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OPINION

Genetics and the conservation of natural populations: allozymes to genomes

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I consider how the study of genetic variation has influenced efforts to conserve natural populations over the last 50 years. Studies with allozymes in the 1970s provided the first estimates of the amount of genetic variation within and between natural populations at multiple loci. These early studies played an important role in developing plans to conserve species. The description of genetic variation in mitochondrial DNA in the early 1980s laid the foundation for the field of phylogeography, which provided a deeper look in time of the relationships and connectivity among populations. The development of microsatellites in the 1990s provided much more powerful means to describe genetic variation at nuclear loci, including the ability to detect past bottlenecks and estimate current effective population size with a single temporal sample. In the 2000s, single nucleotide polymorphisms presented a cornucopia of loci that has greatly improved power to estimate genetic and population demographic parameters important for conservation. Today, population genomics presents the ability to detect regions of the genome that are affected by natural selection (e.g. local adaptation or inbreeding depression). In addition, the ability to genotype historical samples has provided power to understand how climate change and other anthropogenic phenomena have affected populations. Modern molecular techniques provide unprecedented power to understand genetic variation in natural populations. Nevertheless, application of this information requires sound understanding of population genetics theory. I believe that current training in conservation genetics focuses too much on the latest techniques and too little on understanding the conceptual basis which is needed to interpret these data and ask good questions.

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The first two papers describing genetic variation at multiple loci in natural populations were published some 50 years ago (Harris 1966; Lewontin & Hubby 1966). The rush was on to describe genetic variation in as many species as possible species using protein electrophoresis. The term allozyme was introduced in 1969 to refer to allelic forms at the same protein-coding locus detected with electrophoresis (Prakash *et al.* 1969). Those were exciting times when it first became possible to detect and compare the amount of genetic variation at many loci in any species, from *E. coli* to elephants. Charlesworth & Charlesworth (2017) have provided an overview of the theoretical and empirical advances in population genetics over this 50-year period which complements this consideration of the applications of population genetics to conservation.

The absence of empirical data on the amount of genetic variation within and among populations had wide implications for other central problems in ecology and evolution. Ehrlich & Raven (1969) proposed that gene flow in natural populations is highly restricted and that natural selection is the 'primary cohesive and disruptive force in evolution'. They proposed that natural selection, rather than gene flow, determines the amount of differentiation among populations. The study of allozymes made it possible for the first time to test empirically the rich body of population genetics theory that had been developed in the previous 50 years by such intellectual giants as R.A. Fisher, J.B.S. Haldane and Sewall Wright. How much genetic variation is there in natural populations? Do large populations contain more genetic variation than small populations? How genetically divergent are populations in the same species?

Early results made it clear that the 'classical' view that most individuals are homozygous for a single wild-type allele at most loci was incorrect (Lewontin 1974). However, the debate raged for years whether the surprising amount of genetic variation detected with allozymes was maintained primarily by some form of balancing selection or a combination of neutral mutations and genetic drift (King & Jukes 1969; Kimura 1983; Nei 2005). A prescient paper by Kimura & Crow (1964) proposed the possibility that the 'wild-type' allele may in fact be comprised of many nearly identical alleles ('isoalleles') with little effect on fitness.

In this essay, I consider how this ability to detect and describe genetic variation in natural populations has influenced efforts to conserve natural populations. Frankel (1974) was the first to argue that the principles of genetics should be applied to the conservation of species. The field of conservation genetics has matured, and it has come to play an essential role in the conservation and management of species. My career began in 1971 and has spanned the period beginning when there was virtually no understanding of the amount of allele frequency divergence in natural populations to the present when data are available from complete genome sequencing of many individuals in a variety of species.

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Table 1 Timeline showing major advances in the use of genetic variation in natural populations to conserve species

	Empirical approach	Conservation genetic advance
1966	Allozymes	Description of the amount of genetic variation within and between populations
		Detection of sibling species
1979	Mitochondrial DNA	Phylogeography
		Ability to distinguish between male and female gene flow
1990	Microsatellites	More power to describe population structure
		Detection of population bottlenecks
		Estimation of current effective population size
		Individual-based analysis
		Assignment tests
		Landscape genetics
2000	SNPs	More power to describe population structure
		Ability to detect loci that affect fitness
2010	Population genomics	Detection of genomic regions under selection
		Estimation of proportion of the genome identical by descent
		Detection and understanding of inbreeding depression
		Detection and understanding of hybridization

My objectives are to (i) briefly review the primary techniques that have been used over the last 50 years to describe genetic variation in natural populations, (ii) highlight the importance of understanding population genetics theory to interpret observed patterns of genetic variation in natural populations, and (iii) consider past, present and future applications of genetics to conserve natural populations.

From allozymes to genomes

A variety of techniques have been employed over the last 50 years to detect genetic variation in natural populations (Sunnucks 2000; Schlötterer 2004). Here, I consider the major advances associated with each of the primary approaches that have been used in conservation genetics (Table 1).

Allozymes. Empirical population genetics describing genetic variation in a wide variety of species exploded following the initial two papers in 1966. One of the beauties of allozyme analysis was that it required no a priori information about a species (e.g. primer sequences for microsatellites). The same allozyme techniques could be used directly without modification with any fresh tissue sample of animal, plant or microbe because all such tissues contain enzymes:

> A.D. Hershey is reported to have described heaven as "finding an experiment that works and doing it over and over again". Population geneticists too have found heaven. (Lewontin 1974, p. 116)

By 1976, genetic variation at multiple loci had been described in 125 species of animals and eight species of plants (Selander 1976). A comprehensive review in 1984 described genetic variation at a mean of 23 loci in over 1100 species of animals and 75 species of plants (Nevo *et al.* 1984).

Table 2 Comparison of H_T, H_S and F_{ST} estimated with allozymes for different major taxa of animals (Ward *et al.* 1992) and plants classified by their geographical range (Hamrick & Godt 1990, 1996)

Taxa	H_T	H_S	F_{ST}	No. of species
Amphibians	0.136	0.094	0.315	33
Birds	0.059	0.054	0.076	16
Fish	0.067	0.054	0.135	79
Mammals	0.078	0.054	0.242	57
Reptiles	0.124	0.090	0.258	22
Crustaceans	0.088	0.063	0.169	19
Insects	0.138	0.122	0.097	46
Molluscs	0.157	0.121	0.263	44
Endemic plants	0.096	0.063	0.248	100
Regional plants	0.150	0.118	0.216	180
Widespread plants	0.202	0.159	0.210	85

H_T is the total expected heterozygosity in a species; H_S is the mean expected within population heterozygosity averaged over all populations; F_{ST} is the proportion of total heterozygosity in a species due to genetic divergence among populations.

And, as the same suite of enzyme loci were examined in different species, it was easy to compare meaningfully the relative amount of genetic variation within and between populations in different species. Table 2 summarizes the amount of allozyme variation found within and among populations in animals (Ward *et al.* 1992) and plants (Hamrick & Godt 1990, 1996). Some interesting patterns emerged. First, insects tended to have greater mean expected heterozygosity within populations (H_S) than vertebrates. This reflects the tendency for local populations of invertebrates to be larger than vertebrates because of the relationship that species with smaller body size tend to

have larger population size (Cotgreave 1993). An analogous pattern is seen for plants. Species with a wider range, and therefore greater total population size, have greater total mean heterozygosity (H_T) than endemic plants that have a more restricted range.

In addition, those taxa that we would expect to have greater ability for movement and exchange among populations have less genetic divergence among populations (F_{ST}). For example, bird species have the same mean amount of genetic variation within populations (H_S) as fish and mammals, but they have much less genetic divergence among populations. This difference reflects the greater ability of birds for exchange among geographically isolated populations because of flight. In contrast, amphibians, which have limited dispersal capability, have the greatest mean value of F_{ST}. Plant species that are wind-pollinated, or have wind-dispersed seeds, generally have less genetic divergence among populations than species that are insect pollinated or have animal-dispersed seeds (Hamrick & Godt 1996). These results also demonstrated that Ehrlich & Raven (1969) were incorrect in their hypothesis that natural selection is the primary cohesive and disruptive force in evolution. The concordance of F_{ST} over loci argues that gene flow is the primary force affecting the amount of divergence among populations (Allendorf & Seeb 2000).

Little genetic divergence was found between even geographically distant populations of highly mobile species. For example, Elliott *et al.* (1994) found that orange roughy (*Hoplostethus atlanticus*) from Scotland and southern Australia, 22 000 km apart, had almost no genetic divergence ($F_{ST} < 0.01$). On the other extreme, some single species were discovered to consist of genetically distinct sibling species that are nearly morphologically identical. For example, Shaklee & Tamaru (1981) collected what was then considered a single species of bonefish (*Albula vulpes*) off the coast of Hawaii. Allozyme analysis revealed that this single sample actually comprised two distinct species that had diagnostic alleles at 58 of 84 loci. Minor, but consistent, differences in morphology were uncovered when the two groups of fish were distinguished with genetic analysis. They estimated that these two lineages have been reproductively isolated for at least 10 Myr. Analysis of mitochondrial DNA (mtDNA) sequence divergence from a more comprehensive geographical collection of bonefish samples revealed that what was considered to be a single species may consist of as many as eight species (Colborn *et al.* 2001)!

This abundance of empirical information was quickly applied to conservation (e.g. Brown 1978). One of the primary insights provided by this information was the ability to identify small population size or recent population bottlenecks. For example, Bonnell & Selander (1974) found a complete lack of genetic variation in northern elephant seals (*Mirounga angustirostris*) as a result of severe bottleneck in population size that the species suffered in the last century. An early influential paper reported that two populations of cheetah (*Acinonyx jubatus*) from South Africa were completely homozygous at 47 allozyme loci because of a population bottleneck or two (O'Brien *et al.* 1983). The conclusion of the authors that cheetahs were more vulnerable to extinction because of this lack of genetic variation soon became very controversial (May 1995). Critics pointed out that the greatest threat to the cheetah was the loss of habitat and other human activities, not lack of genetic variation. As with many such controversies, both arguments are somewhat correct. The loss of habitat and other human activities were, and are, the greatest threat to the cheetah. Nevertheless, the reduced genetic variation does make the cheetah more vulnerable because of increased genetic load of deleterious recessive alleles and reduced disease resistance. A recent genomic study has provided intriguing insight into this controversy (see below, Dobrynin *et al.* 2015).

The most intensive application of allozymes to conservation was almost certainly the management and conservation of anadromous Pacific and Atlantic salmon (*Oncorhynchus* spp., Waples *et al.* 1990; *Salmo salar*, Ferguson *et al.* 1995). Anadromous salmon spawn in freshwater, migrate to the ocean where they spend most of their lives, and return to freshwater to spawn. They have enormous cultural and economic value, and they are harvested in mixed-populations aggregations. The homing tendency has resulted in many local subpopulations of salmon species having substantial genetic differences within river systems. For example, the Alaska Department of Fish & Game has described genetic variation in 294 genetically distinct subpopulations of sockeye salmon (*O. nerka*) contributing to the Bristol Bay sockeye salmon fishery (Dann *et al.* 2012).

All in all, it is likely that nearly a million Pacific salmon were genotyped at allozyme loci over the years in North American laboratories (L. W. Seeb, J. E. Seeb & R. S. Waples, personal communication). These estimates include the five species of Pacific salmon native to the west coast of North America and anadromous rainbow trout (*O. mykiss*). This information has been used to identify the subpopulation composition of mixed stocks of salmon captured in the ocean and freshwater (Utter & Ryman 1993). For example, Seeb *et al.* (2000) genotyped 27 allozyme loci in all major spawning populations of sockeye salmon from the upper Cook Inlet in Alaska and found substantial differentiation among subpopulations (e.g. $F_{ST} = 0.075$). The salmon from these major populations are harvested in a mixed-stock aggregation in upper Cook Inlet. A mixed-stock genetic analysis allowed estimation of the proportion of individuals from each subpopulation in the pool of harvested fish. The genotyping and statistical analysis was conducted within 48 h after harvest and allowed real-time monitoring of harvest. It allowed managers to close the fishery if too many fish were harvested from any single breeding population. This is crucial to help prevent overfishing, longer term closures of fishing and the extinction of a major source population.

These genetic analyses of salmon also have played an important role in conservation. The description of genetic population structure with allozymes was essential in designating over 50 distinct population segments (DPSs) as

'species' under the United States Endangered Species Act (ESA) of the six salmon species above (NOAA 2016). Twenty-eight of these DPSs are currently listed as either threatened or endangered under the US ESA (NOAA 2016).

Mitochondrial DNA. The next major innovation in the description of genetic variation in natural populations occurred in 1979 when two independent groups published the first reports of genetic variation in mtDNA from natural populations. Avise *et al.* (1979a, b) used restriction enzyme analysis of mtDNA to describe sequence variation and the genetic population structure of mice (*Peromyscus*) and pocket gophers (*Geomys pinetis*). Brown & Wright (1979) used the maternal inheritance of mtDNA to determine the sex of two lizard species (*Cnemidophorus*) that originally had hybridized to produce parthenogenetic species.

Mitochondrial DNA provided a different perspective of the genetic structure of natural populations because of its maternal inheritance and general lack of recombination between mtDNA molecules. Unlike nuclear DNA, the historical genealogical record of descent is not 'shuffled' by recombination between different mtDNA lineages during gamete production, as occurs in nuclear DNA during meiosis. The ability to reconstruct the phylogeny of mtDNA molecules led to the creation of the new field of phylogeography, the study of the spatial arrangements of genealogical lineages within conspecific populations (Avise 2000). This field has since blossomed and has made many valuable contributions to the understanding and protection of biodiversity (Avise *et al.* 2016).

The lack of recombination, which makes mtDNA especially valuable to reconstruct phylogenies, reduces its utility for describing patterns of gene flow between populations within species (Wilson *et al.* 1985; Ballard & Whitlock 2004). The primary problem is that the entire mtDNA genome acts as a single locus because there is no recombination. There can be substantial differences between loci in the patterns of genetic variation just by chance alone (Slatkin & Barton 1989). It is inappropriate to use any single locus to describe patterns of gene flow among populations because of the variability between loci. In addition, mtDNA capture can cause discordance between the geographical patterns of genetic structure in the nuclear and mitochondrial genomes, which makes it imperative to not use mtDNA alone to describe units of conservation (Toews & Brelsford 2012).

In addition, gene flow brought about by males is not detectable because of the maternal inheritance of mtDNA. For example, Pardini *et al.* (2001) examined mtDNA and microsatellite genotypes in great white sharks (*Carcharodon carcharias*) collected off the coasts of South Africa, Australia and New Zealand. Sharks from South Africa were nearly fixed for different mtDNA haplotypes compared to sharks from Australia and New Zealand (F_{ST} = 0.85). In striking contrast to this result, no allele frequency differences were found at five nuclear microsatellite loci between these regions. Pardini *et al.* (2001) concluded that female great white sharks are philopatric and that males undertake long transoceanic movements. However, study of transoceanic movement with electronic tags and photographic identification indicates that females, as well as males, make transoceanic movements between these areas (Bonfil *et al.* 2005). Therefore, the difference between divergence of mtDNA and nuclear markers is apparently not based on differences in transoceanic migrations of males and females, but result from whether these migrants become reproductively integrated into the recipient population.

These limitations make mtDNA unsuitable for estimating gene flow between populations and describing units of conservation without incorporating information from nuclear markers. Unfortunately, many papers over the last 30 years have done just this. This journal enacted a policy in 2011 (L. H. Rieseberg, personal communication) which currently states 'authors of single species phylogeographic studies must base their inferences on multiple loci' to address this problem. Mitochondrial DNA alone has been used commonly to identify both evolutionarily significant units and management units (MUs). In fact, the initial criterion used to delineate MUs was proposed by Moritz (1994) who defined MUs as '... populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles'. The wording 'significant divergence' has generally been inferred to mean the statistical rejection of panmixia, and was commonly used as the yardstick when designating MU status from population genetic data (Palsbøll *et al.* 2007). This is not a useful approach because virtually any pair of population samples will have statistically significant differences in mtDNA frequencies with large sample sizes because of the greater divergence expected at loci with smaller N_e such as mtDNA. On the other hand, the maternal transmission of mtDNA can provide important demographic information for the determination of MUs when used in combination with nuclear markers. A local population for which females are isolated, but there is substantial male gene flow, can still be at major risk because males provide no demographic rescue effect.

Microsatellites. The third major innovation in applying molecular genetics to conservation was the development and application of microsatellites (Goldstein & Pollock 1997). Microsatellites provided two major advantages over allozymes as a nuclear marker. First, the ability to screen many microsatellite loci and select a suite of highly variable loci made it possible to examine a large number of polymorphic loci in virtually any species. In comparison, the low amount of polymorphism made allozymes unusable in species with little genetic variation. Second, the use of the polymerase chain reaction made it possible to genotype microsatellites without destructive sampling in a variety of amazing sources: faeces, hair left on trees, host blood in ticks, a single pollen grain and even in the breath of dolphins (Matsuki *et al.* 2007; Frère *et al.* 2010). Thus, there were now no

limitations in describing genetic variation in any species of interest.

The presence of many alleles at microsatellite loci provided a valuable opportunity to test for recent reductions in population sizes (i.e. bottlenecks) caused by human activities with a single sample in time (Beaumont 1999). This was extremely valuable for conservation. Estimates of genetic variation at allozyme loci uncovered many species with low amounts of genetic variation (e.g. northern elephant seals and cheetahs). However, it was difficult to determine whether this low amount of genetic variation was caused by recent anthropogenic effects or such species had historically small population size. This distinction is crucial because populations going through recent bottlenecks are more likely to contain deleterious recessive alleles at high frequency (Charlesworth & Willis 2009).

Bottlenecks result in loss of both heterozygosity and allelic diversity. However, heterozygosity is relatively insensitive to the effects of bottlenecks (Allendorf 1986). In contrast, even fairly large bottlenecks can result in substantial loss of allelic diversity (Allendorf *et al.* 2014). Genetic bottleneck tests have been valuable because they can detect population decline even with a sample at a single point in time, yet reflect demographic history over multiple generations (Peery *et al.* 2012). Single-sample population bottleneck detection methods detect deviations from expectations under mutation–drift equilibrium. The two most common approaches based on multilocus microsatellite genotypes have been the heterozygosity-excess test (Luikart *et al.* 1998) and the M-ratio test (Garza & Williamson 2001).

The ability to examine many loci made it possible to estimate effective population size using a single sample by estimating the amount of gametic (linkage) disequilibrium that is generated by genetic drift. Hill (1981) provided a method for doing this, but it was not possible to implement this method in natural populations until it became possible to genotype individuals at many loci (Waples & Do 2010). The application of this technique has played a major role in the conservation of many species (Waples 2002).

Genotyping many microsatellite loci makes it possible to identify individuals on the basis of their multiple-locus genotypes, and this has had a variety of valuable applications in conservation. For example, assignment tests have been used to detect dispersal of individuals between populations (e.g. Berry *et al.* 2004). Assignment tests also have been used to identify the geographical origins of poached elephants on the basis of genotyping samples of illegal ivory (Wasser *et al.* 2015). In addition, a variety of methods have been developed to estimate the census size of populations based upon identifying individuals on the basis of their multiple-locus genotype (Luikart *et al.* 2010).

The use of individual genotypes as the unit of analysis rather than estimating 'population' frequencies in arbitrary groups of individuals collected from the same geographical area gave rise to the field of landscape genetics (Manel *et al.* 2003). This approach uses individual-based genotypes to describe genetic patterns (e.g. spatial discontinuities) to identify population boundaries or to group individuals into subpopulations using software such as STRUCTURE (Pritchard *et al.* 2000). This is done by clustering together individuals with similar genotypes and minimizing departures from Hardy–Weinberg proportions and gametic disequilibrium within subpopulations. This approach has been used very effectively to identify cryptic genetic populations, as well as identifying apparent migrants among populations.

In addition, the virtually unlimited number of microsatellite loci present in many genomes allowed hundreds of microsatellite loci to be developed and mapped in some species. This opened the door to population genomic approaches in conservation. For example, Jaari *et al.* (2009) mapped 117 microsatellite loci in the Siberian jay (*Perisoreus infaustus*) onto nine autosomes and the Z-chromosome. Initial population studies with these markers revealed unexpectedly high amounts of gametic disequilibrium throughout the genome (Li & Merilä 2010).

Single nucleotide polymorphisms. The latest major innovation in detecting genetic variation in natural populations was the application of single nucleotide polymorphisms (SNPs; Morin *et al.* 2004). SNPs provide the opportunity to study an unlimited number of loci genomewide. This has allowed greatly improved power to estimate genetic and population demographic parameters (e.g. gene flow and effective population size) important for conservation. In addition, a variety of approaches are now available to detect fitness effects that are potentially involved in local adaptation.

A variety of potential pitfalls need to be carefully considered when trying to find the genetic basis of adaptation (Hoban *et al.* 2016). Two recent papers have considered the difficulties surrounding how this information can best be used to inform conservation (Shafer *et al.* 2015; Pearse 2016). I share their concerns about the difficulties involved with the application of individual adaptive genes to conservation. I also agree that focusing on the preservation of the adaptive variation that we can detect but ignoring the vast majority that we cannot detect is a real danger (Pearse 2016). Perhaps my major concern is that in conservation we should be more concerned about predicting the potential for future adaptation than in identifying the effects of natural selection in the past. Nevertheless, understanding the spatial distribution of adaptive genetic variation is important to make sure that we conserve adaptively divergent populations (Funk *et al.* 2012). These could be important sources of future adaptive genetic variation under environmental change.

Single nucleotide polymorphisms have replaced the use of allozymes to analyse the genetic composition of mixed-stock salmon fisheries. For example, Dann *et al.* (2013) genotyped 12 582 sockeye salmon at 38 SNP loci caught in the Bristol Bay fishery during the years 2006–2010. These 38 loci were selected because they had shown to provide genetic discrimination based upon the baseline genetic

data. Test-fishery caught samples were genotyped within 2–3 days of capture and stock composition estimates were provided to fishery managers within 3–4 days of capture. This analysis detected abundances that were unanticipated based upon preseason fishery forecasts, fishing effort was shifted accordingly, and the fleet and local economies benefited while the risk of overharvesting weaker stocks was minimized. This approach has been developed for many other salmon fisheries on the west coast of North America; perhaps as many as 500 000 salmon are genotyped per year in these efforts (J. E. Seeb & L. W. Seeb, personal communication).

Genetic monitoring. Taking multiple temporal samples over time makes it possible to monitor genetic changes to test for genetic changes associated with anthropogenic effects (Schwartz *et al.* 2007). For example, Rubidge *et al.* (2012; Bi *et al.* 2013) compared modern and historical samples from 100 years ago to test for population genetic effects of a climate-driven elevational range contraction in the alpine chipmunk, *Tamias alpinus*, in Yosemite National Park, USA. This species has reduced its lower elevational limit upslope by more than 500 m, whereas the range of the closely related chipmunk *T. speciosus* did not change. Allelic diversity declined and geographical genetic subdivision increased in *T. alpinus*. In contrast, there were no detectable genetic changes in *T. speciosus* over this period.

Bergner *et al.* (2016) used historical samples to test whether the arrival of Polynesians or the settlement of Europeans in New Zealand caused the low genetic variation currently observed in the kākāpō (*Strigops habroptilus*), a ground breeding parrot endemic to New Zealand. Allelic diversity decreased significantly between pre-European and contemporary kākāpō samples in both microsatellites and mtDNA. Modelling of demographic history indicated a recent population bottleneck associated with European colonization but did not suggest a major decline associated with Polynesian settlement. These results inform kākāpō management by indicating the time frame and possible cause of the bottleneck, and demonstrate the importance of a historical perspective in understanding causes of decline and managing extinction risk in contemporary endangered species.

Population genomics

The term population genomics came into use early in this century. Black *et al.* (2001) defined population genomics as the process of simultaneous sampling of numerous variable loci within a genome and the inference of locus-specific effects from the sample distributions. In this case, population genomics meant sampling more loci than was previously possible. However, very few loci (e.g. 14) were actually genotyped in some of the examples of population genomics presented in this paper. Luikart *et al.* (2003) provided a more comprehensive view of population genomics which they defined as the simultaneous study of numerous loci or genome regions to better understand the roles of evolutionary processes (such as mutation, random genetic drift, gene flow and natural selection) that influence variation across genomes and populations. However, the emphasis in Luikart *et al.* (2003) was also increasing the number of loci rather than applying a conceptually different approach.

Perhaps the first population genomics paper with natural populations was that of Hohenlohe *et al.* (2010) in which they examined genetic variation at 45 000 SNPs in threespined sticklebacks (*Gasterosteus aculeatus*). What distinguished this paper is that they plotted values of heterozygosity and population differentiation (F_{ST}) along the entire mapped genome of 22 chromosomes so that chromosomal regions that stood out from background values could be detected. This is conceptually very different than just looking at more markers. In a more restricted sense, population genomics entails sampling a mapped genome at sufficient density to detect forces affecting any particular genomic region [e.g. runs of homozygosity (ROH) and regions of reduced recombination]; that is, instead of using a representative sample of loci to address the average effect of processes acting across the whole genome, population genomics characterizes variation in those processes along regions of the genome. Perhaps this finally is the death of beanbag genetics (Crow 2001).

A recent genomic investigation of seven cheetahs from Namibia and Tanzania supported the original allozyme finding of low genetic variation (Dobrynin *et al.* 2015). These cheetahs showed substantially less SNP variation than 10 other species, including human, domestic cat, gorilla, lion and the Tasmanian devil. In addition, cheetahs had long stretches of homozygosity throughout the genome. On average, 93% of each cheetah's genome was homozygous. They also found dramatically reduced genetic variation in protein-coding genes, which explains the initial discovery of little genetic variation with allozymes.

Inbreeding depression. Perhaps the most exciting application of genomics to conservation is the detection and understanding of inbreeding depression (Kardos *et al.* 2016). Pedigree-based analyses have traditionally been the basis of studies on individual inbreeding (Pemberton 2008). The pedigree inbreeding coefficient (F_P) predicts the probability of a locus being 'identical by descent' (IBD) based on a known pedigree where the founders are assumed to be unrelated and noninbred (Keller & Waller 2002). However, there is a surprising amount of variability in the actual proportion of the genome IBD of individuals, even with identical pedigrees because of the stochastic effects of Mendelian segregation and linkage (Hill & Weir 2011).

The availability of many thousands of genotyped loci now makes it possible to estimate the proportion of the genome which is IBD by molecular analysis. Several studies have compared estimates of inbreeding depression based on pedigrees and molecular genetic data (Forstmeier *et al.* 2012; Bérénos *et al.* 2016; Huisman *et al.* 2016). These

studies provide compelling empirical evidence that inbreeding depression is more easily detected, and its magnitude more accurately estimated, with genomic measures than with pedigree analysis. Thus, the availability of genomic data means it is now feasible to rigorously study inbreeding depression in many species.

More informative approaches are available to detect and understand inbreeding depression when using mapped loci that allow the detection of continuous genomic regions that are IBD (ROH). The distribution of the lengths of ROH can be analysed to estimate the proportion of an individual's genome IBD, as well as to infer population history (Kirin *et al.* 2010; Pemberton *et al.* 2012). An abundance of very long ROH suggests small N_e recently, and an abundance of very short ROH suggests small N_e in more distant history. Procedures have been developed that will estimate N_e in the recent past on the basis of the distribution of ROH (Browning & Browning 2015).

Palkopoulou *et al.* (2015) applied the ROH method to two historical samples of woolly mammoths (*Mammuthus primigenius*) to test whether reduced genetic variation and inbreeding depression might have contributed to the extinction of this species. They presented complete genome sequences from two woolly mammoths. One was from ~4300 years before the present, representing one of the last surviving individuals on Wrangel Island. The second was obtained from a ~45 000-year-old specimen from the mainland of northeastern Siberia. The Wrangel mammoth had 20% less heterozygosity than the mainland mammoth. In addition, 23% of the genome of the Wrangel mammoth consisted of ROHs, in comparison with <1% for the mainland mammoth. In addition, the ROHs in the Wrangel mammoth were relatively small and widely distributed throughout the genome. ROHs of such length typically occur from background relatedness associated with limited population size in the last dozens of generations rather than due to recent mating of closely related individuals, which would be expected to produce much longer stretches. Thus, the large proportion of ROHs in the Wrangel genome is likely due to a cumulative effect of recurrent breeding among distant relatives, which is consistent with a small Holocene effective population size on Wrangel Island. These authors concluded that the last surviving woolly mammoth population had a small N_e for many generations before its extinction.

Hybridization. Population genomics also provides new approaches to study the effects of hybridization, one of the major problems in conservation (Allendorf *et al.* 2001). A conceptually similar approach to ROH is the search for admixture (or migrant) tracts in genomes (Liang & Nielsen 2014). The length and distribution of such tracts can provide valuable insight into the evolutionary history of populations and species. For example, Fu *et al.* (2014) estimated the time of hybridization between Neanderthals and modern humans on the basis of the length of admixture tracts in a modern human sample from 45 000 years ago. There have been valuable recent advances in applying molecular tools to understanding the role of hybridization in evolution (Abbott *et al.* 2016; Wayne & Shaffer 2016).

The importance of theory

Here, I have focused on the history of applying empirical techniques to describe the patterns and amounts of genetic variation in natural populations. Today, it is relatively easy to obtain and analyse enormous amounts of information on genetic variation in any species. A wide variety of software programmes are available to analyse data and estimate parameters of interest. However, the ease of collecting and analysing data has led to an unfortunate and potentially dangerous reduction in the emphasis on understanding theory in the training of population and conservation geneticists. I am concerned that current training focuses too much on techniques and too little on understanding the conceptual basis needed to interpret these data.

Understanding theory is crucial for correctly interpreting outputs from computer programs and statistical analyses. The most powerful software programs that estimate important parameters, such as effective population size, are not useful if their assumptions and limitations are not understood. For example, I was working with a postdoctoral researcher at a workshop who wanted to estimate effective population size using the gametic disequilibrium approach (Waples & Do 2010). However, his study population contained substantial gametic disequilibrium because of recent hybridization with another population. I was unable to convince him that estimates of N_e using the gametic disequilibrium approach would not be meaningful because this method assumes that all the disequilibrium results from small population size.

Even the most basic aspects of population genetics theory are commonly misunderstood or overlooked (Waples 2015). Testing for Hardy–Weinberg proportions is routine in studies describing genotypic variation in natural populations, but many published papers do not demonstrate understanding of the purposes of these tests or how to interpret the results. Waples (2015) has presented a clear overview of these problems associated with testing for Hardy–Weinberg proportions and gametic disequilibrium.

One solution to this problem is raising the awareness of the need for greater emphasis on understanding theory in the training of conservation geneticists and molecular ecologists. My co-authors and I have emphasized the importance of theory in our text on conservation genetics (Allendorf *et al.* 2013). Eight of the 22 chapters deal with the fundamentals of population genetic theory (Hardy–Weinberg, genetic drift, natural selection, etc.). A highly readable and more advanced treatment of population genetics theory can be downloaded for free (Felsenstein 2015).

The coalescent. One conceptual advance in theoretical population genetics has come about in the last 50 years that deserves special recognition. The development of coalescent theory represents a qualitative conceptual advance

that has revolutionized the interpretation of population genetics data (Kingman 1982; Hudson 1983; Tajima 1983). The coalescent provides a backwards, rather than forwards, approach to modelling genetic changes in which the probability that a given pair of genes 'coalesce' into a common ancestral allele in some previous generation. The coalescent approach allows more powerful interpretation of sequence variation which can be used to obtain crucial estimates of the genetic and demographic history of a population (Rosenberg & Nordborg 2002; Wakeley 2009).

The future

Most of the applications of molecular genetics to conservation do not require a population genomics approach. Nevertheless, it is interesting to speculate what advances the ability to sequence whole genomes of many individuals could bring to conservation. Telenti *et al.* (2016) recently reported high-quality sequences of 10 545 human genomes, and they suggest that recent technological advances will shortly make this possible for relatively little expense per genome. They identified some variable sites in regions of the genome that are highly intolerant to variation, and they suggested that such variable sites are more likely to affect the health of carriers. In addition, the software tool Protein Variation Effect Analyzer (PROVEAN; Choi & Chan 2015) predicts which sequence variants carried by individuals are likely to affect protein function. Yoshida *et al.* (2017) have used this tool to detect deleterious mutations in the Japan Sea stickleback (*Gasterosteus nipponicus*). Such approaches could be used to estimate the genetic load carried by different individuals or populations which could be very useful in setting conservation priorities and captive breeding programmes.

There has been much recent anticipation and speculation about the use of biotechnology, such as genome-editing (CRISPR-Cas9), for conservation. There have been several recent papers that deal with these possibilities and their dangers that I recommend (Webber *et al.* 2015; Corlett 2017; Johnson *et al.* 2016). I believe, however, that sound understanding of population genetics theory is needed to use these tools effectively and safely in natural populations. For example, the introduction of adaptive variants to prevent extinction (e.g. Thomas *et al.* 2013) is not likely to be of general use for conservation (Hedrick *et al.* 2013). However, CRISPR-based gene drives, where offspring of an edited parent have two copies of the edited genes, could be used to spread deleterious genes to control harmful invasive species or disease vectors (Champer *et al.* 2016). Nevertheless, I am concerned that such applications could have unanticipated effects. Gene drives have the potential to cause extinctions and may affect nontargeted populations or species. My work in conservation genetics has been fuelled by my passion for understanding population and evolutionary genetics. I have long believed that some of the most interesting basic questions in population and evolutionary genetics are of crucial importance for conservation (e.g. the genetic basis of inbreeding depression). I am anxious to see how these powerful technical tools are applied by the next generation of conservation geneticists.

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