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MOLECULAR ECOLOGY

OPINION

Fifty Years of Conservation Genetics: A Personal Perspective

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Received: 2 September 2024 | **Revised:** 17 November 2024 | **Accepted:** 5 December 2024

Handling Editor: Michael M. Hansen

Funding: The author received no specific funding for this work.

Keywords: allozymes | microsatellites | mtDNA | neutral theory | SNPs | theory

ABSTRACT

I provide my personal account of the application of genetics to conservation. I began graduate school shortly after the first description of genetic variation in natural populations. The use of allozymes uncovered an unexpected amount of genetic variation in a wide variety of species. During this same period, Motoo Kimura proposed the Neutral Theory of Evolution. Understanding the adaptive significance of allozyme variation became the major focus of population genetics. The utility of population genetic data for conservation and management was questioned because if the observed patterns were determined primarily by selection, then they could not be used to estimate gene flow or genetic drift. The study of mitochondrial DNA next provided a different view of genetic variation by allowing the overlaying of genealogical information on the locations of sampled individuals (phylogeography). The introduction of microsatellites allowed the study of a large number of nuclear markers. The many loci and large number of alleles at microsatellites were valuable for detecting bottlenecks and identifying relationships of individuals. The use of single nucleotide polymorphisms (SNPs) next opened the door to genomic analysis that allowed sampling a mapped genome to detect forces affecting particular genomic regions instead of using a representative sample of loci. For example, using runs of homozygosity has revolutionised our understanding of the effects of inbreeding and the detection of inbreeding depression. Current techniques provide unprecedented power to study genetic variation in natural populations. Nevertheless, the application of this information requires a sound understanding of population genetics theory.

1 | Introduction

My career has spanned the entire period that molecular genetics has been applied to conservation. Here, I provide a personal account of this history. I describe the major breakthroughs of applying molecular genetics to conservation, and how they influenced the field and my career over this time. This approach is inspired by Mike Wade's excellent book describing his experiences in striving to understand the evolution of adaptation in metapopulations throughout his career (Wade 2016).

I began graduate school in 1971 at the University of Washington (UW), just 5 years after the first two papers describing molecular genetic variation in natural populations with allozymes were published (Harris 1966; Lewontin and Hubby 1966). My graduate school years were in the heyday of the 'find'em and grind'em' period of population genetics (Lewontin 1991). Only eight papers describing allozyme variation in natural populations were published before 1971 (Nevo et al. 1984). The dam was about to break; 100 papers were published during the 4 years that I was in graduate school.

The ability to analyse data was as primitive as the ability to generate data at that time. I remember walking into Joe Felsenstein's office in 1972, and he showed me with great excitement his new hand-held HP-35 calculator, which is

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generally recognised as the first scientific calculator. My data analysis was done with a similar calculator or by writing my own software in FORTRAN to do basic analysis (estimating allele frequencies, testing for Hardy–Weinberg proportions, etc.) using the UW mainframe computer. I would drop off my punch cards in the afternoon at the computer centre and pick up the output the next morning.

In this paper, I address three major issues with regard to applying molecular genetics to conservation. First, I describe the long-standing debate surrounding the application of the Neutral Theory to population genetics and conservation. Second, I describe what I believe to be the major advances in genetic markers over this time. Finally, I describe my experiences translating research into practical conservation.

2 | Allozymes: Detecting Genetic Variation

Allozymes are different molecular forms of an enzyme encoded by different alleles at the same genetic locus (Lewontin 1974). Allozymes were genotyped using electrophoresis, in which the supernatant from fresh or frozen tissue was placed on a gel medium (potato starch in our lab) through which an electric current ran (similar to gel electrophoresis in DNA sequencing). Different allozymes of an enzyme migrate at different rates because they have different amino acid compositions and therefore different electrical charges, sizes and shapes.

Allozymes were identified with a stain specific for a particular enzyme, and the bands were marked by their relative position on the gel (e.g., aconitate hydratase, Figure 1). Genotypes were scored by the presence of bands associated with different alleles. For example, the first three individuals in Figure 1 have the following genotypes: *110/110*, *100/110* and *100/100*. Alleles were identified by the relative mobility of their protein products, and the most common allele was generally scored as *100*.

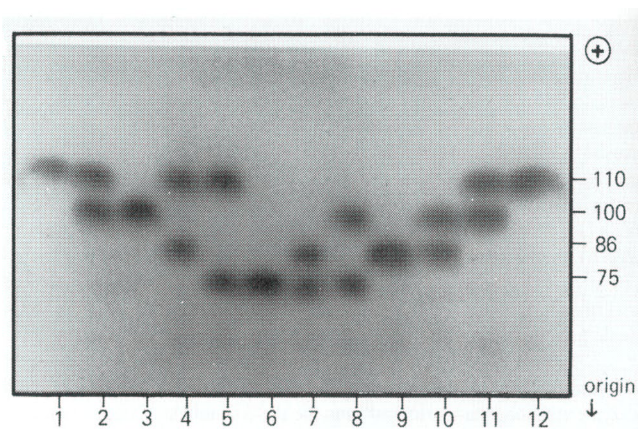

FIGURE 1 | Starch gel electrophoresis of the enzyme aconitate hydratase in livers of 12 Chinook salmon (*Oncorhynchus tshawytscha*; Allendorf et al. 2022, 55). The relative mobilities of the allozymes encoded by four alleles at this locus are on the right. The genotypes of all 12 individuals are (1) *110/110*, (2) *100/110*, (3) *100/100*, (4) *110/86*, (5) *110/75*, (6) *75/75*, (7) *86/75*, (8) *100/75*, (9) *86/86*, (10) *100/86*, (11) *110/100* and (12) *110/110*.

In general, 25 or so different allozyme loci would be scored for each individual. However, only roughly 10%–20% of these loci were polymorphic in a single population. In the system we used when I was a graduate student, I could genotype 40 individuals in a single day at 32 loci. This was a very low-tech procedure. All of the components were home-made from glass or plastic, and we used power supplies manufactured by Heathkit. The gels were scored by hand.

These early results made it clear that molecular genetic variation was abundant in natural populations. In fact, the absence of genetic variation became noteworthy. Bonnell and Selander (1974) published a paper in *Science* that found no allozyme variation at 24 loci in northern elephant seals (*Mirounga angustirostris*). They concluded that this was the result of a bottleneck resulting from commercial overharvest during the 19th century. It is thought that the species consisted of as few as 20 individuals during the 1890s. A recent genomic study confirmed the early results with allozymes. Hoelzel et al. (2024) estimated that approximately 88% of the genome-wide heterozygosity in this species was lost during the bottleneck.

One of the beauties of allozyme analysis was that it required no a priori information (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 the same enzymes. My fellow graduate student, Allyn Johnson, used the same allozyme technique to describe genetic variation in 26 species of vertebrates and invertebrates from Puget Sound (Johnson and Utter 1976). Lewontin (1974, 116) described this as Hershey's Heaven based on a report that A.D. Hershey described heaven as 'finding an experiment that works and doing it over and over again'. Population geneticists had found heaven.

The assumption was made that the allozyme variation had a simple genetic basis, largely on the basis of fitting Hardy–Weinberg proportions. Fairbairn & Roff (1980) demonstrated that the test for Hardy–Weinberg proportions has little power, and they presented examples where the described variation did not have a genetic basis. I was fortunate working with trout and salmon because it was relatively easy to test for Mendelian inheritance. I would collect ripe males and females from local hatcheries, genotype the fish, and add the sperm and eggs together in the desired combination of parents. Testing for inheritance is especially crucial for trout and salmon because of their tetraploid ancestry. Some allozyme loci show a complex mixture of disomic and tetrasomic inheritance (Allendorf and Danzmann 1997).

3 | The Neutral Theory

3.1 | The Controversy

In the 1970s, the field of population genetics was dominated by the question of the adaptive significance of the newly discovered molecular variation. The field was divided into camps of selectionists and neutralists.

The Neutral Theory of Evolution was first proposed by Motoo Kimura (1968) and independently by King and Jukes (1969). The concept of the Neutral Theory was actually first suggested as a possibility by Kimura and Crow (1964):

> With hundreds of nucleotides, each presumably capable of base substitutions and with additional permutations possible through sequence rearrangements, gains, and losses, the number of possible gene states becomes astronomical. It is known that a single nucleotide substitution can have the most drastic consequences, but there are also mutations with very minute effects and there is the possibility that many are so small as to be undetectable.

This paper was published just 11 years after Watson and Crick (1953) described the structure of DNA. It did not take long for theory to catch up with advances in molecular genetics.

The alternate view to the Neutral Theory was presented by Christopher Wills (1973) in his provocatively titled paper 'In Defense of Naive Pan-Selectionism'. Wills argued that the Neutral Theory can never be 'proved' for any locus because hidden selective pressures may be undiscovered. This view was shared by many selectionists at the time. I recall my student Robb Leary telling me about a disagreement that he had at a meeting with an ardent selectionist. The selectionist argued that selection is the appropriate null hypothesis, while Robb argued that selection should not be the null hypothesis because it can never be rejected, as Wills had argued. Both of my mentors, Joe Felsenstein and Fred Utter, were in the neutralist camp.

The general consensus at that time was that the two primary possible mechanisms for maintaining genetic variation were heterozygous advantage (overdominance) and the drift–mutation balance of neutral alleles. In fact, these were the only two mechanisms considered by Kimura and Crow in their 1964 paper.

My view of the maintenance of genetic variation was expanded by a year I spent with Bryan Clarke in England in 1978–79. Bryan was one of the leading selectionists at that time (Clarke 1970). Bryan did not convince me to become a selectionist, but he did open my eyes to the importance of frequency-dependent selection in maintaining genetic variation (Clarke 1979; Clarke and Allendorf 1979). English population geneticists tended to be in the selectionist camp following their tradition of ecological genetics (R.A. Fisher, E.B. Ford and A.C. Cain). In his obituary of Arthur Cain (Clarke 2008), Bryan wrote the following:

> On occasion Arthur's enthusiasm to make this argument led him apparently to imply that the null hypothesis should be selection rather than neutrality, a strategy that is of course impracticable.

The controversies surrounding the Neutral Theory have continued (Kern and Hahn 2018). Jensen et al. (2019) have provided a valuable history of the Neutral Theory over the last 50 years. In their words, the Neutral Theory 'continues to serve as the basis of modern evolutionary genomics' (Jensen et al. 2019).

3.2 | The Neutral Theory and Conservation

My graduate student career was spent in the laboratory of Fred Utter at the National Marine Fisheries Service, a short walk from the UW campus. This laboratory was founded in the 1950s to develop genetic techniques to identify the geographical origin of salmon caught in the ocean (Hodgins 1972). The earliest attempts to use genetics to identify populations used blood groups (de Ligny 1969). However, these efforts were quickly abandoned when allozymes became available.

It was clear from the beginning that an understanding of the genetic population structure of a species is crucial for conservation. The conservation of distinct populations is required to maximise evolutionary potential and to minimise the long-term extinction risks of a species. Moreover, the loss of populations throughout a species' range could have substantial and widespread ecological effects, even though the species itself is relatively safe from extinction in a part of its former range. Luck et al. (2003) have considered the effects of population diversity on the functioning of ecosystems and so-called ecosystem services. They argued that the relationship between biodiversity and human well-being is primarily a function of the diversity of populations, not species.

The identification of reproductively isolated populations is especially important for harvested species. Harvest of mixed populations is common in migratory waterfowl, marine mammals, ungulates and many other species. Virtually all harvested species have local breeding groups (subpopulations) that are somewhat reproductively isolated. Harvesting a group of individuals that is a mixture of several subpopulations can result in the overharvest and extirpation of one or more subpopulations. This will not be recognised unless the subpopulations are identified separately and individuals from population mixtures are assigned to subpopulations.

Utter and Hodgins (1969) reported that an allele at a lactate dehydrogenase locus in Pacific hake (*Merluccius productus*) was at a frequency of 0.01 along the Pacific coast from Washington to California, but this allele was at a frequency of 0.25 in Puget Sound. On this basis, they concluded that the coastal and Puget Sound populations were reproductively isolated and should be recognised as separate demographic units for harvest. This conclusion was based on the assumption of selective neutrality. If natural selection was acting on this locus, then the difference between the coastal and Puget Sound fish could be the result of differential selection in the ocean versus Puget Sound. More extensive investigation (29 allozyme loci and 10 microsatellite loci) support the conclusions of Utter & Hodgins (Iwamoto et al. 2004, 2015).

Using the amount of genetic divergence between populations to determine demographic connectivity is not straightforward (Waples and Gaggiotti 2006). Genetic connectivity depends primarily on the absolute number of migrants exchanged among populations per generation (Allendorf and Phelps 1981). In contrast, demographic connectivity depends on the migration rate per generation and the relative contribution of migrants to population growth (Waples and Gaggiotti 2006). I worked with my good friend and colleague Winsor Lowe, a population ecologist, to understand this relationship. We presented approaches for estimating genetic and demographic connectivity, highlighting the value of combining genetic data with measures of movement to understand the complex role of migration in natural populations (Lowe and Allendorf 2010).

3.3 | Selectionists

The use of genetic differences to identify reproductively isolated populations was questioned by some who argued that genetic differences between populations were due primarily to natural selection rather than the combined effects of gene flow and genetic drift. Williams et al. (1973) reported significant clinal genetic differences of the American eel (*Anguilla rostrata*) at three loci that they concluded was the result of natural selection. In this species, random mating (panmixia) occurs in the Sargasso Sea, and the planktonic larvae passively drift with the currents to the eastern North American coast from Florida to Labrador. These authors concluded that their results 'indicate that the recognition of separate Mendelian populations on the basis of significantly different allele frequencies in commercially important species, is highly questionable' (Williams et al. 1973). Genomic studies with American eels (Gagnaire et al. 2012) did not find similar clinal patterns of differentiation, but they did find some evidence for a few regions of the genome possibly under selection.

Other voices at that time argued with great passion (and little evidence) that allele frequency differences could not be used to identify genetically isolated populations because of natural selection. The loudest voice at that time was Bob Gauldie, who argued that allozyme variation could not be used to identify genetic population structure because of natural selection (Gauldie 1991). Fred Utter had devoted his career to applying genetic population structure to manage populations; his face would turn red, and he would start yelling whenever anyone mentioned Bob Gauldie in his presence.

Gauldie 1991 paper provoked a well-reasoned response by Paul Bentzen (1992) who described a number of fatal flaws in Gauldie's analysis. As expected, Bentzen's comment evoked a strong response from Gauldie (1992):

> Neutralist views of population genetics have proved to be so distant from experimental reality that they have been characterized by Cain (1983) as "Textbook myths… asserted on no direct evidence whatever, supported by a display of mathematical theorizing so simplistic that it could not possibly apply to real organisms, and protected from exposure by an avoidance of any consideration of the probable biology of the variants…" The selection versus neutralist issue is central to the idea of genetically isolated stocks. Any biological character whose distribution is controlled by selection cannot be used in the identification of genetically isolated stocks.

The controversy slowly faded as new techniques were developed to describe genetic population structure. In general, different types of markers detected the same patterns of variation, suggesting that natural selection was not a major influence on these patterns. For example, we found similar values of F_{ST} for 36 nuclear loci using different techniques, as well as mitochondrial DNA (mtDNA), in sockeye salmon (*Oncorhynchus nerka*; Allendorf and Seeb 2000). Clearly, however, some loci included in studies of genetic population structure are affected by natural selection. Larsson et al. (2007) found little divergence (F_{ST} < 0.002) at 11 allozyme and 8 microsatellite loci in Atlantic herring (*Clupea harengus*) from the North and Baltic Seas. However, they found a microsatellite locus with much higher divergence ($F_{ST} = 0.033$), apparently resulting from divergent selection associated with salinity (Gaggiotti et al. 2009).

Jonathan Pritchard (2023, 166) summarised the current view of using genetic markers to understand genetic population structure in his human genetics book:

> It is no longer controversial that most new mutations are neutral and that, of those that are not neutral, most are selected against. These properties allow us to use genetic variation as a tool for studying population structure and history while largely ignoring the role of selection.

4 | Advances in Molecular Markers

A variety of molecular genetic markers have been developed that have been used in conservation genetics. Thirty years ago, *Molecular Ecology* published a special issue on Conservation Genetics. In his introduction, Terry Burke (1994) stated that a special issue on Conservation Genetics would not have been justified just 2 years earlier. All the empirical papers in this special issue studied animals with the following distribution of marker types: DNA fingerprinting 5, mtDNA 4, allozymes 1, microsatellites 1 and RAPDs (randomly amplified polymorphic DNA) 1. It is gratifying to see how far the field has come in the last 30 years.

4.1 | Mitochondrial DNA

In 1979, two independent groups published the first reports of genetic variation in mtDNA from natural populations. Avise, Giblin-Davidson, et al. (1979) and Avise, Lansman, and Shade (1979) used restriction enzyme analysis of mtDNA to describe sequence variation and the genetic population structure of mice (*Peromyscus* spp.) and pocket gophers (*Geomys pinetis*). Brown and Wright (1979) used the maternal inheritance of mtDNA to determine the parental sexes of two lizard species (*Cnemidophorus* spp.) that hybridised to produce a parthenogenetic species.

Several characteristics of animal mtDNA make it valuable for some conservation genetic applications (Moritz 1994). MtDNA is haploid and maternally inherited in most species. MtDNA is especially valuable for reconstructing phylogenies because there is generally no recombination between mtDNA molecules. Unlike nuclear DNA, the historical genealogical record of descent is not 'shuffled' by recombination.

A number of papers followed the initial description of mtDNA to describe genetic population structure. Nevertheless, the lack of recombination, which makes mtDNA especially valuable in reconstructing phylogenies, reduces its value for describing genetic population structure (Ballard and Whitlock 2004). The primary problem is that the entire mtDNA genome acts as a single locus because there is no recombination. In addition, maternal inheritance makes it especially inappropriate to be used for describing units of conservation since patterns of divergence are not influenced by the genetic contributions of males (Bowen and Karl 2007). In addition, the smaller effective population size (N_e) of mtDNA results in greater expected genetic divergence than nuclear loci.

These limitations make mtDNA unsuitable for estimating gene flow between populations and describing units of conservation without accompanying information from nuclear markers. Unfortunately, many papers over the last 40 years have done just this. *Molecular Ecology* enacted a policy in 2011 (L. H. Rieseberg, personal communication) which states 'authors of single species phylogeographic studies must base their inferences on multiple loci' to address this problem. This policy was primarily aimed at studies using only mtDNA or chloroplast DNA (Loren Rieseberg, personal communication).

The pocket gopher paper by Avise, Lansman, and Shade (1979) introduced a conceptually new approach for understanding the genetics of natural populations. They used six restriction enzymes to detect mtDNA sequence differences in 87 pocket gophers from across their range in the southeastern United States. They then constructed a phylogenetic network of these haplotypes and superimposed this network upon the geographic locations of the sampled individuals (Figure 2).

This was the beginning of the discipline of phylogeography (Avise et al. 1987). Avise (1998) defined phylogeography as 'the spatial distributions within and among populations of alleles whose phylogenetic relationships are deduced'. Today the term phylogeography is often used in a much more general sense to mean the study of the geographical distribution of genetic variation within a species.

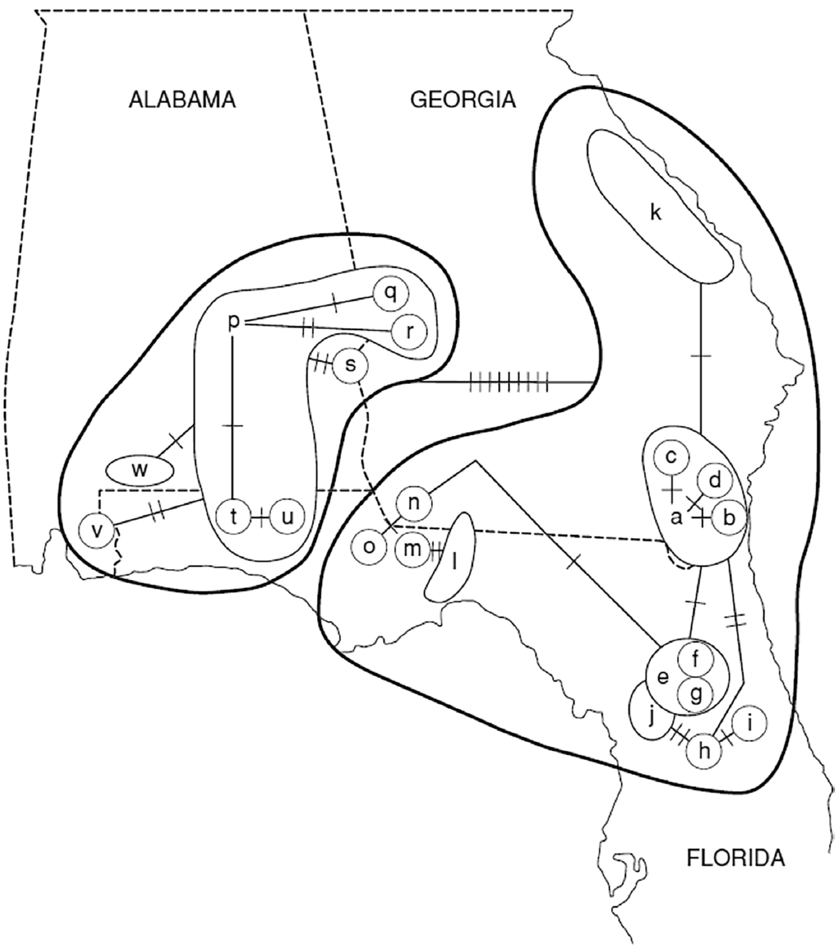

FIGURE 2 | Phylogeographic network of mtDNA for 87 pocket gophers (Avise, Giblin-Davidson, et al. 1979). Genotypes are represented by lower case letters and are connected by branches in a parsimony network (Allendorf et al. 2022, 470). Slashes across branches are substitutions. Nine mutations separate the two major mtDNA clades encircled by heavy lines.

I conducted an informal survey of my colleagues, and I was surprised to discover that about half of them accept the more general meaning of phylogeography. John Avise himself suggested that phylogeography 'could be expanded to include allele frequencies or any other information pertinent to understanding historical aspects of the population genetic structure of conspecific populations' (John Avise, personal communication). In contrast, his former student Brian Bowen replied that 'The difference between population genetics and phylogeography is that the latter *requires* genealogical information' (Brian Bowen, personal communication).

Allan Wilson et al. (1985) had previously explained that genealogical information and the distribution of allele frequencies are alternative ways to study and understand evolutionary genetics. The Wilson lab was a leader in the use of mtDNA to study evolutionary genetics. Allan had a profound effect on my career when I interacted with him while I was on sabbatical at UC Davis in 1984. Allan helped us incorporate mtDNA into our description of hybridisation between trout species (Gyllensten et al. 1985). He also suggested that we could use hybrid swarms between trout species to test for phenotypic effects of mtDNA. We examined three hybrid swarms between Westslope Cutthroat Trout (*Oncorhynchus lewisi*) and Rocky Mountain Cutthroat Trout (*O. virginalis*), but we could not detect any phenotypic effects of mtDNA (Forbes and Allendorf 1991).

My focus later returned to the fitness effects of mtDNA in a collaboration with Neil Gemmell, whom I met while on sabbatical in New Zealand. At the time, Neil was working on the role of mtDNA in human sperm function and male fertility. Deleterious mtDNA mutations that affect only males, such as those that impair sperm function, will not be subject to natural selection because mitochondria are generally maternally inherited and could reach high frequencies in populations if the mutations are not disadvantageous in females. Neil came up with the term 'Mother's Curse' for this effect (Gemmell et al. 2004). It occurred to me that this effect could be especially important in conservation because of the small effective population size of mtDNA (Gemmell and Allendorf 2001). A recent review concluded that evidence for the Mother's Curse is limited to a few taxa, with the strongest support coming from *Drosophila* (Edmands 2024). Most evidence comes from studies of fertility, which is expected to be particularly vulnerable to male-harming mitochondrial mutations.

4.2 | Microsatellites

The introduction of the polymerase chain reaction to detect genetic variation revolutionised conservation genetics. For the first time, any genomic region could be amplified and analysed in many individuals without cloning or isolating large amounts of genomic DNA. A bewildering number of techniques were introduced: RAPDs, ISSRs, IRAPs, AFLPs, etc. Some of these techniques were initially popular because they do not require a priori knowledge about primer sequences in the target species. For example, we used AFLPs to map the pink salmon genome (*Oncorhynchus gorbuscha*; Lindner et al. 2000). A major drawback was that AFLPs are dominant, but we circumvented this problem because we were able to artificially produce gynogenic haploid progeny.

RAPDs (Welsh and McClelland 1990) became popular because they were easy and could be used without any a priori information. However, it eventually became clear that this technique was not reliable (Pérez et al. 1998), and the use of RAPDs was discouraged by many journals (e.g., *Molecular Ecology*). I contacted Paul Sunnucks about RAPDs when we were working on the 2nd edition of our book (Allendorf et al. 2022). This was Paul's reply: 'There was a brief crazy period where in desperation people thought that it might be useful to go amplifying crappy, unreproducible, unidentified bands that 25% of the time don't even come from their organism, but then microsatellites came along so most people stopped thinking this way' (Sunnucks, personal communication).

Microsatellite DNA markers quickly became the primary nuclear marker of choice to study genetic variation in natural populations (Schlötterer 1998). Microsatellites are often highly polymorphic, even in small populations, because of their high mutation rate. We found microsatellite mutation rates as high as nearly 10^{-2} at some loci in pink salmon (Steinberg et al. 2002). The high mutation rate of microsatellites results in greater heterozygosity and greater allelic diversity than allozymes so that even a small number of microsatellite loci can be quite informative for some purposes. The large number of alleles present at some microsatellite loci makes them especially valuable for estimating relatedness (Blouin et al. 1996) and detecting bottlenecks with a single sample (Luikart et al. 1998). In addition, there are thousands of repeats in most genomes that are suitable for microsatellite analysis, which allowed constructing linkage maps of complete genomes. For example, Jaari et al. (2009) mapped 117 loci in the genome of the Siberian jay (*Perisoreus infaustus*).

4.2.1 | Detection of Bottlenecks

I attended a workshop on the genetic management of captive populations in 1984 organised by Kathy Ralls and Jon Ballou. Most of the primary workers in conservation genetics at that time attended this workshop, and the proceedings became a valuable resource for this emerging field (Ralls and Ballou 1986). I recall my frustration during the workshop because of the emphasis on heterozygosity without consideration of allelic diversity. This frustration resulted in my contribution to the proceedings on the importance of allelic diversity in conservation (Allendorf 1986). I remained interested in this topic throughout my career, and I recently published an updated treatment of this same topic with my longtime friend and colleague Nils Ryman (Allendorf et al. 2024).

My 1986 paper had two primary points: rare alleles are likely to be lost during bottlenecks and even small bottlenecks will have relatively little effect on heterozygosity. My Australian friend and colleague Mike Johnson published a paper in 1988 describing 11 polymorphic allozyme loci in a land snail (*Theba pisana*) that went through a bottleneck when it was introduced to Western Australia from France. His data clearly demonstrated both of these points (Figure 3). I used this paper as an example in my course in population genetics, and Gordon Luikart, my student

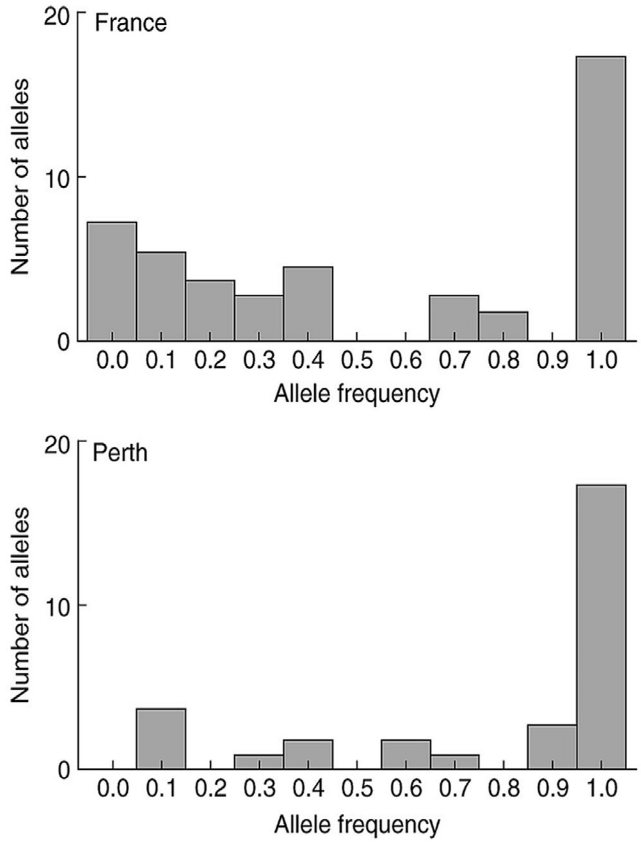

FIGURE 3 | Effects of a bottleneck on the distribution of allele frequencies at 25 allozyme loci in a land snail that was introduced from France into Perth, Western Australia in the 1890s (Allendorf et al. 2022, 122). Data from Johnson (1988).

at the time, became excited about the potential of using these principles to detect bottlenecks with a single sample. Gordon worked on this problem for his dissertation (Luikart 1997), and he developed a number of tests that became widely used to detect bottlenecks (e.g., Cornuet and Luikart 1996; Luikart et al. 1998).

4.3 | SNPs

In the early 2000s, single nucleotide polymorphisms (SNPs) began to be applied to conservation (Morin et al. 2004). This provided greatly improved power to estimate genetic and population demographic parameters (e.g., gene flow and effective population size) important for conservation. However, there was a long lag before SNPs became commonly used in conservation. The first edition of our book (Allendorf et al. 2022) was published in 2007, and the second in 2013. There were not many papers using SNPs that we incorporated into the second edition. The third edition was published in 2022. There was a tsunami of SNP papers during this period so that the third edition was completely dominated by the application of SNPs to conservation.

The application of thousands of SNPs has allowed the identification of loci responsible for adaptive differences between populations (Hoban et al. 2016). However, the best way to incorporate information about adaptive genomic variation into conservation has long been controversial. The controversy goes back to the early 1990s when it was proposed that captive breeding for conservation of vertebrates should focus exclusively on the major histocompatibility complex (MHC) because of its important role in disease resistance (Hughes 1991). This suggestion evoked a strong response from the conservation genetics community (Miller and Hedrick 1991; Vrijenhoek and Leberg 1991). There are a variety of problems associated with following this recommendation. The primary problem is that 'selecting' individuals on the basis of their MHC genotype will reduce genetic variation throughout the rest of the genome.

Several papers have considered the difficulties surrounding how adaptive loci can best be used to inform conservation (e.g., Pearce 2016; Kardos and Shafer 2018). 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 (Pearce 2016). Perhaps my major concern is that in conservation we should be more concerned about predicting the potential for future adaptation rather than identifying the effects of natural selection in the past. Nevertheless, understanding the spatial distribution of adaptive genetic variation is important to ensure that we conserve adaptively divergent populations (Funk et al. 2012).

Teixeira and Huber (2021) renewed this controversy by suggesting that the importance of neutral genetic variation has been over-emphasised in conservation genetics. They argued that there is no evidence that genome-wide patterns of neutral genetic diversity are important for either population persistence or adaptive potential. They suggested the application of genetics to conservation should focus on loci that affect fitness.

In response, Kardos et al. (2021) showed that both theory and empirical results indicate that conserving genome-wide genetic variation is the best approach to prevent inbreeding depression and loss of adaptive potential. They showed that focusing conservation efforts on adaptive genetic variation will result in the loss of genome-wide genetic variation, which is crucial for long-term viability.

4.4 | Transition From Genetics to Genomics

The term population genomics came into use early in the 2000s. Initially, population genomics referred to sampling more loci than were previously possible (Black et al. 2001; Charlesworth 2010). As few as 14 loci were genotyped in some of the examples of population genomics presented by Black et al. (2001). However, to me, population genomics is conceptually different from just looking at many markers. Luikart et al. (2018) explain the difference between broad sense (many markers) genomics, which addresses the average effect of processes acting across the whole genome, and narrow sense genomics, which entails sampling a mapped genome at sufficient density to detect forces affecting any particular genomic region (e.g., runs of homozygosity, ROH).

In my view, the first population genomics paper with natural populations was that of Hohenlohe et al. (2010) which examined genetic variation at over 45,000 SNPs in threespine sticklebacks (*Gasterosteus aculeatus*). What distinguished this paper is that they plotted heterozygosity and population differentiation (F_{ST}) along the entire mapped genome of 22 pairs of chromosomes so that chromosomal regions that stood out from background values could be detected.

In this section, I discuss the two primary areas where I believe population genomics has made the greatest contributions to our understanding of conservation genetics.

4.4.1 | Individuals as the Unit of Study

In my experience, the most dramatic and important change in the analysis of genetic population structure was the introduction of software that allowed using individuals, rather than populations, to be the unit of study (e.g., *STRUCTURE*, Pritchard et al. 2000). Before this, genetic population structure was studied by placing individual samples into sometimes arbitrary groups. *STRUCTURE* uses multilocus genotypes to probabilistically assign individuals into subpopulations by minimising departures from Hardy–Weinberg proportions and minimising linkage disequilibrium (LD). Individual-based methods such as this are useful to identify cryptic subpopulations and localise population boundaries on the landscape.

4.4.2 | Inbreeding

A seminal paper in 1979 by Kathy Ralls and her colleagues had a dramatic effect on the application of genetics to the management of wild and captive populations of animals (Ralls et al. 1979). They used pedigrees of 12 mammal species held in zoos to show that progeny from matings between related individuals tended to show reduced survival relative to progeny produced by matings between unrelated parents. I can clearly remember being questioned excitedly in the hallway by our departmental mammalogist who had just received his weekly issue of *Science* and could not believe the data of Ralls and her colleagues.

The pedigree inbreeding coefficient (F_P) traditionally has been used to detect inbreeding depression (Pemberton 2008). F_P is the expected increase in homozygosity for inbred individuals relative to the base population (i.e., the founders of the pedigree). The actual proportion of an individual's genome that is identical by descent (IBD) can now be estimated directly by identifying continuous segments of the genome that are homozygous because of inbreeding (runs of homozygosity; Ceballos et al. 2018). This method is superior to the traditional use of pedigrees to estimate the proportion of the genome IBD for two primary reasons (Kardos et al. 2015). First, the estimation of F_P assumes that the founder individuals are unrelated and not inbred. This is often not the case in conservation. The second reason is that there is great variability in the actual proportion of the genome IBD for individuals with the same pedigree. The amount of this variability depends upon genome size and the amount of recombination. This variability can be seen clearly in Figure 4 which shows the simulated distribution of IBD in the zebra finch (*Taeniopygia guttata*) for matings between full sibs ($F_P = 0.25$; Knief et al. 2017).

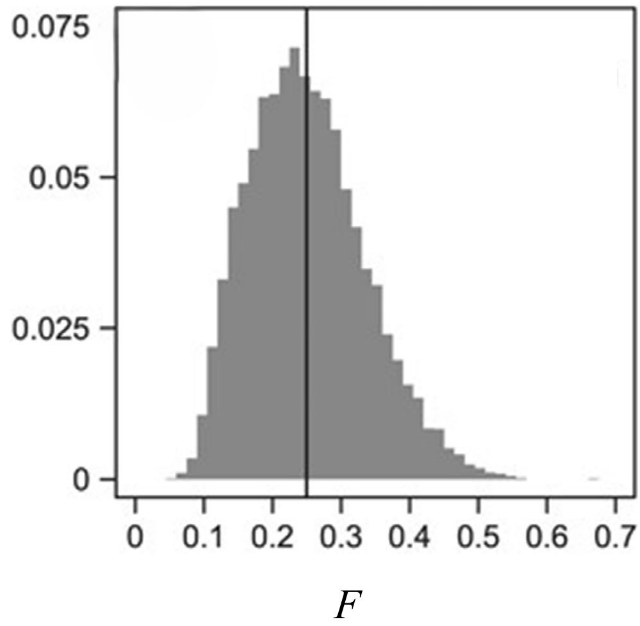

FIGURE 4 | Simulated variability in realised proportion of the genome IBD for progeny of matings between full sibs in the zebra finch (Knief et al. 2017). The black line indicates the pedigree inbreeding coefficient (Allendorf et al. 2022, 380).

The large variability shown in Figure 4 in the proportion of the genome IBD among individuals with the same pedigree was very surprising to me. Actually, this was known from theory for many years. Ian Franklin (of 50/500 fame discussed in Section 5.1) published a paper in 1977 showing the great variability expected in IBD among individuals with the same pedigree (Franklin 1977). I learned about this paper, and almost all I know about runs of homozygosity, from my former student and current colleague, Marty Kardos.

5 | Translating Research Into Application

I have been asked to consider my experience in applying genetics to practical problems in conservation. I suggest reading Chapter 24 of Allendorf et al. (2022). Helen Taylor, the primary author of that chapter, does an excellent job of presenting an overview of this topic. In addition, Mike Schwartz describes in a Guest Box his over 20 years' experience as a geneticist working for a government agency responsible for managing nearly 80 million hectares of public lands (US Forest Service, USFS).

I believe that I have been successful in applying genetics to conservation through close collaboration and support from management agencies. I have been fortunate that my primary taxa of research are also valued resources. As a graduate student, I was supported by the Washington State Department of Game to study the population genetics of steelhead (anadromous rainbow trout, *Oncorhynchus mykiss*). I provided the first study of the genetic relationships among populations of steelhead in the State of Washington, which was used to guide a number of management programmes (Utter and Allendorf 1977). I also helped develop an experiment that used allozymes to genetically mark fish to test the relative fitness of hatchery and wild fish in natural conditions (Chilcote et al. 1986). At the same time, my dissertation itself involved esoteric work on the inheritance of duplicated genes in salmonid fishes, which are derived from a tetraploid ancestor (Allendorf and Utter 1973).

I moved to the University of Montana in 1976, and I immediately began close collaboration with Montana Fish, Wildlife and Parks (MFWP). They supported a graduate student in my lab for many years, and eventually, an MFWP employee became a member of my lab group. Having an MFWP employee as a lab member greatly improved our ability to work effectively with MFWP. Previously, there were always communication issues between our lab and MFWP. Having a MFWP employee as a lab member solved these issues. In my experience, close interaction with management agencies is the key.

5.1 | Population Viability

The National Forest Management Act of 1976 required the USFS 'to maintain viable populations of existing native and desired non-native vertebrate species' on the land they managed. I remember getting a phone call from a regional manager for MFWP saying that he was told by the USFS that they interpreted this to mean there had to be at least one adult male and one adult female in a stream. He asked me to write a letter explaining why this was not a biologically sound interpretation of 'viable'. Unfortunately, I cannot find a copy of the letter that I wrote.

The question of how large populations must be to be genetically viable was first addressed by Ian Franklin (1980). He suggested that for an isolated population, the effective population size (N_e) should be greater than 50 to avoid the harmful effects of inbreeding depression in the short term, and N_e should be greater than 500 to maintain sufficient genetic variation to allow adaptation to environmental changes in the long term.

The appropriateness of the 50/500 rule has been debated (Jamieson and Allendorf 2012; Frankham et al. 2014; Franklin et al. 2014), but Franklin's original suggestion of 50/500 currently has strong support (Hoban et al. 2020; Laikre et al. 2020), and it remains a useful guideline for conservation practitioners. Several recent papers have recommended that the 50/500 rule be incorporated into the assessment procedures of the International Union for Conservation of Nature (IUCN) Red List of Threatened Species (e.g., Garner et al. 2020; Hoban et al. 2022).

The $N_e > 500$ guideline has played an important role in the conservation of grizzly bears (*Ursus arctos horribilis*) in the Greater Yellowstone area. This population has been completely isolated from other grizzly bear populations for over 100 years (Miller and Waits 2003; U.S. Fish and Wildlife Service 2023). The US Fish & Wildlife Service has failed in attempts to delist this population from the US Endangered Species Act (ESA) largely because of genetics. For example, a federal judge ruled in 1995 that the US Fish & Wildlife Service had failed to meet its ESA recovery planning obligation because they paid insufficient attention to connectivity and the risk of genetic isolation (Miller and Waits 2003). The primary genetic argument for not considering Greater Yellowstone grizzly bears recovered under the ESA is that they are isolated and their N_e is below 500 (Allendorf 2019).

6 | Final Thoughts

6.1 | Importance of Theory

A sound understanding of population genetics theory is essential for interpreting genomic data (Karl et al. 2012). I was fortunate to be trained by Joe Felsenstein. I highly recommend the text for Joe's population genetic course, which is available from his website (Felsenstein 2019). In the 1970s, population genetics courses consisted primarily of theory because there was so little empirical data available. Today, I am concerned that teaching the underlying theory has been overwhelmed by the need to present the enormous amount of empirical data.

Even the most comprehensive genomic data sets can be misleading if the underlying theory is not applied correctly. vonHoldt et al. (2024) described the population genetic structure of 85,195 'statistically neutral and unlinked' SNPs in the grey wolf (*Canis lupus*) from North America. I do not understand what is meant by 'unlinked'. Clearly, many of the 85,195 SNPs must be linked in a genome with 39 pairs of chromosomes. It appears that the criterion for being 'unlinked' was actually linkage disequilibrium, not linkage.

Kardos and Waples (2024) have pointed out several problems with the data interpretation of this paper. vonHoldt et al. (2024) estimated effective population size using an LD method which assumes sampling a single local random mating population. However, their estimates of N_e were based on lumping samples from across the western United States that had previously been shown to contain several genetically differentiated areas. Such admixture will generate LD and cause a downward bias in the estimated N_e. Based on their results, vonHoldt et al. (2024) make the important observation for conservation that heterozygosity in Rocky Mountain wolves rapidly declined between 1991 and 2020, and that N_e declined over the last 50 generations. The re-evaluation of Kardos & Waples brings these conclusions into question.

6.2 | Miscommunication

Population genetics is complicated, and clear communication is essential. One source of confusion is misnomers, which have concerned me throughout my career. They cause confusion and hinder clear communication (Manikat and Chopra 2023).

The clearest example of a misnomer in population genetics is '*linkage disequilibrium*'. This term is misleading because unlinked loci can be in LD. Things are complicated enough without using a misnomer that leads to additional confusion when considering multilocus models. The term gametic disequilibrium is a much more descriptive and appropriate term to use in this situation. See Box 10.1 of Allendorf et al. (2022) for the history of the use of linkage and gametic disequilibrium.

Not all miscommunication problems result from misnomers. Sometimes confusion results because the meaning of words changes over time. As discussed previously, the meaning of the term '*phylogeography*' has changed since its introduction. Its original meaning has been lost, so the term no longer has a clear meaning.

Mike Lynch et al. (1993) introduced the term '*mutational meltdown*' to refer to extinction of small populations caused by the effect of new, not standing, mutations that increase in frequency because in very small populations 'genetic drift completely overwhelms the ability of natural selection to eradicate incoming deleterious mutations, and the population goes extinct'. However, some papers now use mutational meltdown to include both standing and new mutations. In this sense, mutational meltdown is not different from inbreeding depression. I contacted Mike some years ago, and he said that he accepted this new broader definition.

Robin Waples and I were discussing vonHoldt et al. (2024) while I was preparing this paper. We ended up in a long back-and-forth exchange about the term '*linkage*' Robin defined linkage as two loci being on the same chromosome. I defined linkage as two loci that do not assort independently, and I made the point that the word '*syntenic*' was introduced to describe two loci that are on the same chromosome, whether or not they assort independently (Renwick 1971).

Novitski and Blixt (1978) discussed this issue and explained that my definition is the original one, but that linkage is now used by some to mean on the same chromosome. It is truly amazing that two people who have worked in population genetics for almost a combined 100 years disagree on the definition of such a basic term! And, to make things even more confusing, syntenic is now often used to refer to gene loci in different organisms located on a chromosomal region of common evolutionary ancestry (Passarge et al. 1999).

6.3 | Fini

My primary interest and research focus over the last 50 years has been the application of population genetics to conservation. I have operated under the guiding principle that some of the most interesting basic questions in population genetics are also the most important applied questions for conservation. My work on the fitness effects of mtDNA (i.e., the Mother's Curse) discussed in Section 4.1 is an example of this. In addition, we have published over ten papers in basic and applied journals on the adaptive significance of heterozygosity in populations (e.g., Allendorf and Leary 1986; Thelen and Allendorf 2001; Kardos et al. 2014). Operating in the contact zone between basic and applied research has been extremely rewarding for me and has contributed to the usefulness of my research in conservation.

Acknowledgements

I was extremely fortunate to have had Fred Utter and Joe Felsenstein direct my graduate program. I give special thanks to M.M. Hansen for his help throughout the preparation of this paper. Additional thanks to S.N. Aitken, J.C. Avise, C.S. Baker, B.W. Bowen, J.F.Y. Brookfield, W.C. Funk, W.S. Grant, S.B. Heard, M. Kardos, W.H. Lowe, M. Lynch, B.P. May, S.D. Miller, J.B. Mitton, C. Moritz, E. Nevo, L.H. Rieseberg, M.K. Schwartz, J.E. Seeb, P. Sunnucks, M.J. Wade, and R.S. Waples. I thank three not-so-anonymous reviewers for their helpful suggestions. I am grateful to my wife Diane Haddon for her helpful suggestions and support.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

The author has nothing to report.

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