American Genetic Association

Journal of Heredity, 2020, 227–236
doi:10.1093/jhered/esaa001
Original Article
Advance Access publication March 10, 2020

OXFORD

Original Article

Population Genomics Training for the Next Generation of Conservation Geneticists: ConGen 2018 Workshop

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Received May 17, 2019; First decision July 4, 2019; Accepted January 6, 2020.

Corresponding Editor: Jill Pecon-Slattery

Abstract

The increasing availability and complexity of next-generation sequencing (NGS) data sets make ongoing training an essential component of conservation and population genetics research. A workshop entitled "ConGen 2018" was recently held to train researchers in conceptual and practical aspects of NGS data production and analysis for conservation and ecological applications. Sixteen instructors provided helpful lectures, discussions, and hands-on exercises regarding how to plan, produce, and analyze data for many important research questions. Lecture topics ranged from understanding probabilistic (e.g., Bayesian) genotype calling to the detection of local adaptation signatures from genomic, transcriptomic, and epigenomic data. We report on progress in addressing central questions of conservation genomics, advances in NGS data analysis, the potential for genomic tools to assess adaptive capacity, and strategies for training the next generation of conservation genomicists.

Subject areas: Conservation genetics and biodiversity, Genomics and gene mapping
Keywords: adaptive capacity, conservation genetics pedagogy, effective population size, evolutionary significant units, population genomic data analysis

Informing conservation efforts is one of the most important and challenging needs of the genomic era (Allendorf 2017; Lewin *et al.* 2018; Hunter *et al.* 2018). To help meet this challenge, 16 experts from many areas of genomic data analysis met to discuss and teach recent analytical approaches at the 10th International Population Genetics Data Analysis Workshop for Conservation ("ConGen"),

held at Flathead Biological Station in September of 2018. The goal of the workshop was to train participants to apply rigorous theory and novel molecular and computational approaches in conservation and population genetics.

Since the first ConGen in 2006 (https://cibio.up.pt/congen/index.html), the molecular and computational tools accessible to conservation have grown in number and matured (Andrews and Luikart 2014; Benestan *et al.* 2015; Hendricks *et al.* 2018). ConGen 2018 participants originated from 16 countries and had a wide range of research questions and career stages including undergraduate and graduate (Masters and PhD) students, postdoctoral scholars, university faculty, laboratory technicians, and governmental agency scientists. This diversity of origins and perspectives enriched the questions, comments, discussions, and overall learning experience.

Historically, ConGen and other conservation genetics courses have focused mainly on questions that require and use only ~10–20 well-tested markers (e.g., microsatellites) such as hybridization, inbreeding, population structure, and loss of genetic diversity (Allendorf 2017). Today, the variety of molecular tools, amount of genetic data, and range of computational approaches have greatly expanded. Conservation genomics can be broadly defined as the application of genome-wide markers and new technologies to address problems in conservation. A more narrow-sense definition requires high-density loci to characterize locus- or gene-specific patterns and address conceptually novel questions that were intractable using traditional approaches (Allendorf *et al.* 2010; Garner *et al.* 2016; Allendorf 2017; Luikart *et al.* 2018).

Throughout this genetics-to-genomics transition, many authors, including those of previous ConGen workshop reviews, have reflected on this paradigm shift. They have noted the best practices for data production and quality control (filtering), experimental design, computational approaches, career guidance, and the increasing roles of women (Andrews and Luikart 2014; Benestan *et al.* 2015; Shafer *et al.* 2017; Hendricks *et al.* 2018). In this meeting review of ConGen 2018, we focus our reflection on training the next generation of researchers in conservation genomics through the novel components of this year's workshop: progress in understanding central concepts including assessing population differentiation and conservation units, estimation of effective population size, molecular data production and analysis for diverse empirical systems (Figure 1), and prospects for understanding genomic vulnerability.

Figure 1. Empirical examples provided by instructors at ConGen 2018 across a broad range of data types, questions, and taxa. (**A**) RAD-Capture and GWAS in characterizing the genetic architecture of disease-related traits in Tasmanian devils (*Sarcophilus harisii*; Margres *et al.* 2018), (**B**) targeted-capture, demographic modeling, and linkage-disequilibrium analysis in understanding the evolutionary history of color polymorphism of the gray wolf (*Canis lupus*; Schweizer *et al.* 2018), and (**C**) RADseq and analysis of population structure in identifying range expansion and hybridization of the tamarisk beetle (*Diorhabda* spp.), a recently introduced biocontrol agent (Bean and Dudley 2018). Photographs by (A) Menna Jones, (B) Marco Musiani, and (C) Ed Kosmicki, respectively, reproduced with permission. See online version for full colors.

Progress in Central Concepts

Populations, ESUs, and CUs: How Do You Identify Them Using Genomics?

Defining biologically meaningful management units within species is challenging (Waples and Gaggiotti 2006; Bradshaw *et al.* 2018; Waples and Lindley 2018). For conservation, an Evolutionarily Significant Unit (ESU) is a distinct population or group of populations that can be protected under the U.S. Endangered Species Act (ESA; USFWS and NMFS 1996; Waples and Lindley 2018). In Robin Waples' (Northwest Fisheries Science Center) lecture on ESUs, he explained that while there is no single or universal definition of a population, the competing definitions of ESUs emphasize 2 criteria: 1) substantial reproductive isolation and 2) an important component of the evolutionary legacy of the species (Waples 1991; Waples and Gaggiotti 2006). Evolutionary legacy refers to having distinct or different adaptations probably important for species persistence. Molecular genetic data have long been used to assess the isolation criterion for identifying ESUs, but prior to the age of genomics, the evolutionary significance of a population was difficult to determine and was largely inferred by ecological observations.

With genomic data, we can now identify loci, alleles, and surrounding chromosomal regions associated with adaptive differentiation, which improves our capacity to define ESUs while taking into account both demographic and selective processes (Funk *et al.* 2012, 2018). Incorporating adaptive variation into ESU listing raises theoretical and practical challenges (Funk *et al.* 2018). Mike Miller's ConGen 2018 lecture on an early-migration phenotype in salmonids demonstrated this challenge, wherein previous studies found little evidence for genetic isolation, but locus-specific analysis and simulation modeling provided strong evidence for this phenotype as an important component of the species' evolutionary legacy (Box 1).

Effective Population Size and Effective Number of Breeders (N_e and N_b)

Effective population size (N_e) is one of the most important concepts and parameters in conservation and evolutionary genetics because it influences the rate of loss of genetic variation, the levels of individual inbreeding, and the effectiveness of natural selection and gene flow (Wang *et al.* 2016). Conservation genetics has long employed estimates of effective population size to help assess and monitor the vulnerability of a population to potentially harmful genetic changes as mentioned above.

Although genomic data provide greater resolution and ability to estimate N_e in a growing diversity of species and scenarios, these data can also present unique challenges in estimating N_e. In his lecture on N_e, Waples discussed the recent advances in theory and computational analysis, which have vastly improved N_e estimation in the genomic era (Waples et al. 2014, 2018a, 2018b; Hollenbeck *et al.* 2016; Zhou *et al.* 2018). The use of thousands of loci, many of which are probably physically linked, will downwardly bias N_e estimates unless physical location (linkage) is taken into account (Waples and Do 2008; Do *et al.* 2014b; Waples *et al.* 2016).

The recently improved LDNe method implemented in the NeEstimator program (as of version 2.1) improves reliability of confidence intervals and reduces bias in estimating N_e by calculating r^2 on locus pairs, employing positional information from assembled loci or, when available, linkage groups or chromosomes (Do *et al.* 2014a). Likewise, the improved capability of NeEstimator to handle missing data, which calculates a fixed inverse variance-weighted harmonic mean at each locus (Peel *et al.* 2013), has been shown to be accurate with up to 50% missing data (Nunziata and Weisrock 2018). Together, these methodological improvements make estimating effective population size more accessible to studies with reduced representation data (i.e., NGS methods that subsample a genome with a restriction enzyme or targeted capture) with or without a reference genome.

Waples and Andrew Whiteley (University of Montana) highlighted N_b, or the number of effective breeders in a cohort, as a promising parameter for genetic and population management because of its intrinsic relationship to N_e and potential relationship with population abundance or environmental conditions (Kamath *et al.* 2015). An advantage of estimating N_b, rather than N_e, is that N_b provides frequent (e.g., yearly) information on population status, rather than having to wait to sample between generations which is often required by temporal estimations of N_e (e.g., Waples and Yokota 2007; Waples *et al.* 2014).

Whiteley's lecture emphasized monitoring population cohorts using a single sample and sib-ship or linkage-disequilibrium methods (Kamath *et al.* 2015; Waples *et al.* 2018b) and demonstrated the nuances of estimating N_b through recent studies of brook trout (*Salvelinus fontinalis*). He cautioned that while estimates of N_b can

Box 1. How will an adaptive locus influence listing of distinct salmonid populations under the Endangered Species Act (ESA) of the United States?

Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*O. mykiss*) have distinct spring (premature) and fall (mature, normal) migratory phenotypes (called runs) in several river basins across western United States. The spring-run phenotype differs substantially in behavior and physiology but has declined in abundance throughout the ranges of both species. Spring-run phenotypes have ecological, economic, and cultural importance, and are valuable to commerce and ecosystems for their greater fat content (Cook 2017). They also have had long histories with indigenous peoples, including documented ritualistic management by the Yurok, Karok, Hupa, Shasta, and Tolowa (Swezey and Heizer 1977). Due to reliance on cool, clean water in the summer, spring-run salmonids are particularly vulnerable to anthropogenic effects and have dramatically declined (Thompson *et al.* 2019).

Low genetic divergence (e.g., $F_{ST} < 0.03$) between premature and mature migrants within local rivers was found by multiple studies (Allendorf 1977; Chilcote *et al.* 1980; Waples *et al.* 2004; Arciniega *et al.* 2016). Based on these findings, premature migrant forms did not meet the first criterion for ESU status, sufficient reproductive isolation (Waples and Lindley 2018). However, recent genomic studies by Prince *et al.* (2017) and Thompson (2019) have identified a single locus that has a major effect on the migration phenotype and highlighted the potential for the loss of allelic variation at this locus to have significant ecological consequences, leading to legal action (Hess *et al.* 2016; Prince *et al.* 2017; Micheletti *et al.* 2018; Narum *et al.* 2018; NMFS 2018; Thompson *et al.* 2019). Prince *et al.* (2017) conducted a genome-wide association study that identified a single genetic locus (*GREB1L*) associated with premature migration. Further phylogenetic analyses suggested that the *GREB1L* alleles determining the premature migrant phenotype arose only once in each species, and subsequently spread through dispersal and positive selection.

Thompson *et al.* (2019) further examined selection against the premature migrant phenotype of Chinook salmon in the Rogue River in Oregon after the construction of a dam. They estimated the strength of selection needed to explain the change in allele frequencies at *GREB1L* under multiple dominance scenarios and predicted allele frequencies in future populations. Results suggested that the premature migration allele is probably codominant with respect to fitness and may be lost from the population if the current selection pressure continues (Figure 2B).

Together, these findings suggest that the premature migration phenotype (and allele) is vulnerable to loss and unlikely to reappear for a long time if lost from a population. Populations where *GREB1L* early-migration alleles are prevalent may deserve special legal protection. Based on these results, the Karuk Tribe submitted a petition to list the Klamath premature Chinook under the ESA (NMFS 2018). In February 2018, National Oceanic and Atmospheric Administration (NOAA) Fisheries announced a finding of substantial scientific evidence indicating the creation and listing of a new ESU as threatened or endangered may be warranted. At the time of writing, the National Marine Fisheries status review of the Upper Klamath and Trinity River Chinook salmon was still pending. The decision on whether to list Klamath premature Chinook could have wide-reaching implications for conservation (Waples and Lindley 2018).

Box 1. Continued

These studies and the resultant legal action have recharged debate over whether, when, and how species should be managed for single genes (Kardos and Shafer 2018). A concern is that as genomics continues to make it easier to find adaptive genetic variation, management units could be over-split as more and more important loci and alleles are identified. As this case study in Pacific salmon shows, iterative and focused genomic studies have the power to identify crucial adaptive variation and to inform long-standing debates.

track abundance in some species (Ferchaud and Hansen 2016), which may supplement or allow demographic-based monitoring, it is unlikely to closely track abundance for species with high variance in reproductive success and limited reproductive habitat. For example, for brook trout that spawn in available habitat patches N_b estimates had no association with yearly abundance in 2 populations; however, they provided important information about environmental conditions (Whiteley *et al.* 2015). A comparison among several brook trout populations showed that N_b was the largest at intermediate flow conditions, which is consistent with biological hypotheses (Whiteley *et al.* 2017).

The theory and application of N_b was presented mainly in the context of aquatic organisms. Nonetheless, N_b is easier to estimate than N_e for most taxa (beyond aquatic organisms), requiring only a single sample per generation (Waples 2005; Waples *et al.* 2013; da Silva *et al.* 2018). Whiteley's example demonstrated the importance of incorporating detailed biological information in the study design, analysis, and interpretation of effective population size estimates and its relationship to census size (Waples 2005; Waples

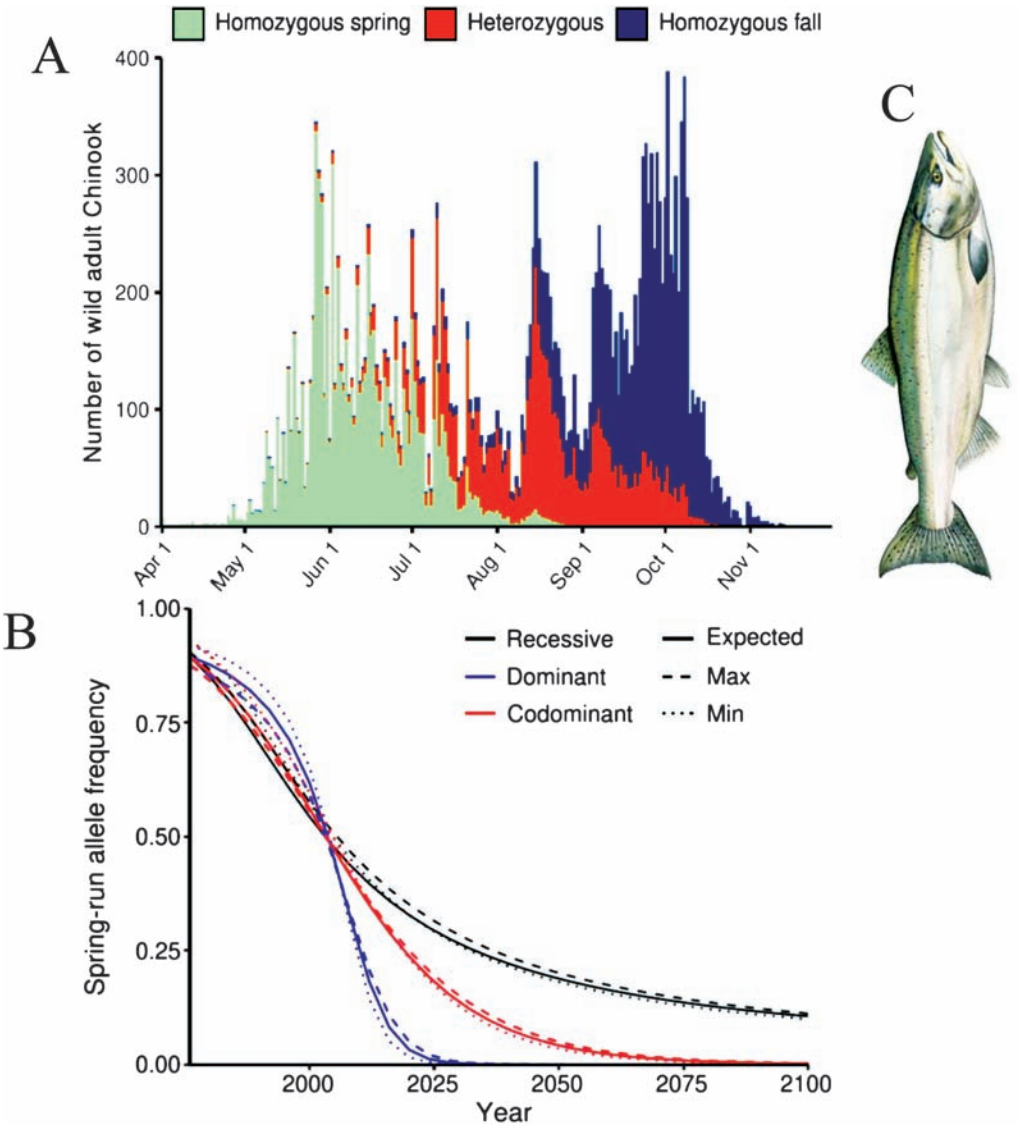

Figure 2. (**A**) Stacked bar graph representing the number of wild adult Chinook salmon passing Gold Ray Fish Counting Station on the Rogue river in 2004; colors represent estimated proportion of each GREB1L locus genotype. (**B**) Selection modeling in Rogue Chinook. Curves representing the decline (or loss) of the spring-run allele frequency over time under a recessive, dominant, or codominant scenario. Spring-run alleles are thought to be codominant and predicted to be lost by ~2075. The modeling assumes random mating and no genetic drift. (**C**) Image of a Chinook salmon. Figure modified from Thompson *et al.* (2019). See online version for full colors.

Box 2. How will changes in DNA methylation influence adaptation to artificial environments in hatchery fish?

A common goal of captive breeding programs is to support declining wild populations (i.e., genetic rescue); however, there is concern that rearing in artificial conditions may inadvertently reduce fitness. In conservation salmonid hatcheries, there is mounting evidence that tank-rearing conditions can induce developmental plasticity and impact life-history traits. To examine the role of epigenetic changes in hatchery-reared steelhead trout (*Oncorhynchus mykiss*), Gavery *et al.* (2019) raised steelhead in an artificial stream and small simulated hatchery tank for 2 years, well past germ cell differentiation, then sampled individuals and performed reduced representation bisulfite sequencing (Meissner *et al.* 2005) to determine methylation patterns. After accounting for familial relationships influencing methylation patterns, they were able to discern up-methylated and down-methylated gene differences between their 2 conditions (artificial stream vs. tank). Although family relatedness had the largest effect, environmental differences also caused significant changes in the methylation pattern. If these epigenetic changes occur at an early stage in development in response to environmental pressures, they may not only affect the organism's growth, but will continue to persist well past the time when those environmental pressures are no longer present. This has implications for conservation of salmonids and other species if environmentally induced epigenetic shifts are transmitted to offspring and grand offspring. For example, if hatchery-adaptive epigenetic changes are transmitted to wild fish, the fitness of wild fish could decline (Christie *et al.* 2016; Le Luyer *et al.* 2017). There is substantial evidence of maladaptive introgression in wild populations (Rodriguez *et al.* 2019), though more work must be conducted to determine whether epigenetic changes can persist, be transmitted across multiple generations, and spread within and among natural populations (Charlesworth *et al.* 2017).

et al. 2013). Simulations, such as those conducted by ConGen 2018 participants with EasyPop (Balloux 2001) and those implemented in tools, such as AGENE (Waples *et al.* 2013), NeOGen (Blower *et al.* 2019), and Neff (Grimm *et al.* 2016), can be employed to determine an appropriate sampling scheme, implement sensitivity analysis, and corroborate empirical results (Waples *et al.* 2013).

Molecular Genomic Data Generation and Analysis

Training the next generation of conservation genomicists includes empowering participants to evaluate and incorporate a wealth of diverse molecular genetic methods. The first ConGen meetings in 2006–2009 were focused on microsatellites. Since 2010, genomic techniques such as restriction-site associated DNA-sequencing (RADseq) have increasingly been the main focus (Andrews and Luikart 2014). Of 33 participants at ConGen 2018, 27 participants had RADseq data, 4 participants had exon capture data, and 5 participants had whole-genome sequencing (WGS) data. Several participants reported having multiple types of molecular data.

At ConGen 2018, methods both currently applied widely and those only recently employed in conservation genomics were discussed. Paul Hohenlohe (University of Idaho) reviewed the many variations and utility of RADseq (Andrews *et al.* 2016), Stefan Prost (Senckenberg Museum) presented a guide to de novo genome assembly (Fuentes-Pardo and Ruzzante 2017; Hendricks *et al.* 2018), and Rena Schweizer (University of Montana) highlighted the practical and conceptual considerations regarding exon capture (Bi *et al.* 2012; Schweizer *et al.* 2016). Here, we highlight advances in RAD-capture, transcriptomics, and epigenomics.

Rapture: A Hybrid Reduced Representation Approach

Lectures by Hohenlohe and Seth Smith (University of Montana) demonstrated the utility of Rapture (RAD-capture; Ali *et al.* 2016), a reduced representation technique that combines an improved RADseq library preparation protocol (informally referred to as bestRAD) with an in-solution sequence probe capture to enrich sequencing libraries for a subset of RADseq loci (e.g., polymorphic loci, loci in or near genes, diagnostic loci for species identification or admixture analysis, and/or loci with high heterozygosity or high F_{ST}, all on the same capture array). The major improvements prescribed by the bestRAD protocol are the ability to reduce the proportion of PCR duplicates, efficiency in using smaller starting quantities of DNA, and efficiency in scaling from hundreds to thousands of samples (Ali *et al.* 2016). We encourage interested readers to see Meek and Larson (2019) for a detailed review of sequence capture techniques and their utility in conservation. Here we focus on the details each individual researcher must weigh in respect to each individual project: cost, PCR duplication rate, and computational approaches.

Because individual (indexed) samples are pooled early in the bestRAD protocol, the cost of the library preparation kit and capture reaction scales well for large sample sizes. For instance, up to 96 uniquely indexed individual samples are pooled prior to adding sequencing adapters and amplifying the library using a commercially available kit. Seth Smith estimated that bestRAD libraries can be generated for <$5.00 per individual after the cost of bestRAD adapters is amortized. The per sample cost for the hybridization capture reaction was ~$0.50, assuming the above multiplexing scheme and a bait panel of up to 20 000 loci. This cost could vary substantially depending on the vendor used for supplies (e.g., the capture array) and does not include labor for the data production, which is often the majority of the cost. The cost of sequencing depends on the desired coverage. The number of samples that can be multiplexed per sequencing lane is a function of the number of targeted loci, the PCR duplication rate, and the proportion of reads that do not that align to targeted loci. He cautioned that the PCR duplication rate and proportion of off-target reads are expected to vary depending on the proportion of RAD loci targeted for capture and the total number of loci in the original RAD library which can be influenced by sample quality and PCR duplicate rates, and are typically 20–30% but can be >80% (e.g., Margres *et al.* 2018).

Following sequencing, Rapture data can be analyzed with any method applicable to RAD-type data (Andrews *et al.* 2016). Among these, Stacks (Catchen *et al.* 2013) is commonly used for population genomics with RADseq and has been covered at ConGen since 2011. At ConGen 2018, Amanda Stahlke (University of Idaho) taught de novo and reference-based locus assembly and genotyping in Stacks version 2.3, which has several major changes from the original implementation (Rochette *et al.* 2019). Participants examined the

effects on *F*-statistics of removing PCR duplicates and aligning to a reference or not. These choices depend on genetic and financial resources available, local laboratory expertise, and the study question. Useful sensitivity frameworks for assessing RAD locus assembly, the benefits of a reference genome, and the effects of PCR duplication have been described elsewhere (Ebbert *et al.* 2016; Paris *et al.* 2017; Shafer *et al.* 2017; Euclide *et al.* 2020). For example, low-coverage sequencing can be a cost-effective and powerful approach (Maruki and Lynch 2017) but is also the most sensitive to the effects of PCR duplicates (Euclide *et al.* 2020).

As one of the most widely used software pipelines for genotyping RADseq data and population genomic analysis, the Stacks program (Catchen *et al.* 2013) has been discussed and used at the ConGen course for several years. Here we highlight some key changes in the recently released Stacks 2 (Rochette *et al.* 2019) taught at the 2018 course. For users with bestRAD data (Ali *et al.* 2016), the addition of the *--bestrad* flag to process_radtags reorients paired fastq files such that bestRAD indexes and the remainder of restriction cut-sites are always located at the beginning of the first read, eliminating the requirement of an external script to reorient the reads prior to input.

In Stacks 2, users also have the ability to input paired-end reads and assemble local RAD contigs with data produced by protocols with a randomly sheared end (e.g., Ali *et al.* 2016) or random oligos in ddRAD (Schweyen *et al.* 2014). Instead of concatenating forward and reverse reads as previously recommended (Rochette and Catchen 2017), paired-end reads are incorporated through the new tsv2bam and gstacks, the new genotyping module, yielding major improvements in memory usage and genotype-calling frameworks (Rochette *et al.* 2019).

Novel genotype-calling algorithms have also been implemented in gstacks, including the diploid Maruki and Lynch (2017) maximum likelihood genotyping model which can incorporate population-level genotype frequencies (the "low-coverage model") and error-rates with Bayes' theorem. In gstacks, users may increase *--alpha* to require a greater statistical threshold for calling genotypes, instead of setting a redundant minimum stacks depth flag in the population module (*-m* is deprecated). These advances in Stacks hold promise to advance RADseq analysis in conservation genomics by yielding more accurate genotypes and longer haplotypes (Rochette *et al.* 2019).

Transcriptomics and Epigenomics

Transcriptomics and epigenomics, the high-throughput studies of transcribed products and epigenetic modifications of the genome, respectively, can be used to disentangle mechanisms of local adaptation (i.e., plasticity vs. Darwinian adaptation) across biological and temporal scales (Hendricks *et al.* 2018; Kelly 2019), though the application of understanding these mechanisms in conservation is still developing (Christie *et al.* 2016; Le Luyer *et al.* 2017). Recent technological advances in library preparation which better accommodate degraded and low input DNA have made transcriptomic analysis more accessible to systems of conservation concern (Schuierer *et al.* 2017). RNAseq, the high-throughput sequencing of synthesized cDNA fragments (Wang *et al.* 2009), has been used to identify the molecular basis for resilience to changing environment in corals (Barshis *et al.* 2013; Pratlong *et al.* 2015; Bay *et al.* 2017) and redband rainbow trout (*Oncorhynchus mykiss gardieri*; Garvin *et al.* 2015; Chen *et al.* 2018).

Still, there are surprisingly few studies that employ these techniques to inform conservation. Perhaps this is due to fewer labs having the capacity to produce and analyze these potentially tissue- and time-specific data, the actual and perceived conflicts in evolutionary paradigms, or the ongoing discussion regarding the role of plasticity in long-term population persistence (Kelly 2019). Regardless, transgenerational gene expression and epigenetic changes can underlie an adaptive response to environmental change (e.g., corals).

At ConGen 2018, participants gained exposure and experience to transcriptomics through an interactive lecture on data production and hands-on analysis of differential gene expression led by Joanna Kelley (Washington State University). Participants learned how to functionally annotate variants of interest and perform enrichment analysis with instructor Mackenzie Gavery (University of Washington) and an epigenomic data set. Here we highlight Gavery's lecture demonstrating the potential utility of epigenomics in conservation with a recent study of DNA methylation of cytosine residues at CpG sites induced by hatchery conditions (Gavery et al. 2018, 2019; Box 2).

Understanding Adaptive Potential and Genomic Vulnerability

Genomic methods now allow researchers to determine the genetic basis for variation in fitness, quantify adaptive capacity, and predict potential outcomes for natural populations facing environmental change (Funk *et al.* 2018). Adaptive potential can be defined as the capacity of species or populations to respond to stressors (e.g., environmental change) by genetically based changes (Nicotra *et al.* 2015; Funk *et al.* 2018). Rachael Bay (University of California Davis) and Christen Bossou (Colorado State University) demonstrated the exciting potential for *genomic vulnerability*, which is an estimate of the extent to which allele frequencies of wild populations must change to maintain current genotype–environment associations in the future (Fitzpatrick and Keller 2015; Box 3).

Box 3. How will genomic vulnerability of yellow warblers influence their evolutionary response to climate change?

In their workshop lecture, Bay and Bossou invited participants to assess genomic vulnerability of the yellow warbler (*Setophaga petechia;* Figure 3), a migratory songbird distributed across much of North America (Bay *et al.* 2018). First, participants identified the environmental variables that best explained variation at a subset of genome-wide SNPs using gradient forest analysis, a regression tree-based machine learning approach (Ellis *et al.* 2012). Then, genomic vulnerability was calculated as the difference between current versus predicted gradient forest-transformed climate variables. A significant negative association was found between genomic vulnerability and current population trends, suggesting that populations with high genomic vulnerability may have already been affected (Bay *et al.* 2018). This approach provides a useful starting point to incorporate evolution into models that predict the effects of climate change on biodiversity. Important future extensions of the model could include incorporating additional evolutionary components, such as gene flow and population sizes. Predictive modeling, such as the strategy taught by Bay and Bossou, will become increasingly useful for conservation as it incorporates both local adaptation and projected environmental conditions.

Figure 3. The wide breeding range of the yellow warbler (*Setophaga petechia*), pictured here, and recent population declines in some regions motivated the hands-on tutorial of Bay and Bossou. Photograph by Daniel Karp reproduced with permission.

The Next Generation: Developing Theoretical, Empirical, and Analytical Skills

Conservation genomics is a multidisciplinary field, requiring practitioners to have a working knowledge of population genetic theory and molecular biology while developing the computational skills necessary to apply novel and conventional analyses to increasingly large data sets. These challenges, raised by Allendorf *et al.* (2010), Garner *et al.* (2016), and Shafer *et al.* (2016), remain relevant and were discussed by participants and instructors alike at ConGen 2018. Conservation genomicists often need to navigate social (e.g., legal), ecological, and molecular dimensions, sometimes in the most challenging of field conditions (Groom *et al.* 2006).

Researchers must also be able to effectively communicate with stakeholders, including agency managers, NGOs, policy makers, and the public (Hand *et al.* 2018). The diversity of topics covered during lectures, discussions, and hands-on activities during ConGen 2018 demonstrates the importance of taking a holistic approach when tackling questions in conservation genomics. One recommendation from managers at ConGen to help conservation geneticists ensure their data is used for conservation management was to design a study with a manager who has plans in place (e.g., including permits, policy, etc.) to use the genetic data once it is available to make management decisions (Boyer M, personal communication). This recommendation is an important consideration for future discussion in conservation and genetics workshops where open forums and group conversations can be organized. Other big-group discussion topics ranged from the best programming languages for population genomics (e.g., R and shell scripting), to career choices.

Theory in population genetics has a long and rich history, and yet, is still developing with effective population size concepts and empirical estimation methods among the most important areas (e.g., Waples *et al.* 2014; Ceballos *et al.* 2018; Beaumont and Wang 2019). The importance of theory, and specifically effective population size, is exemplified by the following quotes: "Nothing in evolution makes sense except in light of population genetics" (Lynch and Walsh 2007) and "Nothing in population genetics makes sense except in light of effective population size," which Robin Waples at ConGen 2018 said was a quote from Fred Allendorf (University of Montana). For example, when testing for genotype–phenotype associations, knowing the effective population size is helpful because N_e influences the extent of linkage-disequilibrium along chromosomes, which in turn determines the density of markers and molecular methods needed to conduct a powerful genome-wide scan (e.g., Kardos *et al.* 2016).

The increasing diversity and complexity of analysis also requires that code be well annotated and highly reproducible. A number of instructors shared version-controlled worksheets and R code via Github including Racheal Bay, Eric Anderson (Southwest Fisheries Science Center), Joanna Kelley, and Brenna Forester (Colorado State

University). Kelley, for example, provided instruction and materials for transcriptome assembly and quantifying differential gene expression (https://github.com/jokelley/congen-2018). Also of discussion was the increasing availability of R packages to efficiently analyze and visualize NGS data sets and results and the importance this has in increasing reproducibility and reliability, and lowering the barrier on bioinformatics and data analysis in general (Paradis *et al.* 2017).

Summary and Conclusions

In conclusion, major conceptual advances discussed at ConGen 2018 include estimating the effective population size per cohort or generation (e.g., N_b with age structure, using thousands of loci), assessing population genomic vulnerability, and using adaptive genetic information to identify conservation units. New approaches have emerged for cheaper genome-wide data production (e.g., Rapture) and data analysis (e.g., major updates in Stacks). Emphasis in recent years at ConGen including the use of tools becoming more cost-effective and available to conservation genomics including DNA capture, transcriptomics, epigenomics, genome-wide, and reference-genome-based work. The purpose of ConGen remains to introduce recent novel techniques and approaches to a wide range of participants from different career paths, institutes, and countries. Recent work by ConGen workshop instructors and other researchers has expanded the types of data used in conservation genomics at large (e.g., see transcriptomics and epigenomics and Forester *et al.* 2018). A researcher now often has multiple data types that may include everything from *de novo* genome assemblies to RADseq to differential gene expression among populations and more. Although the amount of genomic data production grows exponentially, the continuing challenge for genomicists remains in obtaining a solid foundation in population genetics theory, data filtering, and computational analysis. Through training and experiences such as those available at workshops like ConGen 2018, the modern conservation and population genomicist will be able to examine a wide range of central questions, evaluate the appropriate tools for data production and analysis, and integrate across different data types from RADseq to whole-genome resequencing, RNAseq, and more. As population genomics continues to evolve, we hope this review of ConGen 2018 will help serve as a benchmark, motivation, and starting point for information and references for readers from world-wide to advance multiple disciplines including conservation, ecology, and evolutionary genomics.

Funding

This work was supported by National Science Foundation grant DEB-1655809 to P.A.H. and National Science Foundation grant DEB-1639014, DOB and NASA grant NNX14AB84G to B.K.H. and G.L.

Acknowledgments

The authors thank the many instructors and participants of ConGen 2018 for invaluable discussion throughout ConGen 2018, as well as the 3 anonymous reviewers whose insight improved this manuscript. We sincerely appreciate R. Waples who is the only instructor to have lectured at all 10 ConGen courses (plus helped organize them) since its inception in 2006; he also was awarded the prestigious 2018 Molecular Ecology Prize while at ConGen 2018. We especially thank the American Genetics Association (AGA) for a "Special Events Award" supporting student participation and making this ConGen course possible.

References

Ali OA, O'Rourke SM, Amish SJ, Meek MH, Luikart G, Jeffres C, Miller MR. 2016. RAD capture (Rapture): flexible and efficient sequence-based genotyping. *Genetics*. 202:389–400.

Allendorf FW. 1977. Genetic variability in a species possessing extensive gene duplication: genetic interpretation of duplicate loci and examination of genetic variation in populations of rainbow trout. PhD Thesis. Seattle (WA): University of Washington. p. 98.

Allendorf FW. 2017. Genetics and the conservation of natural populations: allozymes to genomes. *Mol Ecol*. 26:420–430.

Allendorf FW, Hohenlohe PA, Luikart G. 2010. Genomics and the future of conservation genetics. *Nat Rev Genet*. 11:697–709.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. *Nat Rev Genet*. 17:81–92.

Andrews KR, Luikart G. 2014. Recent novel approaches for population genomics data analysis. *Mol Ecol*. 23:1661–1667.

Arciniega M, Clemento AJ, Miller MR, Peterson M, Garza JC, Pearse DE. 2016. Parallel evolution of the summer steelhead ecotype in multiple populations from Oregon and Northern California. *Conserv Genet*. 17:165–175.

Balloux F. 2001. EASYPOP (version 1.7): a computer program for population genetics simulations. *J Hered*. 92:301–302.

Barshis DJ, Ladner JT, Oliver TA, Seneca FO, Traylor-Knowles N, Palumbi SR. 2013. Genomic basis for coral resilience to climate change. *Proc Natl Acad Sci USA*. 110:1387–1392.

Bay RA, Harrigan RJ, Underwood VL, Gibbs HL, Smith TB, Ruegg K. 2018. Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*. 359:83–86.

Bay RA, Rose N, Barrett R, Bernatchez L, Ghalambor CK, Lasky JR, Brem RB, Palumbi SR, Ralph P. 2017. Predicting responses to contemporary environmental change using evolutionary response architectures. *Am Nat*. 189:463–473.

Bean D, Dudley T. 2018. A synoptic review of Tamarix biocontrol in North America: tracking success in the midst of controversy. *BioControl*. 63:361–376.

Beaumont M, Wang J. 2019. Conservation genetics. In: Balding D, Moltke I, Marioni J, editors. *Handbook of statistical genomics*. Hoboken (NJ): John Wiley & Sons.

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L. 2015. RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). *Mol Ecol*. 24:3299–3315.

Bi K, Vanderpool D, Singhal S, Linderoth T, Moritz C, Good JM. 2012. Transcriptome-based exon capture enables highly cost-effective comparative genomic data collection at moderate evolutionary scales. *BMC Genomics*. 13:403.

Blower DC, Riginos C, Ovenden JR. 2019. neogen: a tool to predict genetic effective population size (N_e) for species with generational overlap and to assist empirical Ne study design. *Mol Ecol Resour*. 19:260–271.

Bradshaw PJ, Broderick AC, Carreras C, Fuller W, Snape RTE, Wright LI, Godley BJ. 2018. Defining conservation units with enhanced molecular tools to reveal fine scale structuring among Mediterranean green turtle rookeries. *Biol Conserv*. 222:253–260. doi:10.1016/j.biocon.2017.12.014

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool set for population genomics. *Mol Ecol*. 22:3124–3140.

Ceballos FC, Joshi PK, Clark DW, Ramsay M, Wilson JF. 2018. Runs of homozygosity: windows into population history and trait architecture. *Nat Rev Genet*. 19:220–234.

Charlesworth D, Barton NH, Charlesworth B. 2017. The sources of adaptive variation. *Proc R Soc B Biol Sci*. 284:20162864.

Chen Z, Farrell AP, Matala A, Hoffman N, Narum SR. 2018. Physiological and genomic signatures of evolutionary thermal adaptation in redband trout from extreme climates. *Evol Appl*. 11:1686–1699.

Chilcote MW, Crawford BA, Leider SA. 1980. A genetic comparison of sympatric populations of summer and winter steelheads. *Trans Am Fish Soc*. 109:203–206.

Christie MR, Marine ML, Fox SE, French RA, Blouin MS. 2016. A single generation of domestication heritably alters the expression of hundreds of genes. *Nat Commun*. 7:10676.

Cook L. 2017. *Upstream: searching for wild salmon, from river to table*. New York: Random House.

da Silva FM, Miño CI, Izbicki R, Del Lama SN. 2018. Considerations for monitoring population trends of colonial waterbirds using the effective number of breeders and census estimates. *Ecol Evol*. 8:8088–8101.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014a. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour*. 14:209–214.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014b. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour*. 14:209–214.

Ebbert MT, Wadsworth ME, Staley LA, Hoyt KL, Pickett B, Miller J, Duce J, Kauwe JS, Ridge PG; Alzheimer's Disease Neuroimaging Initiative. 2016. Evaluating the necessity of PCR duplicate removal from next-generation sequencing data and a comparison of approaches. *BMC Bioinformatics*. 17 (Suppl 7):239.

Ellis N, Smith SJ, Pitcher CR. 2012. Gradient forests: calculating importance gradients on physical predictors. *Ecology*. 93:156–168.

Euclide PT, McKinney GJ, Bootsma M, Tarsa C, Meek MH, Larson WA. 2020. Attack of the PCR clones: rates of clonality have little effect on RAD-seq genotype calls. *Mol Ecol Resour*. 20:66–78.

Ferchaud AL, Hansen MM. 2016. The impact of selection, gene flow and demographic history on heterogeneous genomic divergence: three-spine sticklebacks in divergent environments. *Mol Ecol*. 25:238–259.

Fitzpatrick MC, Keller SR. 2015. Ecological genomics meets community-level modelling of biodiversity: mapping the genomic landscape of current and future environmental adaptation. *Ecol Lett*. 18:1–16.

Forester BR, Landguth EL, Hand BK, Balkenhol N. 2018. Landscape genomics for wildlife research. In: Hohenlohe PA, Rajora OP, editors. *Population genomics: wildlife*. Cham, Switzerland: Springer International Publishing AG. doi:10.1007/13836_2018_56

Fuentes-Pardo AP, Ruzzante DE. 2017. Whole-genome sequencing approaches for conservation biology: advantages, limitations and practical recommendations. *Mol Ecol*. 26:5369–5406.

Funk WC, Forester BR, Converse SJ, Darst C, Morey S. 2018. Improving conservation policy with genomics: a guide to integrating adaptive potential into US Endangered Species Act decisions for conservation practitioners and geneticists. *Conserv Genet*. 20:1–20.

Funk WC, McKay JK, Hohenlohe PA, Allendorf FW. 2012. Harnessing genomics for delineating conservation units. *Trends Ecol Evol*. 27:489–496.

Garner BA, Hand BK, Amish SJ, Bernatchez L, Foster JT, Miller KM, Morin PA, Narum SR, O'Brien SJ, Roffler G, *et al*. 2016. Genomics in conservation: case studies and bridging the gap between data and application. *Trends Ecol Evol*. 31:81–83.

Garvin MR, Thorgaard GH, Narum SR. 2015. Differential expression of genes that control respiration contribute to thermal adaptation in redband trout (Oncorhynchus mykiss gairdneri). *Genome Biol Evol*. 7:1404–1414.

Gavery MR, Nichols KM, Berejikian BA, Tatara CP, Goetz GW, Dickey JT, Van Doornik DM, Swanson P. 2019. Temporal dynamics of DNA methylation patterns in response to rearing juvenile steelhead (*Oncorhynchus mykiss*) in a hatchery versus simulated stream environment. *Genes*. 10:356.

Gavery MR, Nichols KM, Goetz GW, Middleton MA, Swanson P. 2018. Characterization of genetic and epigenetic variation in sperm and red blood cells from adult hatchery and natural-origin steelhead, *Oncorhynchus mykiss*. *G3 (Bethesda)*. 8:3723–3736.

Grimm A, Gruber B, Hoehn M, Enders K, Henle K. 2016. A model-derived short-term estimation method of effective size for small populations with overlapping generations. *Methods Ecol Evol*. 7:734–743.

Groom MJ, Meffe GK, Carroll CR. 2006. *Principles of conservation biology*. Sunderland (MA): Sinauer Associates Sunderland.

Hand BK, Flint CG, Frissell CA, Muhlfeld CC, Devlin SP, Kennedy BP, Crabtree RL, McKee WA, Luikart G, Stanford JA. 2018. A social–ecological perspective for riverscape management in the Columbia River Basin. *Front Ecol Environ*. 16:S23–S33.

Hendricks S, Anderson EC, Antao T, Bernatchez L, Forester BR, Garner B, Hand BK, Hohenlohe PA, Kardos M, Koop B, Sethuraman A, Waples RS, Luikart G. 2018. Recent advances in conservation and population genomics data analysis. *Evol Appl*. 11:1197–1211.

Hess JE, Zendt JS, Matala AR, Narum SR. 2016. Genetic basis of adult migration timing in anadromous steelhead discovered through multivariate association testing. *Proc R Soc B Biol Sci*. 283:20153064.

Hollenbeck CM, Portnoy DS, Gold JR. 2016. A method for detecting recent changes in contemporary effective population size from linkage disequilibrium at linked and unlinked loci. *Heredity (Edinb)*. 117:207–216.

Hunter ME, Hoban SM, Bruford MW, Segelbacher G, Bernatchez L. 2018. Next-generation conservation genetics and biodiversity monitoring. *Evol Appl*. 11:1029–1034.

Kamath PL, Haroldson MA, Luikart G, Paetkau D, Whitman C, van Manen FT. 2015. Multiple estimates of effective population size for monitoring a long-lived vertebrate: an application to Yellowstone grizzly bears. *Mol Ecol*. 24:5507–5521.

Kardos M, Husby A, McFarlane SE, Qvarnström A, Ellegren H. 2016. Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Mol Ecol Resour*. 16:727–741.

Kardos M, Shafer ABA. 2018. The peril of gene-targeted conservation. *Trends Ecol Evol*. 33:827–839.

Kelly M. 2019. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. *Philos Trans R Soc Lond B Biol Sci*. 374:20180176.

Le Luyer J, Laporte M, Beacham TD, Kaukinen KH, Withler RE, Leong JS, Rondeau EB, Koop BF, Bernatchez L. 2017. Parallel epigenetic modifications induced by hatchery rearing in a Pacific salmon. *Proc Natl Acad Sci USA*. 114:12964–12969.

Lewin HA, Robinson GE, Kress WJ, Baker WJ, Coddington J, Crandall KA, Durbin R, Edwards SV, Forest F, Gilbert MTP, *et al*. 2018. Earth BioGenome project: sequencing life for the future of life. *Proc Natl Acad Sci USA*. 115:4325–4333.

Luikart G, Kardos M, Hand BK, Rajora OP, Aitken SN, Hohenlohe PA. 2018. Population genomics: advancing understanding of nature. In: Rajora O., editor. *Population genomics*. Cham (Switzerland): Springer.

Lynch M, Walsh B. 2007. *The origins of genome architecture*. Sunderland (MA): Sinauer Associates.

Margres MJ, Jones ME, Epstein B, Kerlin DH, Comte S, Fox S, Fraik AK, Hendricks SA, Huxtable S, Lachish S, *et al*. 2018. Large-effect loci affect survival in Tasmanian devils (*Sarcophilus harrisii*) infected with a transmissible cancer. *Mol Ecol*. 27:4189–4199.

Maruki T, Lynch M. 2017. Genotype calling from population-genomic sequencing data. *G3 (Bethesda)*. 7:1393–1404.

Meek MH, Larson WA. 2019. The future is now: Amplicon sequencing and sequence capture usher in the conservation genomics era. *Mol Ecol Resour*. 19:795–803. doi:10.1111/1755-0998.12998

Meissner A, Gnirke A, Bell GW, Ramsahoye B, Lander ES, Jaenisch R. 2005. Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis. *Nucleic Acids Res*. 33:5868–5877.

Micheletti SJ, Hess JE, Zendt JS, Narum SR. 2018. Selection at a genomic region of major effect is responsible for evolution of complex life histories in anadromous steelhead. *BMC Evol Biol*. 18:140.

Narum SR, Di Genova A, Micheletti SJ, Maass A. 2018. Genomic variation underlying complex life-history traits revealed by genome sequencing in Chinook salmon. *Proc R Soc B Biol Sci*. 285:20180935.

Nicotra AB, Beever EA, Robertson AL, Hofmann GE, O'Leary J. 2015. Assessing the components of adaptive capacity to improve conservation and management efforts under global change. *Conserv Biol*. 29:1268–1278.

NMFS. 2018. Endangered and threatened wildlife; 90-day finding on a petition to list Chinook salmon in the Upper Klamath-Trinity Rivers Basin as

Threatened or Endangered Under the Endangered Species Act. *Fed Reg*. 83:8410–8414.

Nunziata SO, Weisrock DW. 2018. Estimation of contemporary effective population size and population declines using RAD sequence data. *Heredity (Edinb)*. 120:196–207.

Paradis E, Gosselin T, Goudet J, Jombart T, Schliep K. 2017. Linking genomics and population genetics with R. *Mol Ecol Resour*. 17:54–66.

Paris JR, Stevens JR, Catchen JM, Johnston S. 2017. Lost in parameter space: a road map for stacks. *Methods Ecol Evol*. 8:1360–1373.

Peel D, Waples RS, Macbeth GM, Do C, Ovenden JR. 2013. Accounting for missing data in the estimation of contemporary genetic effective population size (N_e). *Mol Ecol Resour*. 13:243–253.

Pratlong M, Haguenauer A, Chabrol O, Klopp C, Pontarotti P, Aurelle D. 2015. The red coral (*Corallium rubrum*) transcriptome: a new resource for population genetics and local adaptation studies. *Mol Ecol Resour*. 15:1205–1215.

Prince DJ, O'Rourke SM, Thompson TQ, Ali OA, Lyman HS, Saglam IK, Hotaling TJ, Spidle AP, Miller MR. 2017. The evolutionary basis of premature migration in Pacific salmon highlights the utility of genomics for informing conservation. *Sci Adv*. 3:e1603198.

Rochette NC, Catchen JM. 2017. Deriving genotypes from RAD-seq short-read data using Stacks. *Nat Protoc*. 12:2640–2659.

Rochette NC, Rivera-Colón AG, Catchen JM. 2019. Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. *Mol Ecol*. 28:4737–4754.

Rodriguez DB, Verspoor E, Sobolewska H, Coulson M, Consuegra S. 2019. DNA methylation changes in the sperm of captive-reared fish: a route to epigenetic introgression in wild populations. *Mol Biol Evol*. 36:2205–2211.

Schuierer S, Carbone W, Knehr J, Petitjean V, Fernandez A, Sultan M, Roma G. 2017. A comprehensive assessment of RNA-seq protocols for degraded and low-quantity samples. *BMC Genomics*. 18:442.

Schweizer RM, Robinson J, Harrigan R, Silva P, Galverni M, Musiani M, Green RE, Novembre J, Wayne RK. 2016. Targeted capture and resequencing of 1040 genes reveal environmentally driven functional variation in grey wolves. *Mol Ecol*. 25:357–379.

Schweizer RM, Durvasula A, Smith J, Vohr SH, Stahler DR, Galaverni M, Thalmann O, Smith DW, Randi E, Ostrander EA, *et al*. 2018. Natural selection and origin of a melanistic allele in North American gray wolves. *Mol Biol Evo*. 35:1190–1209. doi:10.1093/molbev/msy031

Schweyen H, Rozenberg A, Leese F. 2014. Detection and removal of PCR duplicates in population genomic ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters. *Biol Bull*. 227:146–160.

Shafer ABA, Peart CR, Tusso S, Maayan I, Brelsford A, Wheat CW, Wolf JBW, Gilbert M. 2017. Bioinformatic processing of RAD-seq data dramatically impacts downstream population genetic inference. *Methods Ecol Evol*. 8:907–917.

Shafer AB, Wolf JB, Alves PC, Bergström L, Bruford MW, Brännström I, Colling G, Dalén L, De Meester L, Ekblom R, *et al*. 2015. Genomics and the challenging translation into conservation practice. *Trends Ecol Evol*. 30:78–87.

Shafer ABA, Wolf JBW, Alves PC, Bergström L, Colling G, Dalén L, De Meester L, Ekblom R, Fior S, Hajibabaei M, *et al*. 2016. Reply to Garner *et al*. *Trends Ecol Evol*. 31:83–84.

Swezey SL, Heizer RF. 1977. Ritual management of salmonid fish resources in California. *J Calif. Anthropol*. 4:6–29.

Thompson TQ, Bellinger MR, O'Rourke SM, Prince DJ, Stevenson AE, Rodrigues AT, Sloat MR, Speller CF, Yang DY, Butler VL, *et al*. 2019. Anthropogenic habitat alteration leads to rapid loss of adaptive variation and restoration potential in wild salmon populations. *Proc Natl Acad Sci USA*. 116:177–186.

USFWS and NMFS. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. *Fed. Reg*. 61:4722.

Wang Z, Gerstein M, Snyder M. 2009. RNA-Seq: a revolutionary tool for transcriptomics. *Nat Rev Genet*. 10:57–63.

Wang J, Santiago E, Caballero A. 2016. Prediction and estimation of effective population size. *Heredity (Edinb)*. 117:193–206.

Waples RS. 1991. Pacific salmon, Oncorhynchus spp., and the definition of" species" under the Endangered Species Act. *Mar Fish Rev*. 53:11–22.

Waples RS. 2005. Genetic estimates of contemporary effective population size: to what time periods do the estimates apply? *Mol Ecol*. 14:3335–3352. doi:10.1111/j.1365-294X.2005.02673.x

Waples RS, Antao T, Luikart G. 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics*. 197:769–780.

Waples RS, Do C. 2008. ldne: a program for estimating effective population size from data on linkage disequilibrium. *Mol Ecol Resour*. 8:753–756.

Waples RS, Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol Ecol*. 15:1419–1439.

Waples RS, Grewe PM, Bravington MW, Hillary R, Feutry P. 2018a. Robust estimates of a high N_e/N ratio in a top marine predator, southern bluefin tuna. *Sci Adv*. 4:eaar7759.

Waples RK, Larson WA, Waples RS. 2016. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. *Heredity (Edinb)*. 117:233–240.

Waples RS, Lindley ST. 2018. Genomics and conservation units: the genetic basis of adult migration timing in Pacific salmonids. *Evol Appl*. 11:1518–1526.

Waples RS, Luikart G, Faulkner JR, Tallmon DA. 2013. Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc Biol Sci*. 280:20131339.

Waples RS, Scribner K, Moore J, Draheim H, Etter D, Boersen M. 2018b. Accounting for age structure and spatial structure in eco-evolutionary analyses of a large, mobile vertebrate. *J Heredity*. 1:15.

Waples RS, Teel DJ, Myers JM, Marshall AR. 2004. Life-history divergence in Chinook salmon: historic contingency and parallel evolution. *Evolution*. 58:386–403.

Waples RS, Yokota M. 2007. Temporal estimates of effective population size in species with overlapping generations. *Genetics*. 175:219–233.

Whiteley AR, Coombs JA, Cembrola M, O'Donnell MJ, Hudy M, Nislow KH, Letcher BH. 2015. Effective number of breeders provides a link between interannual variation in stream flow and individual reproductive contribution in a stream salmonid. *Mol Ecol*. 24:3585–3602. doi:10.1111/mec.13273

Whiteley AR, Coombs JA, O'Donnell MJ, Nislow KH, Letcher BH. 2017. Keeping things local: Subpopulation Nb and Ne in a stream network with partial barriers to fish migration. *Evol Appl*. 10:348–365. doi:10.1111/eva.12454

Zhou Y, Tian X, Browning BL, Browning SR. 2018. POPdemog: visualizing population demographic history from simulation scripts. *Bioinformatics*. 1:2.