafter by this latter name. By including geographic and environmental criteria, along with life history traits, these units can be considered in the context of adaptive phenotypes and the underlying evolutionary processes, which is an important aim of conservation biology (Moritz 2002). We therefore present these ESUs to help guide future research and conservation work on this species with the goal of conserving biodiversity and protecting the species from future threats.

While our selection of units were supported in large part by genetic differentiation, additional hierarchical levels might also be relevant for evolutionary or management perspectives. For example, Sakhalin Island populations can be genetically clustered (data not shown) into eastern Sakhalin, whose rivers drain into the waters of Sea of Okhotsk (populations 1–16 in Fig. 1), and western Sakhalin with the Sea of Japan as a drainage basin (populations 21–25). As suggested by Fig. 3 and the heterogeneity of multiple samples from some watersheds, these ESUs could be delineated into sub-units. Moreover, the between-population genetic differentiation of Sakhalin taimen is highly significant even within ESUs, which demands that each population be considered as genetically unique (Table A7). On the other hand, the degree of between-ESU reproductive isolation and limited potential for dispersal mainly within ESUs through short-distance migration makes these ESUs particularly sensitive to anthropogenic impacts and environmental shifts.

Genetic studies on rare, endangered species may often result in small sample sizes; for some species, sampling even ten individuals from a population might be difficult. Such small population samples lead to poor estimates of allele frequencies and richness and increase the likelihood of inflated F_{ST} -values, as well as estimates of other parameters such as migration rates and effective population/breeder sizes, etc. This can lead to statistical outliers

due to false positive or false negative associations of genotypic variants with environmental gradients, and thus logically similar populations may appear in different genetic clusters. For example, small samples drawn from single populations from Iturup, Kunashir and Hokkaido islands likely prevented their assignment to a separate genetic cluster. In order to obtain reliable information on a species of interest, one should collect samples from a large number of populations across the species range, use unbiased estimates of population parameters, sample- or location-based informative priors for individual assignment, pay attention to statistically significance comparison tests, rely on confidence intervals obtained by bootstrapping over loci, or other statistics and statistical tools. Especially in such circumstances, sampling design for detecting spatial environmental effects is one of the important issues of field molecular-ecological studies (Andrew et al. 2013).

The ESU concept can help in designing conservation units as it relies on both geographic-ecological subdivision of a given species range and genetic criteria of population divergence (Waples et al. 2001). For example, genetic data suggest a uniqueness of each taimen population between and even within ESUs. Gene flow between the taimen ESUs seems to be a rare event: such a strong reproductive isolation might have evolved under ESU-specific selection patterns. Declines in population size could further diminish the between-ESU exchange by migrants. The two most common cited reasons for such a decline in Sakhalin taimen populations are habitat destruction and unregulated fishing (Rand 2006; Safronov et al. 2006; Safronov and Sukhonos 2006; Semenchenko and Zolotukhin 2011; Fukushima et al. 2011). Genetic data also suggest historic decline in effective size of the species overall (Fig. B3). Moreover, some populations that are easily accessible to fisherman appear to have very low effective population sizes (e.g., Kuibyshevka, Tym, Onorka, Uljanovka, Kievka rivers and Lebedinoe and Tainoe lakes—Table A9). Our population assignment exercise demonstrated how locally captured Sakhalin taimen can enter the market. In our case, fish likely originating from Tumnin and neighboring Ulika rivers were offered for sale in the Khabarovsk city fish market—both of these rivers are easily accessible from the city by vehicle. Any future conservation strategy needs to incorporate the understanding that some populations, particularly ones close to urban centers, are under substantially higher threat from fishing.

Hierarchical structure, strong between-population genetic differentiation, low level of gene flow, low effective population sizes, and possibly severe consequences of genetic drift can make populations vulnerable to extinction. Protecting evolutionary processes requires preserving multiple populations across different environments, but limited financial resources may dictate the need to

prioritize populations for conservation action (Allendorf et al. 2012). This dichotomy between theoretical and practical perspectives can be in part resolved by considering population subdivision across differing hierarchical levels. Here we propose a two-level conservation strategy, which aligns with the basic two-level hierarchy inferred with use of the EGU approach to defining ESUs. Below we outline policy measures to help carry out this strategy.

Between-ESU policy

Each ESU should be protected independently in order to preserve unique population groups. Artificial introduction into different eco-geographic region through the transplantation of individuals or fertilized eggs or any other genetic material should be prohibited.

Within-ESU policy

Within ESUs, protection plans should take into account both ecological and environmental features to minimize risks to the ESU. Restoration of a particular population should be based on the genetic resources of that target population (or subpopulation if appropriate). As an exception, under conditions when no other donors are available, we recommend donors from another population within the same ESU, preferably a genetically similar population that best mimics the target population in terms of environmental and ecological conditions.

Any conservation policy should also explicitly account for different life histories expressed within a given ESU. In this study we were able to discriminate different life history types based on the distribution of biomass across different habitat types. Some of these life histories have been identified based on observed stomach contents (e.g. consumed marine species suggesting anadromy, Edo et al. 2005), telemetry (e.g. Honda et al. 2012), and migratory reconstructions based on otolith microchemistry (e.g. Zimmerman et al. 2012). This is the first study to describe patterns across a large portion of the species' range, providing a new perspective on how these fish adapt to their environment. As has been documented in other species, Sakhalin taimen appear to exhibit partial anadromy, where individuals in the same river population exhibit a variety of different migratory behaviors, from freshwater residence to anadromy. As in past studies, these migratory types cannot be distinguished reliably based on microsatellite markers alone. Our study does suggest that a particular migratory strategy is strongly selected for in certain rivers and regions (e.g. semi-anadromy in rivers with lagoons). While the factors driving this selection of different life histories is still unclear, we feel it is important to consider them

separately and closely examine the threats unique to each migratory phenotype.

Our data on the critically endangered Sakhalin taimen is an example of how effectively ecological and geographic variation can be used to divide a species into evolutionarily significant units (ESUs) using the three-axes concept (Waples et al. 2001) via the EGU approach. The latter gives templates for selection of ESUs and can be specifically applied to rare species with low population sizes and presumably low rates of gene flow, because genetic drift in a reproductively isolated population can greatly change allele frequencies and small sample sizes may further inflate allele frequencies and prevent direct characterization of ESUs. To define ESUs in such circumstances, we suggest to: (1) select population groups (EGUs) based on ecological/life history and geographic/environmental variables, then (2) justify and modify these units with genetic characteristics, (3) analyze within-EGU population differentiation and produce estimates of gene flow rates, population effective sizes, and other relevant population genetic parameters.

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