

The Use of Population Genetics in Endangered Species Act Listing Decisions

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In recent years the federal agencies that administer the Endangered Species Act have increasingly relied on genetic data to decide which species and populations merit protection. Because the analysis of genetic data is highly technical and unfamiliar to the majority of those concerned with the Act, agency decisions are in danger of becoming less transparent, insulated by the language of genetics and the seeming surety of its associated statistics. In this paper, I attempt to provide a resource for lawyers and other non-biologists faced with understanding the genetics that underlie many modern claims under the Endangered Species Act. I do so by describing the types of data federal agencies routinely use and the analyses those data drive. I then scrutinize the agencies' treatment of the primary data in two particular agency decisions under the Act, and conclude with suggestions to improve the accessibility of this kind of data, making public input more effective. As technical data inform an ever larger percentage of decisions about species protection, this paper provides basic information to improve citizens' ability to understand and engage in the administration of the Endangered Species Act. The continued legitimacy of the agencies and the Act itself depend in part on such citizen engagement, and on the continued transparency of agency decisions.

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INTRODUCTION

Maintaining administrative transparency is increasingly difficult in the face of rapid technological specialization, as ever smaller fractions of the public are equipped to evaluate the details of a given proposed rule. One area of particular concern is the federal agencies' use of genetic data in response to an Endangered Species Act (ESA)¹ listing petition. As listing claims more frequently rely upon genetics to support taxonomic distinctions and identify discrete populations, genetic data analysis has played an increasingly critical role in applying the ESA.² Given the highly technical nature of these analyses, there is a danger that the listing process will become less transparent as genetics comes to the fore, burying reasoning in statistical jargon and disguising judgment calls as indisputable science.

Biological data can be thorough and persuasive, but data never speak for themselves. Contrary to pervasive perceptions of scientists as mere generators of unambiguous information, a critical portion of a scientist's job is interpreting patterns in the data. The ESA brings the human element of biological data into sharp focus by demanding that federal agencies make categorical decisions, such as whether or not a particular species' population is discrete and requires separate protection, based on continuous underlying data. In reality, biological populations exist on a continuum from completely isolated to completely connected, yet the ESA requires agencies to assign biological populations into discrete conservation categories.

Every ESA listing decision therefore requires the agency to exercise substantial judgment in weighing and interpreting the available data. To protect against arbitrary or unreasonable decisions, the Act itself includes some procedural safeguards: the requirement that agencies base decisions on the best available science,³ for example, and that they hold public comment periods before changing federal regulations.⁴

1. Endangered Species Act of 1973, 16 U.S.C. §§ 1531–1539 (2006).

2. For example, Sylvia M. Fallon, *Genetic Data and the Listing of Species under the U.S. Endangered Species Act*, 21 CONSERVATION BIOLOGY 1186 (2007), reviews final agency decisions on ESA claims based on genetic data between 1996 and 2006. The frequency of genetics-based claims per year during this period trend has an exponential best-fit line ($R^2=0.49$), *id.* at 1190, underscoring the common-sense suggestion that the trend will continue to accelerate in the future as the costs of generating genetic data continue to plummet.

3. See § 1533(b)(1)(A) (2006).

4. See § 1533(b). The Administrative Procedure Act, Pub. L. No. 79-404, 60 Stat. 237 (codified as amended in scattered sections of 5 U.S.C.), also provides protections in the form of public participation (through rulemaking requirements), and judicial review.

But these protections depend in large part on informed external oversight if they are to be effective. As in any area of administrative law, the ESA sets up a tension between agency expertise and independence on the one hand, and public accountability on the other. Without sufficient decision-making transparency to allow citizens to meaningfully challenge proposed rules, the ESA's procedural safeguards fail. Agency transparency thus increases the likelihood that agencies will make the necessary judgment calls in an informed and rational way, a hallmark of legitimate government action.

The process of moving from data collection to rulemaking necessarily sheds information with each step, as the biologists must simplify the raw data into conclusions, which the agency must simplify into rules.⁵ But preserving the information lost along the way is important both as part of the administrative record of the ESA listing decision (subject to judicial review under the Administrative Procedure Act (APA)),⁶ and as a way of promoting transparency in the administrative process.⁷ This transparency, in turn, reinforces the political legitimacy of the relevant agencies by increasing public participation and agency accountability.⁸

The challenge, then, is to maintain openness by documenting even fairly technical agency judgments, but to do so in a way that avoids burdening the two federal agencies that administer the ESA. Meeting this

5. See, e.g., Holly Doremus, *Science and ESA Listing Decisions*, 75 WASH. U. L. REV. 1029, 1079 (1997) (discussing the internal data-gathering and handling processes of the Fish and Wildlife Service).

6. See 5 U.S.C. § 706 (2006). *But see* *Ctr. for Biological Diversity v. Norton*, 336 F. Supp. 2d 1155, 1161–62 (D.N.M. 2004) (holding that scientific recommendations to the Fish and Wildlife Service regarding an ESA listing claim fell under the deliberative or mental process privilege, and thus could be protected from discovery during litigation).

7. See, e.g., Sara A. Clark, *Taking a Hard Look at Agency Science: Can the Courts Ever Succeed?*, 36 ECOLOGY L.Q. 317, 352 (2009) (“Increased transparency also opens up agency science to the scientific community, which, with a clearer picture of agency science, can better review and critique this information to the benefit of both the agency and the scientific community.”).

8. Professor Holly Doremus has written extensively on the need for transparency in agency science, and has suggested mechanisms for improving agency policies. See, e.g., Holly Doremus, *Scientific and Political Integrity in Environmental Policy*, 86 TEX. L. REV. 1601, 1646 (2008); Holly Doremus & A. Dan Tarlock, *Science, Judgment and Controversy in Natural Resource Regulation*, 26 PUB. LAND & RESOURCES L. REV. 1, 31 (2005) (“We suggest that both the scientific and policy advice of agency scientists should be available to the public. This could be achieved by requiring the various judgments of agency scientists to be included in the public record, but also structuring those evaluations to separate scientific from other judgments. Even if agency scientists are just as inclined as agency politicians to hide their political judgments, and just as skilled at doing so, mandating public release of their advice should help expose those judgments. Agency decision makers who must disclose internal scientific advice counter to their ultimate decision will face political and judicial pressures to explain the discrepancy. That will give them incentives to reveal the policy judgments both in their ultimate decision and in the recommendations of their scientists.”).

challenge will require the government and members of the public who engage with the ESA to share the burden of sustaining transparency.

On the part of the agencies, making genetic and other technical information functionally accessible requires that the agency data be both *available* for review by, and *meaningful* to, outside parties. Timeliness is essential to both prongs: if the relevant documents are not reviewable within the public comment period for a proposed rule, effective external review is impossible. A failure to make influential data publicly accessible risks undermining agency legitimacy and accountability because all consequential analysis takes place behind closed doors.⁹

Conversely, it is incumbent upon those who litigate, study, and apply the ESA to understand the basics of the relevant analytical tools that agencies use in making listing decisions in order to ensure meaningful external review of those data. Because an increasingly important part of the relevant analytical framework is genetic, appropriately interpreting agency actions will require practitioners to develop some familiarity with the language and methods of genetics.

This paper provides a baseline of information about genetic data and analysis in the context of the ESA, in an effort to make external review of agency data more effective. I begin with a brief review of the ESA and the relevant procedure for making listing decisions under the Act. Then, in Part II, I summarize the different kinds of genetic data that the two listing agencies have used to inform their decisions, and, in Part III, introduce the most common analyses of those data. In Part IV, I present two case studies of particular ESA listing decisions that illustrate different outcomes of the use of these data and analyses, with an emphasis on the agencies' treatment of the primary data. I close with some normative suggestions for improving agency transparency and ensuring a robust public participatory process in future listing decisions.

9. See *Portland Cement Ass'n v. Ruckelshaus*, 486 F.2d 375, 393 (1973) ("It is not consonant with the purpose of a rule-making proceeding to promulgate rules on the basis of inadequate data, or on data that, [in] critical degree, is known only to the agency."), *superseded by statute on other grounds*, 15 U.S.C. § 793(c)(1), *Am. Trucking Ass'ns, Inc. v. EPA*, 175 F.3d 1027, 48 (D.C. Cir. 1999); see also *United States v. Nova Scotia Food Prods. Corp.*, 568 F.2d 240, 251 (2d Cir. 1977) (reversing dismissal of challenge to agency ruling because "interested parties were not informed of the scientific data, or at least of a selection of such data deemed important by the agency, so that comments could be addressed to the data"); *Ober v. Envtl. Prot. Agency*, 84 F.3d 304, 314 (9th Cir. 1996) (holding agency improperly relied on information submitted after public comment period in making decision). *But see* *Ctr. for Biological Diversity v. Norton*, 336 F. Supp. 2d 1155, 1161–62 (D.N.M. 2004) ("[T]he Magistrate Judge thoughtfully analyzed the [relevant] factors . . . and concluded that the withheld materials . . . need not be disclosed because the extensive administrative record already supplied contained sufficient documentation of the listing decision.").

I. THE ROLE OF GENETIC DATA WITHIN THE STRUCTURE OF THE ENDANGERED SPECIES ACT

A. *The ESA and the Listing Process*

The United States' Endangered Species Act of 1973¹⁰ protects imperiled species from extinction with the goal of assisting their recovery to the point at which they no longer need such protection.¹¹ The Act also aims to protect the ecosystems that protected species depend on.¹²

Two federal agencies share responsibility for identifying endangered and threatened species and for promulgating and enforcing regulations to ensure the survival of listed species. The U.S. Fish and Wildlife Service (FWS, within the Department of the Interior) and the National Oceanographic and Atmospheric Administration (NOAA, under the Department of Commerce) are each responsible for listing species, with the agencies' jurisdictions divided according to the habitat of the individual species. NOAA contains within it the National Marine Fisheries Service (NMFS), which actually implements the ESA for its parent agency.¹³

Agencies list species as threatened or endangered by one of two routes. An agency may evaluate a species' status of the agency's own accord, or any citizen may petition the agency to begin the evaluation process.

When an agency begins a species evaluation on its own initiative, it follows the normal course of a notice-and-comment rulemaking.¹⁴ By contrast, the citizen petition route goes as follows: once a group or individual files a petition with the relevant agency, the agency has ninety days to respond with an assessment of whether the petition demonstrates a threshold amount of evidence warranting a full-scale analysis of the species' status.¹⁵ Roughly speaking, this ninety-day finding is analogous to the 12(b)(6) motion in civil procedure: the agency must decide if the petition warrants an in-depth investigation.

10. Endangered Species Act of 1973, 16 U.S.C. §§ 1531–1544 (2006).

11. *See id.* §§ 1531, 1532(3).

12. *See id.* § 1531(b).

13. The FWS and NMFS differ in that by default, FWS treats endangered and threatened species as equivalent for section 9 "take" purposes, while NMFS regulates section 9 take of threatened species on a species-by-species basis. *See* 16 U.S.C. § 1538 (2006); 50 C.F.R. §§ 17.2(b) (for NMFS), 17.31(a) (2010) (for FWS). Because much of the literature and case law on marine and anadromous species refers to the listing agency as "NMFS," I will use that terminology rather than "NOAA" for consistency. For most purposes, FWS and NMFS apply the ESA identically, and I will treat the two as interchangeable here unless specifically noted.

14. *See* 16 U.S.C. § 1533(b)(4) (2006).

15. *See id.* § 1533(b)(3)(A).

If the ninety-day petition meets the threshold, the agency then has twelve months to determine if listing the species is warranted, “solely on the basis of the best scientific and commercial data available.”¹⁶ At this step, the agency may determine that the listing is “warranted,” is “not warranted,” or is “warranted, but . . . precluded” by higher-priority species and limited resources.¹⁷ This third category functions as a waiting list for imperiled species, and the agency must reassess each species in the category each year.¹⁸ If a species is listed, it is classified as either “threatened” or “endangered.”¹⁹ Listing a species as “threatened” triggers identical protections as an “endangered” designation by default, though the listing agency may designate custom rules relaxing the prohibition on taking threatened—but not endangered—species.²⁰

The listing criteria are broad, giving the agencies wide discretion to determine whether listing a species is warranted.²¹ Because these listing decisions are subject only to the standard, under *Chevron v. Natural Resources Defense Council*, that they not be arbitrary and capricious,²² courts largely defer to agency listing decisions.²³ The rare exceptions occur when a plaintiff shows that the agency did not use the best available scientific data in making its decision, as mandated by the ESA.²⁴

FWS and NMFS can list three classes of entities as endangered or threatened: species, subspecies, and distinct population segments (DPSs).²⁵ Species and subspecies are traditional taxonomic distinctions generally accepted in the scientific community.²⁶ These are human constructs, and though biologists dispute their definitions,²⁷ the federal agencies defer to taxonomic²⁸ designations in common scientific usage.²⁹

16. See *id.* § 1533(b)(1)(A).

17. See *id.* § 1533(b)(3)(B)(iii).

18. See *id.* § 1533(b)(3)(C)(i) (treating warranted-but-precluded finding as equivalent to a new petition for listing, which must be acted upon within 12 months).

19. See *id.* § 1531(a).

20. See 16 U.S.C. § 1533(d) (allowing Secretary’s discretion in making regulations to protect threatened species); see also *supra* note 13.

21. See 16 U.S.C. § 1533(a)(1)(A)–(E) (2006).

22. See *Chevron v. Natural Res. Def. Council*, 467 U.S. 837, 844 (1984).

23. See, e.g., *Home Builders Ass’n of N. Cal. v. U.S. Fish & Wildlife Serv.*, 529 F. Supp. 2d 1110, 1117 (N.D. Cal. 2007) (granting deference to agency in listing California tiger salamander as threatened).

24. See 16 U.S.C. § 1533(b)(1)(A); see, e.g., *Ctr. for Biological Diversity v. Lohn*, 296 F. Supp. 2d 1223, 1238 (W.D. Wash. 2003) (“When the best available science indicates that the ‘standard taxonomic distinctions’ are wrong . . . NMFS must apply that best available science.”), *vacated on other grounds*, 511 F. 3d 960 (9th Cir. 2007).

25. See 16 U.S.C. § 1532(16).

26. See *id.* (“The term ‘species’ includes any subspecies of fish or wildlife or plants, and any distinct population segment of any species of vertebrate fish or wildlife which interbreeds when mature.”). This definition corresponds with the “biological species concept,” one commonly-used idea in the scientific community for delineating species.

27. The scientific literature surrounding species concepts is voluminous, and features overlapping and conflicting ideas of what a species is. For reviews of the debate, see, e.g., Joel

DPSs, by contrast, are more legal constructs than biological. Originally applicable to any species, the 1978 ESA amendments restricted the DPS category to vertebrates alone.³⁰ The DPS designation offers protection to imperiled, isolated populations of a species, though the entire species itself may not be in danger of extinction. The DPS is the main area of overlap between population genetics and the ESA: while taxonomic species and named subspecies tend to be genetically distinct from all other such groups, and thus easier to diagnose, populations within a species exist in a continuum from highly isolated to completely connected. Population-level genetic data are hugely valuable to the listing agencies because they bear on the degree of discreteness of a population.

Confusingly, once an entity is listed, it becomes a “species” for the purposes of the ESA whether it was originally listed as a taxonomic species, subspecies, or DPS. Where necessary for clarity, I thus distinguish between a “taxonomic species” (a species with a generally accepted Latin binomial, such as the cougar, *Puma concolor*), and an “ESA species,” which is by definition any listed entity. Once listed, all such entities are entitled to the same legal protections, and the listing agency must review the status of each every five years.³¹

A hybrid concept called the evolutionarily significant unit (ESU) links the biological concepts of the population and the species to the legal concept of the DPS.

B. *The Evolutionarily Significant Unit and the Distinct Population Segment*

The ESU concept arose not out of taxonomy, law, or biological systematics, but rather as a practical response to the particular challenges of conservation.³² Robin S. Waples codified the ESU in the administrative context in 1991, defining it as a population unit that, first, “[i]s substantially reproductively isolated from other conspecific population

Cracraft, *Species as Entities of Biological Theory*, in WHAT THE PHILOSOPHY OF BIOLOGY IS 31 (Michael Ruse ed., 1989); Jody Hey, *The Mind of the Species Problem*, 16 TRENDS IN ECOLOGY & EVOLUTION 326 (2001); Kevin de Queiroz, *Ernst Mayr and the Modern Concept of Species*, 102 PROC. NAT'L ACAD. SCI. 6600 (2005).

28. Here “taxonomic” refers to the system of scientific names used to classify species. The example I use in the main text, *infra*, is *Puma concolor*, commonly known as the cougar.

29. The ESA, after all, is the Endangered Species Act, presumably written under the assumption that species are discrete and real entities. For a discussion of how species definitions impact ESA implementation, see generally Anna L. George & Richard L. Mayden, *Species Concepts and the Endangered Species Act: How a Valid Biological Definition of Species Enhances the Legal Protection of Biodiversity*, 45 NAT. RESOURCES J. 369 (2005).

30. See H.R. REP. NO. 95-1804, at 2 (1978) (Conf. Rep.); Endangered Species Act Amendments of 1978, Pub. L. No. 95-632, § 2, 92 Stat. 3751, 3752 (1978).

31. See 16 U.S.C. § 1533(c)(2) (2006).

32. See Oliver A. Ryder, *Species Conservation and Systematics: The Dilemma of Subspecies*, 1 TRENDS IN ECOLOGY & EVOLUTION 9 (1986).

units,” and, second, “[r]epresents an important component in the evolutionary legacy of the species.”³³ Though the biological literature has elaborated the ESU into several related concepts, Waples’s 1991 definition has remained in force at NMFS.³⁴

Genetically unique and isolated populations represent independent evolutionary units that contribute important diversity to the species as a whole, and thus merit individual protection. Genetic data plainly underlie the ESU; such information can simultaneously estimate the degree of reproductive isolation and evolutionary distinctiveness. Genetic data are not, however, a prerequisite for ESU identification. If direct observation or geographic separation indicates reproductive isolation and evolutionary distinctiveness, for example, the agency can designate an ESU with no genetic data at all.³⁵

NMFS adopted the ESU concept in 1991, marrying the ESU to the Endangered Species Act in a formal sense.³⁶ In a policy that originally applied only to Pacific salmonid fishes, the agency made the DPS equivalent to the ESU by declaring, “A salmon stock will be considered a distinct population, and hence a ‘species’ under the ESA, if it represents an evolutionary [*sic*] significant unit (ESU) of the biological species.”³⁷ The policy then defined the ESU using Waples’ definition above.³⁸

The idea of defining a DPS operationally via the ESU criteria gained traction, and in 1996 FWS and NMFS issued a joint notice applying a similar policy to all vertebrates.³⁹ Though the term “ESU” does not itself appear in the joint policy, the agencies use the substantive ESU criteria to diagnose distinct population segments, requiring a DPS to be discrete, significant, and either threatened or endangered in order to be eligible for listing.⁴⁰

The policy defines a population’s discreteness by “marked separation” from other such populations.⁴¹ Several intrinsic factors, including genetic or morphological distinctiveness, may be used as

33. Robin S. Waples, *Pacific Salmon, Oncorhynchus spp., and the Definition of “Species” under the Endangered Species Act*, 53 MARINE FISHERIES REV. 3, 12 (1991).

34. See Dylan J. Fraser & Louis Bernatchez, 10 MOLECULAR ECOLOGY 2741, 2742 (2001); Robin S. Waples, *Distinct Population Segments*, in 2 SCOTT ET AL., THE ENDANGERED SPECIES ACT AT THIRTY 127 (2006).

35. See Waples, *supra* note 33, at 15 (citing phenotypic and life history traits as relevant to the distinctness inquiry).

36. See Policy on Applying the Definition of Species under the Endangered Species Act to Pacific Salmon, 56 Fed. Reg. 58,612 (Nov. 20, 1991).

37. *Id.* at 58,612.

38. *Id.*

39. See Policy Regarding the Recognition of Distinct Vertebrate Population Segments under the Endangered Species Act, 61 Fed. Reg. 4722 (Feb. 7, 1996) [hereinafter DPS Policy].

40. See *id.* at 4725.

41. *Id.* at 4725.

evidence of a population's degree of discreteness.⁴² If discrete, the agency will then evaluate the population's "importance to the taxon to which it belongs"⁴³ to determine its significance.⁴⁴ Among other factors the agency may consider in determining significance is the degree of genetic difference between the focal population and the rest of the species.⁴⁵

Genetic data thus play a critical role in defining DPSs, which, as noted above,⁴⁶ receive the same protection as full taxonomic species under the ESA. Genetically distinct populations may simultaneously satisfy the discreteness and significance prongs of the DPS policy,⁴⁷ becoming eligible for listing so long as they are themselves endangered or threatened.

C. Genetic Data and the Endangered Species Act

The goal of the ESA is the long-term survival of species.⁴⁸ "[E]volutionary flexibility is essential for long-term survival. Thus, the preservation of diversity at the species level is intrinsically dependent on the maintenance of genetic diversity *within* species."⁴⁹

Hence, the ESA's success depends upon protecting not just the taxonomic species in danger of extinction, but also the vast amount of biological diversity species' distinct populations contain. Such diversity is

42. *See id.* Populations may also be discrete by virtue of existing on either side of an international boundary. *See id.*

43. *Id.* at 4725.

44. Here, "taxon" refers to the entire named, taxonomic species. Note that in the scientific literature, "taxon" refers more generally to a named unit of biological organization, whether a species, genus, family, etc. The INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE, the formal rules for naming animals, provides a yet more general definition: "A taxonomic unit, whether named or not: i.e. a population, or group of populations of organisms which are usually inferred to be phylogenetically related and which have characters in common which differentiate (q.v.) the unit (e.g. a geographic population, a genus, a family, an order) from other such units. A taxon encompasses all included taxa of lower rank (q.v.) and individual organisms." *Glossary*, INTERNATIONAL CODE OF ZOOLOGICAL NOMENCLATURE, <http://www.nhm.ac.uk/hosted-sites/iczn/code/index.jsp?booksection=glossary&nfv=true> (last visited Oct. 3, 2010).

45. *See* DPS Policy, *supra* note 39, at 4725.

46. *See supra* Part I.A.

47. *See* DPS Policy, *supra* note 39, at 4725. Note that a population may be discrete but not significant, even in the face of genetic data. *See, e.g.*, Twelve-Month Finding on a Petition to List the Coaster Brook Trout as Endangered, 74 Fed. Reg. 23,376, 23,386 (May 19, 2009) (to be codified at 50 C.F.R. pt. 17) (noting that while the "coaster brook trout are a discrete population segment," this population segment is not significant to the larger brook trout taxon because the coaster brook trout "co-occur with and are a subset of the same population as other brook trout types (stream residents) in the upper Great Lakes"); *infra* Part IV.B (discussing the cactus pygmy owl).

48. *See* NAT'L RESEARCH COUNCIL, SCIENCE AND THE ENDANGERED SPECIES ACT 8 (1995).

49. *Id.* at 184 (emphasis added).

critical to ecosystem function and resilience,⁵⁰ two characteristics inherently required for ecosystem conservation, the first aim of the ESA.⁵¹

Genetic data provide a high-resolution assessment of this population-level biological diversity. In administering the ESA, the agencies employ genetics in at least three capacities: to substantiate existing taxonomic species as distinct from other such species; to evaluate the degree to which a vertebrate population is discrete and significant and thus constitutes a DPS; and as evidence that a species is endangered or threatened with extinction. Because the second of these categories is by far the most common use of genetic data, I will focus on DPS listing claims, but will also use examples from the other two categories where helpful.

Those engaged with the ESA must have a basic grasp of the science animating DPS claims in order to understand and to participate actively in the listing process. My goal here is to provide a baseline of information for interpreting the primary literature that underlies agency listing decisions and species status reviews. Armed with this paper as a reference, an interested reader can begin to evaluate the quality of the data independently, and make his or her comments on agency actions more effective and productive.⁵²

II. CATEGORIES OF GENETIC DATA

A. Basic Molecular Biology

Nearly every cell of every living thing contains deoxyribonucleic acid, DNA, the genetic blueprint from which biological complexity springs. Genetics is the study of DNA, inheritance, and the effects thereof. The vocabulary surrounding the discipline can be daunting, and a

50. See generally Gary W. Luck, Gretchen C. Daily & Paul R. Ehrlich, *Population Diversity and Ecosystem Services*, 18 *TRENDS IN ECOLOGY AND EVOLUTION* 331 (2003); Thorsten B.H. Reusch & A. Randall Hughes, *The Emerging Role of Genetic Diversity for Ecosystem Functioning: Estuarine Macrophytes as Models*, 29 *ESTUARIES & COASTS* 159 (2006); Thomas Elmqvist et al., *Response Diversity, Ecosystem Change, and Resilience*, 1 *FRONTIERS IN ECOLOGY & ENV'T* 488 (2003).

51. See 16 U.S.C. § 1531(b) (2006) (“The purposes of this Act are to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved . . .”).

52. Though more accessible information can make science-based policy judgments more politicized, see generally Daniel Sarewitz, *How Science Makes Environmental Controversies Worse*, 7 *ENVTL. SCI. & POL'Y* 385 (2004), greater transparency in decision making surely helps to identify the agency value judgments underlying some decisions.

review of the most basic tenets of molecular biology is worthwhile before delving into its applications in the ESA.⁵³

DNA is a long molecule, made up of a sequence of *nucleotides*, which are also known as *base pairs* or *bases*. Four types of nucleotides—abbreviated “A,” “C,” “G,” and “T”—make up DNA’s genetic code;⁵⁴ their sequence determines much of the variation we see in the world around us. Collectively, the genetic sequence of an organism is its *genome*.

DNA consists of many regions of known function (*genes*),⁵⁵ each of which plays a role in producing a protein product. These protein products, in turn, make up much of what we think of as an individual organism.⁵⁶ Interspersed throughout the genome is a huge amount of DNA sequence of thus-far-little-known function; for the present purposes, what is important is that not all of the genome produces identified protein products.

Most DNA is found in the nuclei of cells. Animals and plants, the organisms covered by the ESA, also have DNA in their mitochondria, which are the main energy-packaging components of the cell. In addition, plants also have DNA in their chloroplasts, the components that function in photosynthesis. Small differences in these auxiliary genomes—those in the mitochondria and chloroplasts—are often useful for population-level study of the kind relevant for ESA determinations.

Most sexually-reproducing organisms receive one copy of each gene from each of their parents, resulting in a twofold, or *diploid*, genome. This describes the organism’s main genome, contained in the nucleus of the cell. However, the auxiliary genomes of the mitochondria and chloroplast are *haploid*, containing only a single copy of each gene and reflecting inheritance from only one parent. Haploid and diploid data have different applications and to some extent follow evolutionarily distinct paths, as discussed in more detail below.

Mutations are random changes that occur in DNA sequences, usually as a result of imperfect DNA replication. For the present purposes, it is important to know that most mutations are *silent*—that is, they do not cause a change in the protein product, even though the sequence of the underlying gene has changed.⁵⁷ Non-silent (also known as

53. A standard (and good) biology textbook that goes into more detail is NEIL A. CAMPBELL ET AL., *BIOLOGY* (9th ed. 2007). A great population genetics reference is DANIEL L. HARTL & ANDREW G. CLARK, *PRINCIPLES OF POPULATION GENETICS* (4th ed. 2007).

54. These letters stand for adenine, cytosine, guanine, and thymine.

55. Genes occur in discrete places on the DNA strand; such genes and other identifiable regions in DNA are often known by the Latin *loci* (singular: *locus*, meaning “place”).

56. Substances as diverse as enzymes, hemoglobin, structural elements of cells, and the active portions of muscles are all different proteins.

57. This is possible because the genetic code is redundant: there is more than one way to make an amino acid, the building blocks of proteins. Silent mutations simply change the

nonsynonymous or *coding*) mutations, by contrast, do change the gene's protein product, and thus are subject to natural selection. The ratio of silent to non-silent mutations is an indicator of the extent to which a gene region is subject to selection, and is therefore useful for identifying adaptive or maladaptive gene regions. Importantly for conservation biology, existing statistics to estimate gene flow between populations must use *neutral* markers (that is, gene regions with largely silent mutations, not under selection.)

Below, I describe the most common types of genetic data that appear in the ESA literature. These kinds of markers each make use of the differences between individuals in DNA sequence (or in the case of allozymes, protein product) to inform the researcher about the demographic and evolutionary forces impacting the population or the species.

B. Allozymes

Allozymes, short for "allelic enzymes," were among the first molecular markers used for measuring population differentiation. Appearing in the 1970s and becoming common by the 1980s,⁵⁸ allozymes are different forms of the same protein, encoded by different versions of the same gene. For example, an individual may have one gene encoding enolase, an enzyme that functions in making usable energy in animal cells. Most gene sequences vary to some degree, and enolase is no exception: within a species, different versions of the gene exist, with individual animals having one or two different versions. These are different *alleles* of the enolase gene. Being diploid, I have two copies of the enolase gene, one from each parent. Those copies might be identical, in which case I have two copies of the same allele. If each of my parents passed on different versions of the gene, I have two different enolase alleles.

Different alleles at the DNA sequence level may produce different forms of the enolase enzyme protein product. It is these protein products that biologists then count as data; by recording the frequency of the different versions of the enzyme (alleles) in different populations of a species, biologists can estimate the level of migration⁵⁹ among a species'

underlying DNA sequence to a slightly different sequence that nevertheless encodes the same amino acids, resulting in the same protein. Silent mutations are also known as *synonymous* mutations.

58. A search on the BIOSIS database, ISI WEB KNOWLEDGE, <http://isiknowledge.com/BIOSIS> (last visited Nov. 3, 2010), reveals only 336 papers in the 1970s with the terms "allozyme" or "allozymes" in the title; the 1980s yielded 1777 such papers.

59. In fact, allele frequency differences imply a more complicated variety of evolutionary phenomena. For the present purposes, migration is equivalent to gene flow among populations, and is the most important demographic parameter in evaluating a DPS. Note that allelic

populations, among other relevant biological information.⁶⁰ A century of genetics work has resulted in equations that predict the number of migrants per generation between two populations, based upon the differences in allele frequencies between the populations.⁶¹ Knowing how many individuals any two populations exchange is precisely the kind of information relevant for determining how discrete a DPS might be under the ESA.

Allozymes have been used widely in conservation biology and are featured in a variety of ESA listing claims.⁶² The use of allozymes has subsided, however, as DNA sequencing has become more affordable and simpler, because DNA sequence data provides vastly more information than allozymes. Whereas a single locus (say, again, enolase) provides only one allozyme data point—an individual diploid organism can only have one or two forms of the enzyme—the DNA sequence of the enolase locus consists of thousands of nucleotides, each of which might vary between individuals.

Consequently, allozymes offer a coarse-grain view of genetic diversity and demographics across a landscape. Researchers generally employ multiple allozyme loci simultaneously—say, looking at enolase alongside fifteen other genes—to improve resolution by gathering more data. Yet even the largest allozyme study will typically feature fewer potential alleles than a similar study featuring DNA sequences.⁶³ Thus, a study based on allozymes can miss fine-grain genetic differences between individuals and between populations, failing to detect patterns that higher-resolution tools would not.⁶⁴

diversity within and among populations may also inform a species' endangered or threatened status: as populations become smaller, they very quickly lose diversity. See DANIEL L. HARTL, A PRIMER ON POPULATION GENETICS 95 (3d ed. 2000).

60. Note that I have elided the difference between sequence alleles (that is, different versions of DNA sequences encoding a given protein) and protein alleles (that is, different protein versions resulting from underlying DNA sequence differences). This distinction is important generally, but not for the purposes here.

61. See, e.g., HARTL, *supra* note 59.

62. See Fallon, *supra* note 2 (analyzing the frequency with which different data types have been used in recent ESA decisions).

63. A representative larger-scale allozyme study, for example, bore on the delisting of the Douglas white-tailed deer DPS, which featured a presumptive thirty-five loci. See Final Rule to Remove the Douglas County Distinct Population Segment of Columbian White-Tailed Deer from the Federal List of Endangered and Threatened Wildlife, 68 Fed. Reg. 43,647 (July 24, 2003) (to be codified at 50 C.F.R. pt. 17) (citing Thomas Gavin & Bernie May, 52 J. WILDLIFE MGMT. 1 (1988)). Of these thirty-five, only seven were variable and informative for the focal species. See Gavin & May, *supra*, at 1. By contrast, even the smallest modern study of DNA sequence data will include several hundred nucleotides. Because each nucleotide site might vary between individuals, DNA sequence data generally provides an opportunity for much higher-resolution data than allozymes, though in practice, even DNA sequence data can be invariant.

64. See, e.g., Alec J. Jeffreys, *Genetic Fingerprinting*, 11 NATURE MED. 1035, 1036 (describing a case in which allozyme evidence could establish *some* familial tie between

C. *Restriction Fragment Length Polymorphisms*

Some naturally occurring enzymes function to cut DNA molecules, fragmenting the otherwise very long-chain molecules into smaller pieces up to several thousand nucleotides long. These enzymes, known as *restriction enzymes*, sever the DNA at known sequence identities; for example, one may cut DNA at the sequence "GATC" and only at that sequence. Another may cut at a different sequence ("TCA," for example).

Such enzymes are commercially available and are useful for distinguishing alleles based on differences in their DNA sequences. A restriction enzyme will cut different sequence alleles at different locations, resulting in fragments of differing lengths: if, for example, a mutation changes the sequences from "GATC" to "GGTC," the restriction cut site will disappear, and the enzyme will leave the DNA strand intact. Researchers may use enzymes to cut individual known genes (for example, to measure variability among individuals with respect to the enolase gene based on the resulting number of fragments), or in less targeted ways (for example, digesting the entire mitochondrial genome with one or more restriction enzymes and using the resulting fragmentation pattern to distinguish among individuals or populations). This technique is known as restriction fragment length polymorphism (RFLP).

The wide availability of DNA sequencing has largely supplanted RFLP analysis for the same reasons it has replaced allozymes: DNA sequences provide higher-resolution data with many more uses.⁶⁵ To use the enolase locus as an example, a hypothetical restriction enzyme might sever a 1000 base pair (bp)⁶⁶ piece of the gene into a maximum of three fragments. The presence or absence of cut sites will define the RFLP alleles: zero cut sites (one fragment), one cut site (two fragments), or two cut sites (three fragments).⁶⁷ Applying several restriction enzymes (cutting at different sequences simultaneously) will increase the number of potentially resolvable alleles, but an RFLP of a given locus will still

individuals in an immigration and parentage dispute, but DNA evidence was required to establish a parent-child relationship specifically).

65. See *Endangered Status for the Peninsular Ranges Population Segment of the Desert Bighorn Sheep in Southern California*, 63 Fed. Reg. 13,134 (Mar. 18, 1998) (to be codified at 50 C.F.R. pt. 17) (discussing RFLP versus DNA sequence data).

66. "Base pairs" is often abbreviated "bp," such that a sequence of DNA 350 base pairs (nucleotides) long is abbreviated "350 bp."

67. We may complicate the picture by hypothesizing that different DNA sequences will result in cut sites occurring in different positions along the fragment, as often happens. This results in different alleles having not only different numbers of cut sites (and thus resulting fragments) but also different sizes of resulting fragments. Nevertheless, the point stands: a RFLP results in far fewer potential alleles than the underlying DNA sequence data themselves.

offer far lower resolution than the 1000bp DNA sequence of the same locus.

Amplified Fragment Length Polymorphism (AFLP) analysis is a related technique, which *amplifies* (creates many copies of) the fragments of DNA generated in the RFLP analysis.⁶⁸ AFLP makes identifying genetic differences between analyzed individuals more precise, and makes a greater degree of automation possible. As in the case of allozymes, RFLP and AFLP are useful techniques for identifying dramatic differences between populations or individuals, but they lack the sensitivity of the higher-resolution tools described below. However, these tools can be cost-effective and accurate diagnostic tools—distinguishing species with known RFLP differences, for example—and are perfectly appropriate if used within their limits.

D. DNA Sequence Data and Related Data Sources

While allozyme and RFLP data can be powerful and informative, most current genetics research features DNA sequence data or subsequent technologies. As noted above, differences in DNA sequence underlie the differences researchers may observe in allozyme frequencies or RFLP fragmentation patterns. Instead of relying on these proxies for primary sequence differences, DNA sequencing provides much higher-resolution data by determining the sequence of DNA's constituent nucleotides themselves.⁶⁹ This set of techniques allows researchers to read along portions of an organism's DNA strand, resulting in data in the form of a string of nucleotide characters (e.g., "ATTACGTCCG . . .") hundreds or thousands of base pairs long. Each of those nucleotides might vary between individuals within a species, though in practice the majority do not. As one would expect, more closely related individuals generally have fewer changes distinguishing them while distantly related species have more highly divergent sequences.

68. See generally Pieter Vos et al., *AFLP: A New Technique for DNA Fingerprinting*, 23 NUCLEIC ACIDS RES. 4407 (1995) (describing the technique).

69. DNA sequencing technology was developed in the late 1970s, and became widely available in the late 1980s and early 1990s. See F. Sanger, S. Nicklen & A.R. Coulson, *DNA Sequencing with Chain-terminating Inhibitors*, 74 PROC. NAT'L ACAD. SCI. U.S.A. 5463 (1977). Sanger won one of his two Nobel Prizes in Chemistry for his work in developing DNA sequencing technology. See Biography of Fredrick Sanger, SANGER INST., <http://www.sanger.ac.uk/about/people/fsanger.html> (last visited Oct. 31, 2010). BIOSIS lists over 64,000 citations to the 1977 paper.

Newer, high-throughput technologies are now becoming widespread and will greatly increase the resolution of available data by sequencing orders of magnitude more DNA and even whole genomes. See, e.g., Jay Shendure & Hanlee Ji, *Next-generation DNA Sequencing*, 26 NATURE BIOTECHNOLOGY 1135 (2008) (reviewing sequencing techniques now becoming available).

The three types of data I introduce below differ from allozymes and RFLP analysis in that they draw on this fundamental DNA sequencing technology.

E. Microsatellites and Minisatellites

Microsatellites are short, repeated nucleotide units that are highly variable with respect to the number of repeats. For example, a microsatellite might consist of the DNA sequence “CA,” repeated a variable number of times: one individual may have “CACACACA” (usually represented as “(CA)₄,” with the subscript indicating the number of repeated units), while another has “CACACACACA” (“(CA)₅”) at the same genetic position. Whereas some regions of DNA have mutation rates that generate alleles only over thousands of generations, microsatellite loci are subject to rapid mutations, creating many alleles within a population and allowing researchers to track the movement of individuals and alleles over short timescales. Because they make it easy to identify individuals that may have originated in a different population, microsatellites are popular for calculating estimated rates of migration and population subdivision within a species. However, precisely because of their very high and variable mutation rates, microsatellites are generally not appropriate for estimating evolutionary relationships among individuals or species.⁷⁰ High mutation rates and many alleles tend to obscure or swamp out deeper evolutionary relationships that are apparent in slower-mutating gene regions. As a general rule, gene regions with slow mutation rates preserve more ancient relationships and are therefore useful for determining relationships among species or groups of species; regions with faster rates are useful for addressing shorter-term questions, such as how often populations exchange migrants.

Minisatellites are the same as microsatellites in concept, except that the repeated units are longer, up to 100 nucleotides in length.

70. See, e.g., Naoko Takezaki & Masatoshi Nei, *Empirical Tests of the Reliability of Phylogenetic Trees Constructed with Microsatellite DNA*, 178 GENETICS 385 (2008) (showing that a very large number of microsatellite loci are required to estimate evolutionary relationships with anything approximating accuracy). For example, to correctly infer a set of evolutionary relationships using F_{ST} , see *infra* Part III.C.1 (discussing Wright’s F Statistics), one would need 700 microsatellite loci. See Takezaki & Nei, *supra*, at 389, tbl.2. Even then, the analysis would result in the correct relationships only 90.4 percent of the time. See *id.* For more realistic datasets of ten microsatellite loci, the same statistic correctly inferred the evolutionary relationships in 2.9 percent of trials. See *id.* As a guiding principle, then, microsatellites are not appropriate for inferring relationships among samples. Nevertheless, authors commonly use microsatellites in this way, despite their demonstrated failures.

F. Mitochondrial DNA Sequences

The most common type of genetic data relevant to ESA listing claims is mitochondrial DNA (*mtDNA*) sequence data. The mitochondrion, found inside most cells, contains one small, circular *chromosome*⁷¹ encoding a handful of genes.⁷² This genetic material is largely independent of the nuclear genome, which contains the vast majority of genetic information.⁷³ Mitochondrial DNA is easily sequenced,⁷⁴ has gene regions with a variety of mutation rates,⁷⁵ and is well understood, making it a convenient target for population geneticists.

The most important drawback of mtDNA is that it is haploid (containing only one set of genetic information), because organisms inherit mtDNA from only one parent. Therefore the marker cannot be used to test for departures from Hardy-Weinberg equilibrium, a traditional measure of evolutionary forces acting on a population.⁷⁶ Nevertheless, mtDNA is extremely useful as an indicator of population subdivision or isolation because mtDNA sequences offer a high-resolution picture of genetic differences across the sampled landscape, making it a reasonably sensitive means of testing for the kind of genetic distinctiveness that is both necessary for an ESA determination and ecologically relevant. As a result, many candidate species for listing feature mtDNA data.⁷⁷

71. Chromosomes are dense aggregations of nucleic acids and proteins that form coherent rod-like structures in the nucleus of eukaryotic cells (that is, cells having a nucleus), and circular structures in bacteria and eukaryotic organelles such as mitochondria.

72. The human mitochondrion, for example, contains thirteen protein-coding genes, two moderately-sized regions encoding ribosomal RNAs, and twenty-two small regions encoding transfer RNAs. See Anderson et al., *Sequence and Organization of the Human Mitochondrial Genome*, 290 NATURE 457 (1981). Other species' mitochondria are similar in size and composition.

73. The human mitochondrion contains about 16,500 bp. See *id.* at 457. By comparison, the human nucleus contains over three billion base pairs. See *The Human Genome Project Completion: Frequently Asked Questions*, NAT'L HUMAN GENOME RES. INST., <http://www.genome.gov/11006943> (last visited Oct. 3, 2010).

74. This is for at least two reasons: one, a lot of existing (known) sequence information exists from which to base amplifications, and two, mtDNA is haploid, containing only one copy of the target gene. Because only one copy is present, it is simple to read clean sequence data from the mitochondrion. Where multiple, different copies of genes exist, conflicting sequence reads can result.

75. A variety of mutation rates in different gene regions makes the mitochondrion rather like a buffet: depending on the timescale of interest, a researcher may collect data from gene regions with faster or slower rates. Faster rates tend to inform shorter-timescale questions. Mutational rates vary within the mitochondrion over about an order of magnitude. See JOHN C. AVISE, MOLECULAR MARKERS, NATURAL HISTORY, AND EVOLUTION 123 (2d ed. 2004) (reviewing relevant literature).

76. See *infra* note 88 (discussing Hardy-Weinberg equilibrium).

77. For example, see the case studies of the beluga whale and pygmy owl, *infra* Part IV.

G. Nuclear DNA Sequences

The cell's nucleus contains the vast majority of genetic information in plants, animals, and fungi. In humans, for example, the nucleus contains over three billion nucleotides of DNA, organized into pairs of linear⁷⁸ chromosomes. Other organisms have nuclear genomes several times this size;⁷⁹ the genome is an enormous cache of information that scientists are only beginning to grasp. For technical and historical reasons,⁸⁰ the amount of nuclear sequence data⁸¹ in the population genetics literature is small relative to that of mtDNA.

Next-generation sequencing technology has recently caused the costs of large-scale sequencing to plummet, though these critical advances have yet to impact the population-level studies that inform ESA claims. Over the coming years, it may be that genome scans of candidate endangered species become standard. For the immediate future and recent past, however, nuclear DNA sequences have had and will continue to have only limited use in ESA claims.

When it has been used, nuclear DNA has served largely the same functions as mtDNA sequence data: reinforcing species delineations or probing the degree of differentiation among populations of a named species. The primary relevant distinction between mtDNA and nuclear DNA sequences is that whereas mtDNA is inherited from only one parent (usually passed exclusively from mother to offspring), both parents pass on nuclear DNA to the offspring. This makes parentage analysis possible through nuclear DNA analysis and provides a more accurate estimate of gene flow among populations, in some cases even allowing researchers to infer the origins of recent migrants to a population,⁸² or to detect hybridization events between species. Such precision is highly relevant to imperiled species, which often have low and declining population sizes, and is especially critical for captive

78. As opposed to the circular chromosome of the mitochondrion and of free-living bacteria.

79. The largest known genome has over 670 billion nucleotides, and belongs to a lowly amoeba-like single-celled animal. See Laura Wegener Parfrey, Daniel J. G. Lahr & Laura A. Katz, *The Dynamic Nature of Eukaryotic Genomes*, 25 MOLECULAR BIOLOGY & EVOLUTION 787 (2008). Clearly, genome size does not scale with an organism's complexity.

80. Technical reasons include multiple copies of the same gene in the nucleus and the difficulty of sequencing different alleles of the same gene simultaneously. Historical reasons include the wide availability of mitochondrial *primers*—critical tools in the PCR and sequencing processes that are based on known sequence data. Hence, there is a positive feedback loop with molecular data; once some sequence information is available, it is easier to focus on the known gene region than to look elsewhere in the genome for data.

81. I distinguish between *sequence* data (that is, the actual sequence of nucleotides in the genetic code) and other forms of data because nuclear DNA is widely used in the other data types I discuss here. Microsatellites and RFLPs, for example, are most often expressions of nuclear DNA data, but do not result in actual *sequence*-level information.

82. See *infra* Part III.A.1 (discussing individual-focused approaches).

breeding programs that aim to resuscitate species on the brink of extinction.⁸³

A second distinction between mitochondrial and nuclear DNA sequences is that while the former is a compact arrangement of gene regions that code for proteins and ribonucleic acids, RNAs,⁸⁴ the latter additionally contains a large amount of noncoding (silent) DNA sequence, often of unknown function.⁸⁵ Most relevantly, nuclear genes frequently occur as piecemeal coding regions, or *exons*, interrupted by silent regions known as *introns*. Because these introns do not code for a protein product, they are subject to lesser selection pressures and consequently accrue mutations much faster than the surrounding coding regions. These higher mutation rates may resolve population-level differences in organisms, and, as a result, researchers may use introns in population genetics work.

H. Expression Data

One further data type now coming to the fore is not nuclear DNA sequences, but rather *expression* data—that is, the extent to which different nuclear genes are turned off or on. Expression is what differentiates, say, your liver from your eyeball: both have the same DNA sequences, but have different genes expressed. Differences in gene expression have the potential to distinguish conservation units within species,⁸⁶ and are a powerful way of assessing the evolutionary pressures different populations may experience.⁸⁷

III. COMMON ANALYSES OF GENETIC DATA

The tools of population genetics offer an unsurpassed means of generating information that bears directly on an ESA determination, such as the degree to which populations are demographically separated, how large their populations might be or might have been in the past, and

83. Captive breeding programs monitor genetic relatedness among individuals, in order to maximize the amount of genetic diversity by making breeding decisions that minimize inbreeding. For the importance of precision in measuring genetic differences in natural populations of listed species, see *infra* Part IV.B (discussing the pygmy owl).

84. The transfer RNA molecules encoded on the mitochondrion are used in protein synthesis.

85. For an accessible and interesting treatment of noncoding DNA and other features of the nuclear genome, see Carl Zimmer, *Now: The Rest of the Genome*, N.Y. TIMES, Nov. 11, 2008, at D1.

86. See generally Michael M. Hansen, *Expression of Interest: Transcriptomics and the Designation of Conservation Units*, 19 MOLECULAR ECOLOGY 1757 (2010).

87. For an example of natural selection acting on genes that are subject to natural selection in the wild, see generally Catherine R. Linnen et al., *On the Origin and Spread of an Adaptive Allele in Deer Mice*, 325 SCIENCE 1095 (2009) (linking selection for a light-colored coat in deer mice to a particular genetic locus, the expression of which results in lighter coloration).

to identify populations or individuals that carry the genetic diversity that is the grist of evolution and resilience. As such, population genetics are reasonable and legitimate grounds for ESA listing decisions, but the complexity of genetic analysis carries the danger of obscuring a decision-making process designed to be transparent. What follows is an overview of the kinds of genetic analyses that have featured in ESA listing decisions in the past, as well as those likely to become more important in the future.

For heuristic purposes, I will divide the relevant analyses into three categories, though this arrangement is imperfect and several analyses span categories. Broadly, the three types of analyses are (1) summary statistics, (2) evolutionary/relationship analyses, and (3) distribution/frequency analyses. To better tie these analyses to the ESA context, I will indicate their common flaws, suggesting ways to challenge agency decisions that depend upon suspect analysis. Future listing petitions also stand to become more rigorous if they can include primary data from the published literature to support their claim that a species warrants listing as threatened or endangered.

A. *Summary Statistics*

Summary statistics are the simplest of analyses, providing a straightforward, just-the-facts view of the genetic dataset. These may be thought of as the vital signs of a population: how many alleles are present, how diverse and how different from one another those alleles are, and the like. One further analytical step compares the alleles present in a particular dataset to those expected for the same population at *equilibrium*—that is, a population of large and constant size, with no mutation, migration, selection, or nonrandom mating.⁸⁸ Deviations from equilibrium indicate that one or more of these evolutionary forces is acting on the population. In short, these deviations indicate that something interesting is going on, which is precisely the kind of information relevant to the question under the ESA of whether a DPS is distinct or significant, and hence warrants protection.

88. These are the basic assumptions of the Hardy-Weinberg equilibrium (HWE). HWE is the baseline—the null hypothesis, in experimental terms—against which observed populations are measured. A significant departure from HWE is therefore evidence that one or more evolutionary forces is acting. Researchers most often interpret violations of HWE as evidence of selection or population subdivision (requiring nonrandom mating). This is necessarily a gross simplification, and many volumes have been written about the subject. See, e.g., HARTL, *supra* note 59.

1. Genetic Distance and Nucleotide Diversity

Genetic distance refers generally to methods that quantify how different one individual is from the next, or how different one population or species is from the next. This is easiest to envision using DNA sequence data, though various distance estimations exist to calculate equivalent statistics for other non-sequence sources of data.⁸⁹

Pairwise difference is the simplest measure of the distance between two individuals. With nucleotide (DNA sequence) data, this distance is expressed as the proportion (or percentage) of sites with nucleotides that differ between the two individuals.⁹⁰ The greater the distance between individuals, the less similar they are. Individuals with identical alleles, conversely, have zero differences between them. Pairwise differences are also used as a rough measure of divergence between species, as with the distance-based tree-building measure described below. Divergence, in turn, closely matches the discreteness prong of the DPS inquiry,⁹¹ such that it would be difficult to argue that two highly divergent populations were not distinct in a legal sense.

2. Genetic Diversity

Genetic diversity is a function of population size and mutation rate. All else being equal, larger, more stable populations will be more diverse than smaller, unstable populations. This is particularly relevant for endangered species because researchers would often like to infer the size of a given breeding population in order to establish either recovery or endangered/threatened status.

The average number of pairwise differences between individuals in a sample is sometimes known as its *nucleotide diversity*, because it captures an aspect of the amount of heterogeneity that exists in the sample.⁹² Other common measures include *allelic diversity* (the probability that two alleles chosen at random from the sample will be different), the raw number of alleles in a sample, and the number of *private alleles* in a

89. See, e.g., Laurent Excoffier, Guillaume Laval & Stefan Schneider, *Arlequin (Version 3.0): An Integrated Software Package for Population Genetics Data Analysis*, 1 EVOLUTIONARY BIOINFORMATICS ONLINE 47 (2005) (providing a means of calculating distances for various data types and for both individual differences (within a species) and differences between groups of individuals); see also L.L. Cavalli-Sforza & A.W.F. Edwards, *Phylogenetic Analysis: Models and Estimation Procedures*, 19 AM. J. HUMAN GENETICS 233 (1967) (discussing the often-used Cavalli-Sforza chord distance measure).

90. See HARTL, *supra* note 59, at 107. This pairwise distance between DNA sequences is known as π (pi). The raw number of pairwise differences may be augmented using a model of molecular evolution; if, for example, a mutation of T \rightarrow C is more probable than T \rightarrow G, weighting genetic distances according to a specific mutational model can account for these probabilities.

91. See DPS Policy, *supra* note 39, at 4725.

92. See HARTL, *supra* note 59, at 111. Sometimes expressed as a lower-case theta (θ).

sample (alleles occurring in only one population and nowhere else). Low measures of diversity may be an indicator of small population size.⁹³

Finally, *heterozygosity* is a classic index of diversity, only calculable for diploid markers (those that have two sets of genetic information). This is the proportion of individuals in a sample that have two different alleles for the same locus (gene); for example, the proportion of people with one copy of the allele that confers attached earlobes, and one copy with the allele for detached earlobes. Heterozygosity is the reciprocal of *homozygosity*, the proportion with two identical alleles for a given gene.⁹⁴ Populations with many alleles at low frequencies will tend to have very high levels of heterozygosity; higher heterozygosity is thus higher diversity.

Diversity reflects how genetically robust a population is. Geographically outlying populations may be more acutely threatened, as they tend to be less genetically diverse and subject to greater inbreeding than their geographically central counterparts.⁹⁵ In the ESA context, such information bears directly on the third prong of the agency's DPS evaluation, which requires that DPSs themselves be endangered or threatened before they qualify for listing.⁹⁶

3. *Hardy-Weinberg Equilibrium*

Another category of summary statistics I will treat here is the test for Hardy-Weinberg equilibrium. Again, many volumes are written about the topic,⁹⁷ but for purposes of the ESA, only the basics are necessary. Violations of the equilibrium speak to both the discreteness and significance prongs of the DPS inquiry.

Hardy-Weinberg equilibrium (HWE) is the default state of a population, or, put differently, the null hypothesis against which a population is measured. This equilibrium state occurs in the absence of

93. Low diversity can also result from a recent population "bottleneck," in which a small number of individuals survives for a period of time, inbreeding and losing alleles, and subsequently increases in number—in which case the population size will have recovered from the bottleneck, but the genetic diversity will not yet have rebounded. The genetic bottleneck is a critical concept in conservation biology, because imperiled species often experience this kind of population depression, but it is not directly relevant to diagnosing a DPS for listing.

94. Note that an individual can be either homozygous or heterozygous for a given diploid locus. Thus the proportion of heterozygotes and the proportion of homozygotes sums to one, for any given diploid locus.

95. See, e.g., Andrew Young, Tim Boyle & Tony Brown, *The Population Genetic Consequences of Habitat Fragmentation for Plants*, 11 TRENDS IN ECOLOGY & EVOLUTION 413 (1996).

96. See DPS Policy, *supra* note 39, at 4725.

97. See, e.g., RICHARD FRANKHAM, JONATHAN BALLOU & DAVID BRISCOE, INTRODUCTION TO CONSERVATION GENETICS 78 (2009).

mutation, migration, selection, genetic drift, and nonrandom mating.⁹⁸ We need not dwell on these concepts here; in short, HWE occurs in the absence of any evolutionary process. Under such a scenario, the allele frequencies of a population remain constant from one generation to the next.⁹⁹ Thus the canonical test for evolution—that is, the test to determine if a population is changing from one generation to the next—is a test for violation of HWE. This test is merely a comparison between the expected allele frequencies (at equilibrium) and the observed frequencies in the sample. An excess of homozygotes over their expected frequency, for example, might indicate nonrandom mating (population subdivision) or inbreeding. This often occurs in rare and endangered species.¹⁰⁰

In order to evaluate the observed and expected frequencies of heterozygotes and homozygotes, however, one needs diploid data (that is, containing two copies of genetic information, one inherited from each parent); haploid data is neither heterozygous nor homozygous because it consists of only one allele. As a result, mitochondrial DNA is inappropriate for tests of HWE.

4. *Bases for Legal Challenges*

Two statutory bases for legal challenge lend themselves to fact-intensive questions such as those found in the ESA context, one arising from the ESA and one from the APA.¹⁰¹ Because the ESA requires the agency to use the best available science,¹⁰² the agency is vulnerable to a challenge where substandard data types or analyses underpin its decisions. Similarly, if the agency's conclusions are unreasonable or contrary to the data on which they were based, a court could find the decisions arbitrary and capricious under the APA.¹⁰³ With respect to

98. Here, "migration" refers to the exchange of individuals between populations, and "genetic drift" refers to chance changes in allele frequency between generations, a factor only in very small populations.

99. See HARTL, *supra* note 59, at 29.

100. For an example of the test for HWE in a candidate species for ESA listing, see Terry D. Beacham, Douglas E. Hay & Khai D. Le, *Population Structure and Stock Identification of Eulachon (Thaleichthys Pacificus), an Anadromous Smelt, in the Pacific Northwest*, 7 MARINE BIOTECHNOLOGY 363 (2005).

101. See The Administrative Procedure Act, Pub. L. No. 79-404, 60 Stat. 237 (codified as amended in scattered sections of 5 U.S.C.).

102. See § 1533(b)(1)(A) (2006).

103. See 5 U.S.C. § 706 (2006); *Earth Island Inst. v. Hogarth*, 494 F.3d 757, 766 (9th Cir. 2007) ("An agency action is not supportable if it did not consider all the relevant factors and if there is no rational connection between the facts found and the determination made.") (citing *Pac. Coast Fed'n of Fishermen's Ass'ns v. Nat'l Marine Fisheries Serv.*, 265 F.3d 1028, 1034 (9th Cir. 2001)); see also *Motor Vehicle Mfrs. Ass'n v. State Farm Mut. Auto. Ins. Co.*, 463 U.S. 29, 43 (1983) ("Normally, an agency rule would be arbitrary and capricious if the agency has relied on factors which Congress has not intended it to consider, entirely failed to consider an important aspect of the problem, offered an explanation for its decision that runs counter to the evidence

summary statistics, these are both uphill battles, as courts generally defer to agency decisions on such fact-based questions.¹⁰⁴ Still, some common data flaws could present fruitful challenges.

Where a higher-resolution dataset (for example, a DNA sequence) exists and is easily available, but the agency bases its decision on lower-resolution data (such as allozymes), it is open to either challenge. Old data also presents a problem for the agencies: where populations are small and rapidly changing due to anthropogenic stressors, data collected in years prior may be actively misleading. For example, if the genetic diversity of species A was high when sampled in the 1980s, that is not necessarily an indication that its diversity is still high if the species has experienced population decline.

Finally, there are some datasets that are incapable of answering the question an agency asks. For example, say researchers sample five panthers from Florida and five from Texas using a mitochondrial DNA marker that is not variable—that is, it is identical in all individuals.¹⁰⁵ The marker is uninformative—it does not say anything about the relationship between the two populations, or the level of connectivity between them. But the agency could correctly state that it failed to find a difference between the Texas and Florida populations, and thus the two would not be discrete with respect to the genetic evidence.¹⁰⁶ Such reliance on a facially insufficient dataset would seem vulnerable to challenge as

before the agency, or is so implausible that it could not be ascribed to a difference in view or the product of agency expertise.”).

104. See, e.g., *Baltimore Gas and Elec. Co. v. Natural Res. Def. Council*, 462 U.S. 87, 103 (1983) (“[A] reviewing court must remember that the Commission is making predictions, within its area of special expertise, at the frontiers of science. When examining this kind of scientific determination, as opposed to simple findings of fact, a reviewing court must generally be at its most deferential.”). In *Baltimore Gas*, the Court deferred to the Nuclear Regulatory Commission’s decision regarding the environmental impact of long-term storage of nuclear material. *Id.* at 104. The Court distinguished, interestingly, between “simple findings of fact” and “scientific determination[s].” *Id.* at 103.

105. Note that the researchers would not know prior to sampling that the gene region is identical in all individuals. This would become apparent only after sequencing, at which point the nucleotide sequence would be the same in each sample. As a result, a challenger could employ this argument only in a situation in which the agency improperly relied on the data after the fact; for example, using the results to demonstrate a lack of discreteness between the two populations, which is plainly not a reasonable interpretation of the data in hand.

106. Note that small sample sizes present this problem even when variable markers are used: the subtler the genetic difference, the larger the sample size needed to detect it. See generally Robin S. Waples, *Separating the Wheat from the Chaff: Patterns of Genetic Differentiation in High Gene Flow Species*, 89 J. HEREDITY 438 (1998). As a result, if a challenger can show a statistical impossibility of finding that two populations are significantly different with the given dataset, a court could find the resulting agency finding arbitrary and capricious. See also Berry J. Brosi & Eric G. Biber, *Statistical Inference, Type II Error, and Decision Making under the US Endangered Species Act*, 7 FRONTIERS ECOLOGY & ENV’T 487 (2009) (discussing statistical power in genetic data in the ESA context).

arbitrary and capricious because the conclusions could not reasonably be drawn from the data in hand.

B. Evolutionary/Relationship Analyses

A second set of analyses describes the relationships among alleles, individuals, or other biological units in the dataset. The central assumption of any such analysis is that the units being analyzed arose from a common ancestor at some point in history. Alleles of the same locus, for example, are assumed to derive from a single ancestral allele.¹⁰⁷ Individuals within a species similarly share a common ancestor, such that the arrangement of their present-day differences reflects the historical/evolutionary relationships among them.

The results of such evolutionary inference are most easily understood graphically, as a tree diagram in which the branches of the tree represent lineages, and nodes represent points of evolutionary divergence.

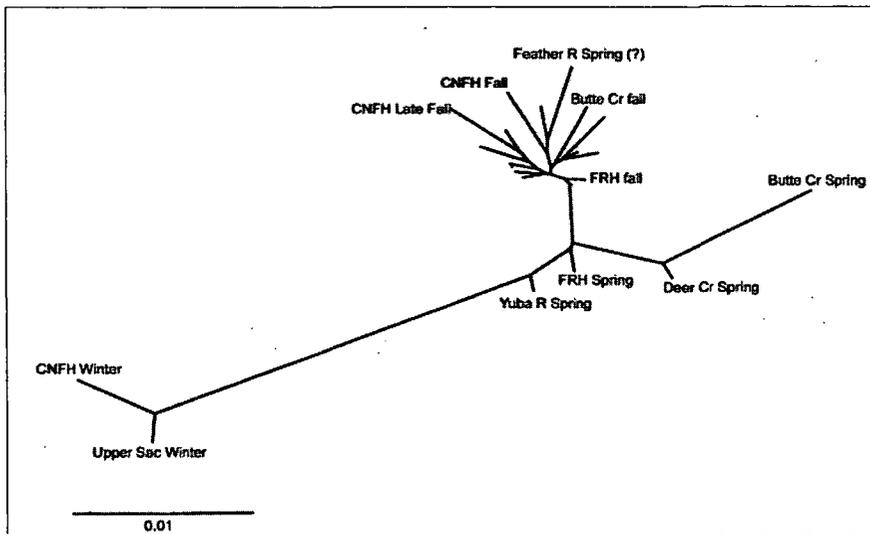


FIGURE 1: A NEIGHBOR JOINING TREE.¹⁰⁸

107. It is possible to avoid making this assumption by using models of evolutionary change that account for mutations that can result in identical alleles arising independently.

108. This tree, estimating the relationships among Central Valley Chinook salmon populations, is used with permission from THOMAS P. GOOD, ROBIN S. WAPLES & PETE ADAMS, NOAA TECHNICAL MEMORANDUM NMFS-NWFSC-66, UPDATED STATUS OF FEDERALLY LISTED ESUS OF WEST COAST SALMON AND STEELHEAD 156 (2005), available at <http://www.nwr.noaa.gov/Publications/Biological-Status-Reviews/upload/SR2005-allspecies.pdf>. This particular tree is based on allozyme data, with nodes indicating different fish populations. Branch length indicates a measure of genetic distance between the populations.

In Figure 1, for example, it is apparent that the populations on the left-hand side of the diagram, “CNFH Winter” and “Upper Sac Winter,” are highly divergent from the other sampled populations. Each terminal line represents a population; not all are labeled in this diagram. Note that one could group “CNFH Winter” and “Upper Sac Winter” into a single putative DPS on the basis that those populations cluster more closely to each other than they do to all other populations. However, one could also argue each population is distinct because no two labeled populations are identical. The level of required distinctiveness is the central judgment call in the discreteness prong of the DPS inquiry.

A network is the other common graphical way to represent a set of relationships; nodes represent the units under analysis (individuals, alleles, etc.), and the connections between nodes represent evolutionary connections, often with intermediate steps inferred.

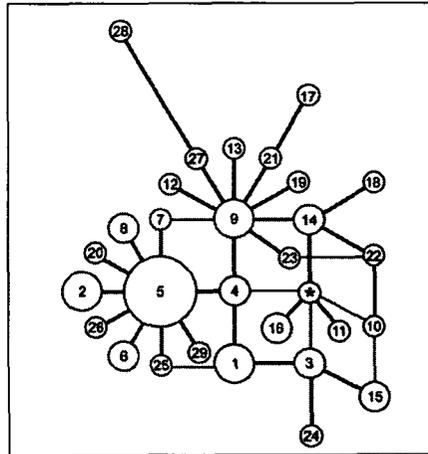


FIGURE 2: A HAPLOTYPE NETWORK.¹⁰⁹

In Figure 2, it is apparent that no one allele dominates the dataset (though allele number 5 is the most common), information that tree diagrams do not convey. Further analysis, which the authors of the data

109. From G. M. O’Corry-Crowe et al., *Phylogeography, Population Structure and Dispersal Patterns of the Beluga Whale Delphinapterus leucas in the Western Nearctic Revealed by Mitochondrial DNA*, 6 *MOLECULAR ECOLOGY* 955, 961 (1997). This haplotype network details the mtDNA patterns of the beluga whale. Each circle, or node, represents a particular allele of mtDNA, with the connections between them representing mutation events. The size of the circle represents the abundance of the allele in the sample. The numbers, used for identifying individual alleles, are arbitrary. Note that a network can represent ambiguous relationships; a given allele can be equally closely linked to multiple others.

paper provide,¹¹⁰ can show where each of the alleles occurs geographically. This is critical for estimating genetic connectivity among geographically distinct populations.

These analyses are powerful, as they imply the power to look back in time and reveal the relationships among the units of analysis. Divergent lineages within a taxonomic species, for example, can represent several DPSs under the ESA. As a result, it is all the more important to understand the basics of the analyses, and to be able to interpret them, before weighing in on their validity. Though tree diagrams often give the impression of being definitive, plainly laying out a set of relationships, they are nevertheless evolutionary *estimates*. They do not represent truth, but rather are hypotheses to be tested against other data.

1. *Tree-Building Algorithms*

Not all tree diagrams are created equal. There exist a great variety of ways to infer relationships among gene sequences or other molecular data, and some are much better than others.¹¹¹ However, all begin with a set of molecular data from a number of individuals and use algorithms that embody some underlying assumptions about what genetic differences or similarities signify, resulting in an inference about how the sampled individuals are related.

I will distinguish between *evolutionary* and *distance-based* tree-building methods at the outset. In a sense, distance-based methods are not evolutionary; they reflect only the pairwise differences between the individuals in the sample. This method only estimates the underlying evolutionary relationships under the assumption that distance is strictly associated with evolutionary history, an assumption the real world nearly always violates. In practice, the resulting *phylogenies* (inferred sets of relationships among individuals) can be similar between evolutionary and distance-based methods, although the underlying math and philosophies are quite different, as I describe below.

In the ESA context, the two most common distance methods for tree-building are the unweighted pair-group method with arithmetic mean (UPGMA) and neighbor joining. Both start with the number of differences between each pair of individuals in the sample and result in quick but suspect trees because (among other shortcomings) the underlying algorithms rely on assumptions that real data routinely

110. *Id.*

111. *See, e.g.,* JOSEPH FELSENSTEIN, *INFERRING PHYLOGENIES* (2004) (exhaustively treating the subject); *see generally* MOLECULAR SYSTEMATICS (David M. Hillis, Craig Moritz & Barbara K. Mable eds., 2d ed. 1996) (an aging, but still widely used, reference for the details of molecular phylogenetic methods); *see also* HARTL, *supra* note 59, at 136–38 (introducing the topic).

violate.¹¹² Yet because they are easy to understand and take little computing time, they are nevertheless common. Distance trees might be better thought of simply as graphical representations of the underlying distance analysis, whereas evolutionary trees are themselves the products of separate analyses. Nevertheless, even substandard trees are extremely effective for graphically representing distance, and because evolutionary relationships are less relevant under the ESA than how distinct individuals are from one another, distance-based trees can be valuable heuristic analyses for evaluating DPS listing claims.¹¹³

Evolutionary methods for tree-building explicitly assume a model of molecular evolution¹¹⁴ in order to interpret the underlying data. These come in two main philosophical groups: *maximum parsimony*, which assumes that the tree with the minimum number of mutational events is the correct one, and *maximum likelihood / Bayesian inference*, which search for the set of relationships with the best probability of generating the observed set of genetic data given the evolutionary model used. The details are not important here, though they are legion.¹¹⁵ In general, these methods produce better estimates than the distance-based methods and have a more legitimate philosophical basis, though they are more computationally intensive.

112. UPGMA sequentially combines the two least-distant individuals in the overall dataset into a higher-order cluster. Once a cluster is formed, its distance to the remaining individuals is the average of the members' distances. See PETER H. A. SNEATH & ROBERT R. SOKAL, *NUMERICAL TAXONOMY: PRINCIPLES AND PRACTICE OF NUMERICAL CLASSIFICATION* 230–34 (1973). Neighbor joining joins the two least-distant individuals, with distances transformed by an equation that incorporates the number of individuals to be joined and the distances between each pair. The algorithm then adds each nearest neighbor in stepwise fashion. See generally Naruya Saitou & Masatoshi Nei, *The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees*, 4 *MOLECULAR BIOLOGY & EVOLUTION* 406 (1987). Note that both methods produce a single tree from a given data matrix, which can give the misleading appearance of having produced the one “true” tree. Note also the vintage of these methods; prior to powerful desktop computers, the speed of a tree-building method was more critical than it is today.

113. This distinction highlights the different focus between the academic biologists that publish the primary data papers and conservationists or agency biologists that may have a particular legal test in mind when evaluating data.

114. Models of molecular evolution effectively weight the relationships among individuals based on a description of how mutations occur in the DNA sequence. The models vary most importantly in their rates and probabilities of character-state changes. Different character-state changes (for example a mutation that changes an “A” to a “T” in the DNA sequence) occur with different frequencies in nature, due in part to different biochemical properties of the nucleotides. While the earliest model of molecular evolution hypothesized that all changes (for example, from “A” to any one of “C,” “G,” or “T”) were equally probable, subsequent models have improved by specifying different rates and probabilities. See Pietro Liò & Nick Goldman, *Models of Molecular Evolution and Phylogeny*, 8 *GENOME RES.* 1233 (1998), for an accessible review.

115. See generally FELSENSTEIN, *supra* note 111, for a detailed discussion of the differences among phylogenetic methods.

No matter which tree-building method is used, however, trees should have an index of *node strength* to help the reader evaluate the robustness of its inferred set of relationships. Many such indices exist—including bootstrap values and posterior probabilities¹¹⁶—but it is critical to have at least some measure of confidence shown in a tree diagram. A reader should view trees without an indication of node strength with suspicion.

The most appropriate type of data for tree-building is DNA sequence data, which provides many nucleotides to function as evolutionary characters that mark change between individuals in fine gradations. Nevertheless, any type of data can yield trees, with varying degrees of confidence. Microsatellites are particularly unsuitable for reconstructing relationships because their very fast mutation rates tend to obliterate evidence of evolutionary relationships, though this has not prevented authors from attempting it.¹¹⁷

2. *Networks*

In networks, alleles are represented as nodes, with connections between them representing mutational steps (see Figure 2). Like trees, networks can effectively illustrate a set of estimated relationships among individuals.¹¹⁸ While a single tree can convey only a single set of relationships, networks can show multiple possible connections among individuals. As a result, networks are a common way to show ambiguous relationships among a small number of samples. Networks, unlike trees, are also able to show that some alleles are actually descendants of other existing alleles. As is often the case in work on rare or endangered species, networks are useful to show a snapshot of all existing alleles, their relative frequencies, and the mutational connections among them.

3. *A Hybrid Approach*

The final relationship analysis common in the genetics work that informs ESA listing claims incorporates elements of tree-building and network-building into a geographically-explicit framework. Nested Clade Analysis (NCA) seeks to wring historical demographic inference from the real-world geographic arrangement of alleles, taking into account the

116. See FELSENSTEIN, *supra* note 111, at 335–62.

117. See Takezaki & Nei, *supra* note 70.

118. An algorithm is still required to infer a set of connections among individuals in a network, but because nodes (individuals) in a network can have multiple connections to other such nodes, the philosophy underlying network-building is probably less important than in tree building. Parsimony criteria are the most often used in building biological minimum spanning networks, such as those discussed here.

evolutionary relationships among them.¹¹⁹ NCA begins with a network as described above,¹²⁰ but then groups individuals hierarchically, such that all individuals separated by a single mutational step are put into the same one-step “clade.”¹²¹ The one-step clades are then themselves nested into two-step clades containing all individuals separated by two mutation events, and so on. Given data about the geographic locations from which the constituent samples were collected, a researcher can then test for a relationship between clades, which represent different hierarchical levels of relatedness, and geography. For example, one might ask whether individuals in a given one-step clade were nonrandomly drawn from the overall geographic sample, indicating nonrandom mating across space. This test functions similarly to other distributional statistics—asking how alleles are distributed across geographic space—but does so in an evolutionary framework, such that one might ask how families of related alleles occur in space. Given assumptions about the geographic spreading of alleles over evolutionary time, the method may also distinguish historical demographic scenarios that other methods cannot; for example, discerning recent range expansion from contemporary gene flow among populations.¹²²

NCA has recently come under fire with what appears to be good justification—the technique may detect patterns that are not real¹²³—but it has been fairly widely used¹²⁴ to probe the connections between geography and genetics in an evolutionary framework.

4. Bases for Legal Challenges

The evolutionary/relationship analyses are difficult grounds on which to challenge a listing decision, given the arcane details that separate a reasonable analysis from an unacceptably poor one. Any challenge is therefore necessarily technical, within the area of agency expertise and

119. See Alan R. Templeton, *Nested Clade Analyses of Phylogeographic Data: Testing Hypotheses About Gene Flow and Population History*, 7 *MOLECULAR ECOLOGY* 381, 381 (1998). For an example of NCA in the context of an endangered species, see K. Shanker et al., *Phylogeography of Olive Ridley Turtles (*Lepidochelys olivacea*) on the East Coast of India: Implications for Conservation Theory*, 13 *MOLECULAR ECOLOGY* 1899 (2004).

120. See *supra* Part III.B.2.

121. “Clade” is a general term in evolutionary biology, referring to any group of individuals more closely related to each other than any other such group.

122. See Templeton, *supra* note 119, at 384 (describing the technique).

123. See generally Rémy J. Petit, *The Coup de Grâce for the Nested Clade Phylogeographic Analysis?*, 17 *MOLECULAR ECOLOGY* 516 (2007) (discussing results that indicate NCA has extremely high false-positive rates of detecting restricted gene flow); see also, L.L. Knowles, *The Burgeoning Field of Statistical Phylogeography*, 17 *J. EVOL. BIOL.* 1, 4 (2004) (criticizing NCA as not accounting for stochastic genetic processes).

124. BIOSIS reports more than 300 articles with NCA in the topic heading.

normally subject to judicial deference. However, one could conceivably challenge an agency finding grounded in manifestly flawed analyses.

For example, a DPS listing based on trees built from microsatellite data, an analysis known to be faulty under real-world conditions,¹²⁵ might be vulnerable. Similarly, the recent evidence undermining the validity of NCA might give some judges pause,¹²⁶ especially if this argument were combined with other analyses the agency dismissed or failed to consider. Finally, neighbor joining and UPGMA trees are plainly not the best available analyses for assessing evolutionary relationships. Where different analyses yield different sets of inferred relationships, this could be a point on which to challenge the listing agency, albeit a weak one given the deference that courts commonly afford agencies' choice of analytical methods.

C. *Distribution/Frequency Analyses*

The analysis of the distribution and frequency of alleles or other biological units may be thought of as classical population genetics. Unlike evolutionary analyses, these statistics generally do not communicate different units' evolutionary relationships. And they differ from summary statistics in that they require a further analytical step, for example, reporting not simply the allelic diversity in the dataset, but how that diversity is apportioned among individuals, populations, or across geographic space.

Such analyses are critical to understanding biological diversity below the species level. While different species will usually have entirely different alleles of a given gene (because one species generally does not generally transmit its alleles to another and two related species rarely retain mutually-shared alleles from a common ancestor), populations *within* a species most often have the same alleles at different frequencies. Significant differences in allele frequency between populations are *prima facie* evidence of an evolutionary force acting within that species. Researchers most often interpret these frequency differences as nonrandom mating as a result of barriers to gene flow and subsequent inbreeding within groups, but they may also signal natural selection or other forces.

These are perhaps the most relevant analyses for the ESA. Species and subspecies tend to be unambiguously distinct from other such species or subspecies, and as a result, genetics only confirms the pre-existing discrete status of a group. By contrast, the ESA's DPS concept nearly

125. See Takezaki & Nei, *supra* note 70.

126. See Petit, *supra* note 123.

requires distributional analyses to inform the agency about how distinct one population is from the next.¹²⁷ Tree-drawing and summary statistics do not adequately address this question,¹²⁸ though they can be useful in identifying distinct groups of alleles that merit further attention.

1. Wright's *F* Statistics

Sewall Wright began his analysis of genetic data in the 1920s, long before Watson and Crick identified DNA as the carrier of genetic information. His *F* statistics, formalized in 1950, employ the degree of inbreeding in a population, *F* (the inbreeding coefficient), to compare levels of genetic variation among individuals, subpopulations, and the total sampled population.¹²⁹ A lack of gene flow *between* populations creates a higher degree of inbreeding *within* populations; this inbreeding creates lower genetic diversity within each population, relative to what would be expected if all individuals were mating at random. By comparing the levels of genetic diversity among different demographic units, then, one can estimate the frequency of gene flow between them, which in turn helps determine how isolated a population is for the purpose of defining a DPS. For example, the common statistic F_{ST} compares the genetic diversity in the subpopulation (denoted with a subscript "S") to that of the total overall sample ("T").¹³⁰

F statistics have proved resilient to subsequent revolutions in our understanding of genetics and in the availability of different types of genetic information. Theoretical geneticists have extended the statistics by analogizing DNA sequence data, microsatellites, RFLP, allozymes, and many other forms of data to heterozygosity calculations.¹³¹ In so doing, they have kept Wright's ideas in the center of population genetics analysis for decades.

F_{ST} and its analogues are the most relevant to the DPS formulation because they do just what the agency does in evaluating a potential DPS: compare one subpopulation to the composition of the overall species.¹³² Different formulations of F_{ST} have proliferated as the statistic has been extended to different data types, but the core concept remains: the statistic varies from zero to one, where $F_{ST} = 0$ indicates a total lack of

127. See *supra* Part I.B (discussing the DPS concept).

128. See *supra* Part III.A.1.

129. See Sewall Wright, *Genetical Structure of Populations*, 166 NATURE 247, 247 (1950).

130. Also common in the scientific literature, and perhaps bearing on the ESA, are F_{IS} , F_{IT} , the heterozygosity of the individual relative to the subpopulation, and the individual relative to the total, respectively.

131. See AVISE, *supra* note 75, at 24; Montgomery Slatkin, *A Measure of Population Subdivision Based on Microsatellite Allele Frequencies*, 139 GENETICS 457 (1995).

132. Here, "subpopulation" is Wright's term and is equivalent to the concept of a population as I've used it.

detected differentiation of the sampled population, and $F_{ST} = 1$ indicates complete genetic differentiation. Generally, a permutation test¹³³ determines the statistical significance of a calculated F_{ST} value.

It is important to note that the F_{ST} calculation does not indicate the absolute magnitude of genetic difference between samples, only the way in which those differences are distributed. For example, a 1-base-pair change between two individuals in a 1000 bp fragment is only a 0.1 percent difference. However, if *all* individuals in one population had the change, and all individuals in another population did not, the resulting pairwise F_{ST} value of the former would equal 1, the maximum amount of genetic subdivision.¹³⁴

It may be counterintuitive that such a small absolute genetic difference can result in an extreme measure of population differentiation, but this reflects a critical property of distributional statistics: what matters is how variation is distributed. Tree-drawing and evolutionary analyses are most informative for genetic changes that accrue over long, evolutionary time scales while distributional statistics are informative over much shorter, ecological time scales. This is another reason why they are of primary importance for the ESA: preserving ecosystems and the species that depend upon them¹³⁵ is an endeavor in ecological real-time.

An important extension of F statistics, and one often found in the primary scientific literature that informs DPS decisions, is the analysis of molecular variance framework (AMOVA). This method applies the statistical analysis-of-variance concept to genetic data, apportioning a sampled species' total variance in genetic diversity into hierarchical levels. In this context, the analogous statistic to F_{ST} is called " Φ_{ST} ," which reports the proportion of the overall variance in genetic diversity that occurs between populations.¹³⁶ When a significant fraction of the variance occurs *between* populations, relative to the variance *within* populations (that is, when Φ_{ST} is high), that is strong evidence for a lack of gene flow or migration among them. Researchers thus most often use the AMOVA approach to represent the overall level of fragmentation among several

133. A permutation test, in general, is one that assesses significance by finding the probability of the observed data (in this example, a given value of F_{ST}) based on an underlying distribution. This "null" distribution is created by permuting the data matrix many times, to approximate a random distribution. Hence, the significance of the observed value is the probability of it occurring by chance alone.

134. The resulting F_{ST} value in fact depends on which version of F_{ST} one might use; for our purposes, a value of one is both illustrative and indicative of the calculation under several formulations of the statistic. Some versions normalize by the overall level of diversity in the sample.

135. See 16 U.S.C. § 1531(b) (2006).

136. See generally L. Excoffier, P.E. Smouse & J.M. Quattro, *Analysis of Molecular Variance Inferred from Metric Distances among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data*, 131 GENETICS 479 (1992).

populations and F_{ST} to represent differentiation between pairs of populations.¹³⁷

There exist versions of F statistics appropriate for any kind of genetic data, making them the workhorses of population genetics.¹³⁸

2. Spatially Explicit Statistics

While F statistics are robust and flexible, they have the particular drawbacks of not accounting for geographic distance between sampling locations and of requiring *a priori* definitions of populations. Several classes of statistics have recently gained traction in addressing these shortfalls, but because they have not yet appreciably contributed to ESA decision making I will mention them only briefly.

The classic test for correlation between geographic and genetic distance is the *Mantel test*.¹³⁹ Each pair of individuals or populations sampled has a calculated genetic distance between them and a measured geographic distance between them. The Mantel test is simply a correlation between the matrix of pairwise geographic distances between individuals and the matrix of pairwise genetic distances between those same individuals. A positive correlation, usually approximating a linear curve, is evidence of limited gene flow along the sampled range—genetic distance is nonrandomly distributed across the geographic extent of the species. A Mantel test can make use of any form of genetic data, so long as one can reliably infer genetic distance.¹⁴⁰

Spatial autocorrelation is increasingly used for estimating the geographic scale over which samples are genetically similar. This method shows the change in the correlation between genetic and geographic pairwise distances over space.¹⁴¹ Like the Mantel test, spatial

137. For one of many examples of F_{ST} , its analogues, and other distributional statistics used in the context of an endangered sea turtle, see B.W. Bowen, A.L. Bass, L. Soares & R.J. Toonen, *Conservation Implications of Complex Population Structure: Lessons from the Loggerhead Turtle (Caretta caretta)*, 14 *MOLECULAR ECOLOGY* 2389 (2005).

138. See, e.g., Excoffier, Laval & Schneider, *supra* note 89.

139. Peter E. Smouse, Jeffrey C. Long & Robert R. Sokal, *Multiple Regression and Correlation Extensions of the Mantel Test of Matrix Correspondence*, 35 *SYSTEMATIC ZOOLOGY* 627 (1986).

140. For an example of the use of the Mantel test in the ESA context, see V.L. Friesen et al., *Population Genetic Structure and Conservation of Marbled Murrelets (Brachyramphus Marmoratus)*, 6 *CONSERVATION GENETICS* 607 (2005).

141. See, e.g., Peter E. Smouse & Rod Peakall, *Spatial Autocorrelation Analysis of Individual Multiallele and Multilocus Genetic Structure*, 82 *HEREDITY* 561 (1999). But see Ryan P. Kelly et al., *A Method for Detecting Population Genetic Structure in Diverse, High Gene-Flow Species*, 101 *J. HEREDITY* 423 (2010) (suggesting that spatial autocorrelation has high false positive and false negative rates of detection for population subdivision). For the use of the method in an endangered species, though with demographic rather than genetic data, see Peter C. Trenham, Walter D. Koenig & H. Bradley Shaffer, *Spatially Autocorrelated Demography and Interpond Dispersal in the Salamander Ambystoma Californiense*, 82 *ECOLOGY* 3519 (2001).

autocorrelation can use a variety of types of data, though in practice researchers most often use microsatellites for their high mutation rates. Because new microsatellite alleles arise often in populations, they are particularly well suited for estimating the connectivity among populations over short time horizons.

Finally, some newer statistics measure the geographic range of individual alleles, which is helpful for tracking the spread of rare alleles or identifying subtle genetic subdivision across a landscape.¹⁴² These newer statistics can also employ a wide range of data types, so long as individual alleles are known.¹⁴³

3. *Individual-Focused Approaches*

A final subset of methods focuses on sampled individuals, rather than on whole populations, though their results have population-level implications. For example, if a researcher determines the probability that a given migrant, collected at location B, came from source population A, she might use that information to estimate the overall migration rate between A and B. These individual-focused methods are likely to be used increasingly in the ESA context, both for identifying and for managing imperiled species and populations.

Parentage analysis is familiar from television crime dramas and the like: it uses multiple diploid markers, often to infer the parents of a given offspring, or the offspring of given parents. In the context of endangered species, such individual attention is often a part of *ex situ* recovery efforts or translocation, rather than a part of diagnosing management units. One can identify the parents of a sampled offspring by systematically excluding those individuals in the population who could not have produced the offspring, by assigning statistical likelihoods to parents given allele frequencies in the overall population, or by reconstructing the likely genotype of parents for a given offspring and surveying for a match.¹⁴⁴

One might think of parentage analysis as a special case of a larger class of methods known as *assignment tests*. With assignment, a

142. See, e.g., M.P. Miller, *Alleles In Space (AIS): Computer Software for the Joint Analysis of Interindividual Spatial and Genetic Information*, 96 J. HEREDITY 722 (2005); Ryan P. Kelly et al., *supra* note 141.

143. Identifying the individual alleles of diploid organisms is not necessarily trivial. Distinguishing the individual alleles of a heterozygote (an organism having two different alleles at the same genetic locus) is known as determining the “phase” of the alleles.

144. For a useful review of these techniques, see Adam G. Jones & William R. Ardren, *Methods of Parentage Analysis in Natural Populations*, 12 MOLECULAR ECOLOGY 2511 (2003). For an analysis of different data types’ performances in parentage analysis, see S. Gerber et al., *Comparison of Microsatellites and Amplified Fragment Length Polymorphism Markers for Parentage Analysis*, 9 MOLECULAR ECOLOGY 1037 (2000).

researcher attempts to discern the source population of an individual—a migrating fish, for example. Parentage is simply a more specific application, in which the source “population” of an individual is its parents.¹⁴⁵ More broadly, biologists increasingly use assignment tests to estimate the origin of a sampled individual, given a background genetic map of potential source populations. Assignment tests also help to describe the underlying genetic landscape of a species by inferring the number of breeding populations (using a clustering algorithm that leverages information from genetic linkage among alleles)¹⁴⁶ or identifying hybridization zones. These powerful tools, just now coming to the fore, will no doubt bear on future ESA listing decisions because they address demographic and ecological questions immediately relevant to imperiled species.¹⁴⁷ Microsatellites are a particularly appropriate data type for assignment tests because the methods require diploid data with identified alleles and fairly high diversity.

4. Bases for Legal Challenges

The most interesting, and legally promising, challenge to a listing decision using distributional analyses focuses on the distinction between legal and statistical significance. Biologists, and researchers in most other fields, generally assign statistical significance to a result when that result (or a more extreme result) is expected to occur *by chance alone* one time out of twenty.¹⁴⁸ This is equivalent to a false-positive rate of 5 percent;¹⁴⁹ if the observed value is likely to occur by chance less than 5 percent of the time, it is called “significant.” This is an arbitrary cutoff, but it is nearly universally used across scientific disciplines for accepting the validity of a test result.

145. For a helpful review, see Stephanie Manel, Oscar E. Gaggiotti & Robin S. Waples, *Assignment Methods: Matching Biological Questions with Appropriate Techniques*, 20 TRENDS ECOLOGY & EVOLUTION 136 (2005). For a review of Bayesian statistical inference in assignment tests, see Mark A. Beaumont & Bruce Rannala, *The Bayesian Revolution in Genetics*, 5 NATURE REV. GENETICS 251, 254 (2004).

146. Particularly relevant to this analysis is a software package called STRUCTURE. See Jonathan K. Pritchard, Matthew Stephens & Peter Donnelly, *Inference of Population Structure Using Multilocus Genotype Data*, 155 GENETICS 945, 956 (2000). See also the related software, *Software*, PRITCHARD LAB, UNIV. CHICAGO, <http://pritch.bsd.uchicago.edu/software.html> (last visited Oct. 30, 2010).

147. For an example of parentage analysis in the ESA context, see Jason Baumsteiger et al., *Use of Parentage Analysis to Determine Reproductive Success of Hatchery-Origin Spring Chinook Salmon Outplanted into Shitike Creek, Oregon*, 28 N. AM. J. FISHERIES MGMT. 1472 (2008). For an example of an assignment test, see Jesse W. Breinholt et al., *Population Genetic Structure of an Endangered Utah Endemic, Astragalus Ampullarioides (Fabaceae)*, 96 AM. J. BOTANY 661 (2009).

148. This rate is an accepted standard for most researchers and journals. Note, however, that this provides no information about the test’s false-negative rate, the probability of finding no significant difference when in fact one exists.

149. Usually denoted $p = 0.05$.

Being so widely accepted in the scientific community, it is unlikely that a judge would find the threshold of statistical significance “arbitrary” in the legal sense. For example, if NMFS found that a new salmonid DPS were discrete by reason of statistically significant genetic differences, and this statistical significance were assessed using the customary five percent cutoff, challenging the significance cutoff as arbitrary would seem unlikely to meet with success: there was a reasonable connection between the data and the agency’s decision.

However, if the agency *declined* to recognize a DPS strictly because it fell just short of significance—say, the observed genetic differences crop up 5.1 percent of the time by chance alone—a challenger might take the opportunity to argue for the DPS listing in either of two ways.¹⁵⁰ First, while the fish may not be discrete in a statistically significant sense, they likely are different in a real-world sense. That is, the agency (and the real world) need not be entirely bound by statistical cutoff points, which are in any case not found in the ESA or its regulations.¹⁵¹ Second, the false-negative rate of the test could mean any distinction between the populations is more probably real than not; a test with a high false-negative rate will fail to detect differences between populations when in fact they do exist. High false-negative rates are especially problematic in small datasets or in datasets with otherwise low statistical power. Because distributional genetic analyses are often full of subtle statistical points, any prospective challenger would do well to read the data papers from which the agencies draw their information in search of this kind of error.

IV. TWO CASE STUDIES OF ESA LISTING ACTIONS USING GENETIC DATA

The kinds of genetic analyses described above feature prominently in many recent ESA listing decisions. In response to a listing petition, FWS

150. Note that this hypothetical works equally well for any agency nonaction on the basis of a failure to find statistically significant results; it is a point about false negative rates, not about erring on the side of listing rather than non-listing.

151. See, e.g., ROBIN S. WAPLES, NOAA TECHNICAL MEMORANDUM NMFS F/NWC-194, DEFINITION OF “SPECIES” UNDER THE ENDANGERED SPECIES ACT: APPLICATION TO PACIFIC SALMON, (1991), available at <http://www.nwfsc.noaa.gov/publications/techmemos/tm194/waples.htm> (“It is important to realize that ‘statistical significance’ is a different concept than ‘evolutionary significance’ as it relates to the Act. . . . [F]ailure to find a statistically significant difference does not disprove the existence of population differences. Power to detect true differences in population means is a function of sample size, so this factor should also be considered in evaluating results of statistical tests.”); see also Brosi & Biber, *supra* note 106, at 489 (“[W]hen the FWS analyzed whether low water flows were correlated with endangered fish mortality in the Klamath River of Oregon and California, the agency required a statistically significant connection between the two before it would commit to action to increase water flows, even though . . . the lack of a statistically significant correlation would provide little or no information about whether such a correlation existed.”) (citing D.J. McGarvey, *Merging Precaution with Sound Science under the Endangered Species Act*, 57 BIOSCIENCE 65 (2007)).

or NMFS may review the published literature for data that bear on the question of whether or not a species or population merits protection, as in the case of the beluga discussed directly below. The listing agency may also solicit data from unpublished or internal sources, as feature in the case of the pygmy owl that follows. In either published or unpublished genetics work, the authors apply, and the agencies interpret, the core techniques of population genetics in an attempt to answer a pivotal question: Is there something about this species that merits protection?

The ESA routinely protects existing taxonomic units that the scientific community recognizes—named species and subspecies—as well as the subspecific units characterized by the DPS criteria. As a result, named species or subspecies normally need no genetic data to distinguish them, although there are examples of genetic data reinforcing the description of a putative species or subspecies for the purposes of listing.¹⁵² As a general matter, however, genetic data bear on the identification and listing of vertebrate DPSs. In practice, agencies wield wildly different amounts of information in listing decisions depending upon the availability of data (often directly related to the commercial or cultural importance of the species) and agency priorities. Where the agencies employ genetic data, the majority of such data comes from existing peer-reviewed academic journal articles whose authors may or may not have contemplated contributing to a listing decision, or else from agency-funded studies carried out for this particular purpose.¹⁵³

No dataset is ideal; every researcher must cope with incomplete sampling and less information than he or she would prefer. Agency datasets are no exception, whether coming from an internal study or the peer-reviewed literature. My purpose in this Part is not to point out the limited nature of genetic datasets in ESA listing decisions; rather, I aim to illustrate the types of data agencies routinely use, the analyses those data drive (and should drive), and the degree to which agency decisions are supported by the primary data.

A. *Beluga Whale*

NMFS's recent listing¹⁵⁴ of the beluga whale (*Delphinapterus leucas*) provides an example of robust genetic support for a listing decision, but a

152. See, e.g., Listing the San Miguel Island Fox, Santa Rosa Island Fox, Santa Cruz Island Fox, and Santa Catalina Island Fox as Endangered, 69 Fed. Reg. 10,335 (Mar. 5, 2004) (to be codified at 50 C.F.R. pt. 17).

153. See Notice of Interagency Cooperative Policy on Information Standards under the Endangered Species Act, 59 Fed. Reg. 34,271 (July 1, 1994) (noting use of academic articles); Notice of Interagency Cooperative Policy for Peer Review in Endangered Species Act Activities, 59 Fed. Reg. 34,270 (July 1, 1994) (noting use of data from wide range of sources).

154. See Endangered Status for the Cook Inlet Beluga Whale, 73 Fed. Reg. 62,919 (Oct. 22, 2008) (to be codified at 50 C.F.R. pt. 224).

near total absence of genetic discussion in the *Federal Register*. The agency addressed the discreteness and significance prongs of the DPS determination in turn. The relevant genetic information for the significance prong led the agency to make the following two findings. First, “[o]f the five stocks of beluga whales in Alaska, the Cook Inlet population was considered to be the most isolated, based on the degree of genetic differentiation and geographic distance between the Cook Inlet population and the four other beluga stocks.”¹⁵⁵ Second, the “Cook Inlet beluga population has been isolated for several thousand years, an idea that has since been corroborated by genetic data.”¹⁵⁶

NMFS’s status reviews¹⁵⁷ of the beluga are more thorough in both their treatment of genetic data and their documentation. The most recent review, from 2008,¹⁵⁸ references the previous status review¹⁵⁹ for the DPS determination information. That document primarily treats genetic data regarding the first DPS prong, discreteness: “the genetic characteristics of this population differ markedly from the other four beluga populations that occur in western and northern Alaska waters.”¹⁶⁰ The authors also lean heavily on the same data in discussing the history of the beluga populations:

Genetic analysis suggests there was a rapid radiation of belugas in the western nearctic after the retreat of Pleistocene ice sheets (over 11,000 years ago) and an early divergence of subpopulations into the Beaufort, Chukchi, and Bering Seas. Geographic barriers to dispersal are few yet genetic results showed little exchange among these populations. Mitochondrial DNA analyses revealed strong site fidelity of mothers and their offspring to the same summering areas which they return to generation after generation. Some interbreeding may occur between summer populations that overwinter in a common area. However, for belugas in Cook Inlet genetic isolation is compounded not only by the geographic barrier of the Alaska Peninsula but also by their year-round residency in the Inlet.¹⁶¹

A 1997 academic paper in the journal *Molecular Ecology* was the primary data source for the status reviews and the *Federal Register*

155. *Id.* at 62,926 (citations omitted) (citing O’Corry-Crowe et al., *supra* note 109).

156. *Id.*

157. Status reviews are required for each listed species every five years. See 16 U.S.C. § 1533(c)(2)(A) (2006).

158. R.C. HOBBS & K.E.W. SHELDEN, NAT’L OCEANIC & ATMOSPHERIC ADMIN., AFSC PROCESSED REPORT NO. 2008-08, SUPPLEMENTAL STATUS REVIEW AND EXTINCTION ASSESSMENT OF COOK INLET BELUGAS (*DELPHINAPTERUS LEUCAS*) (2008).

159. R.C. HOBBS, K.E.W. SHELDEN, D.J. VOS, K.T. GOETZ & D.J. RUGH, NAT’L OCEANIC & ATMOSPHERIC ADMIN, AFSC PROCESSED REP. NO. 2006-16, STATUS REVIEW AND EXTINCTION ASSESSMENT OF COOK INLET BELUGAS (*DELPHINAPTERUS LEUCAS*) (2006). The beluga was a candidate species prior to being listed, and thus the subject of a status review.

160. See *id.* at 37 (internal citation omitted).

161. See *id.* at 10 (internal citations omitted).

entry.¹⁶² That paper details the population genetic structure of belugas using 410 bp of mitochondrial DNA sequence sampled from 324 individuals over a total period of eighteen years.¹⁶³ It is an impressive sampling effort, given its vintage, and the analysis is competently done: the authors include summary statistics (haplotype and nucleotide diversity, and an estimate of migration rates among populations), distributional statistics (F_{ST} and the closely related Φ_{ST}), and an analysis of the evolutionary relationship among sampled alleles presented as a network diagram. It therefore applies most of the types of analyses described above.

Despite consisting of DNA sequence data, O’Corry-Crowe and coauthors’ dataset contains relatively few alleles: though they sampled 410 nucleotides, only 19 of those sites were variable, yielding only 29 distinct alleles.¹⁶⁴ As a result, their information content is limited with respect to evolutionary/relationship analysis because only few characters are available for reconstructing historical relationships. Nevertheless, the distributional signal in the data is incredibly strong, documenting very large values of F_{ST} and Φ_{ST} , suggesting that migration among populations has been severely limited (or nonexistent) for an extended period of time.¹⁶⁵ The authors reinforce this conclusion with field observations as well, and link the observed genetic pattern to historical glaciation in the area, albeit speculatively. As population genetics go, this is a slam dunk—relatively unambiguous evidence of demographic and historical separation among populations.¹⁶⁶

In this case, NMFS treated the data fairly well, capturing the main findings of the source paper’s authors, and applying them in a reasonable fashion to the Cook Inlet population. The main questionable judgment on the part of the agency is one of omission: If the Cook Inlet population of belugas is a DPS, why aren’t the others? The final rule correctly notes that the Cook Inlet is “the most isolated” genetically and geographically,¹⁶⁷ but Cook Inlet is only marginally more genetically distant from other populations than are the Bristol Bay or Eastern Beaufort Sea populations. These other populations are discrete by any

162. See G.M. O’Corry-Crowe et al., *Phylogeography, Population Structure and Dispersal Patterns of the Beluga Whale Delphinapterus Leucas in the Western Nearctic Revealed by Mitochondrial DNA*, 6 MOLECULAR ECOLOGY 955 (1997).

163. See *id.* at 957, 960.

164. See *id.* at 960.

165. See *id.* at 962.

166. Again, this is given the data in hand. Further data could muddy the waters, as it were, but the agency must make decisions based on the available data. Also note that O’Corry-Crowe et al., *supra* note 162, does not discuss the possibility that the observed low genetic diversity is a result of a decline in population size, which is a general concern for imperiled species.

167. See Endangered Status for the Cook Inlet Beluga Whale, 73 Fed. Reg. 62,919, 62,926 (Oct. 22, 2008) (to be codified at 50 C.F.R. pt. 224).

reasonable meaning of the word, given the data, yet they are not discussed in the *Federal Register* or the status review. It seems likely that, because the listing petition only requested the agency evaluate the Cook Inlet population,¹⁶⁸ the agency restricted its evaluation to that population alone. Such narrow focus is perhaps instructive: agencies may understandably act to minimize the political flack a broad listing could generate.

Nevertheless, the case of the beluga whale is an example of an agency's responsible treatment of data in evaluating a DPS. While more data might have been desirable given the limits of mtDNA and the low diversity of this dataset, the agency used the primary literature appropriately to bolster the beluga's DPS listing. However, the primary data would have been difficult for non-agency personnel to find or evaluate had the status reviews, which provide a full references section, not been available online. Neither the final rule nor the agency website made the original data sources accessible or even provided the full citations of the cited references.

B. *Cactus Pygmy Owl*

The cactus ferruginous pygmy owl (*Glaucidium brasilianum cactorum*), which has been the object of an ESA ping-pong match in Arizona for more than a decade, is an example of less responsible treatment of more equivocal genetic data.

The FWS originally listed the owl's Arizona population as an endangered DPS in 1997, without the benefit of genetic data.¹⁶⁹ Rather, the service based the DPS delineation in part on the *potential* genetic differences between the Arizonan and Mexican owl populations.¹⁷⁰ The lack of actual genetic data ultimately undermined the agency's defense of its listing decision, and by the time a court remanded the decision to the agency, FWS had decided the owl did not merit ESA protection.¹⁷¹

In the 1997 listing decision, FWS met the "discreteness" prong of the then-new DPS policy by employing the international border provision,¹⁷² which specifies that a species' population isolated by an international boundary may qualify as discrete due to differential management on

168. See Proposed Endangered Status for the Cook Inlet Beluga Whale, 72 Fed. Reg. 19,854, 19,855 (Apr. 20, 2007) (to be codified at 50 C.F.R. pt. 224).

169. See Determination of Endangered Status for the Cactus Ferruginous Pygmy-owl in Arizona, 62 Fed. Reg. 10,730 (Mar. 10, 1997) (to be codified at 50 C.F.R. pt. 17).

170. See *id.* at 10,731.

171. See Final Rule to Remove the Arizona Distinct Population Segment of the Cactus Ferruginous Pygmy-owl from the Federal List of Endangered and Threatened Wildlife, 71 Fed. Reg. 19,452 (Apr. 14, 2006) (to be codified at 50 C.F.R. pt. 17) [hereinafter Final Arizona DPS Removal Rule].

172. See DPS Policy, *supra* note 39, at 4725.

either side of the border. The agency addressed the “significance” prong in part by finding that losing the Arizona owls “would decrease the genetic variability of the taxon,”¹⁷³ though no genetic data existed to support this finding directly.

Subsequently, the Ninth Circuit struck down the DPS listing in *National Ass’n of Home Builders v. Norton*, finding the listing lacked a rational basis under the FWS/NMFS joint policy of 1996.¹⁷⁴ In particular, the court held that while designating the Arizona populations as discrete was not arbitrary, designating them as significant was.¹⁷⁵ The holding rested in large part on the agency’s lack of evidence for the owl’s supposed genetic differences between the Arizonan and Mexican populations.¹⁷⁶

The court remanded the listing decision to FWS.¹⁷⁷ As a result, the agency faced a decision: it could propose to list the owl DPS on the basis of genetic data that, by 2003, it had had in hand for two years, or it could propose to delist the owl. In the wake of *National Ass’n of Home Builders*, the regional FWS office asked the Arizona Ecological Services Office to evaluate the science surrounding the owl.¹⁷⁸ In an internal white paper, the Ecological Services Office described several grounds for discreteness and significance, and presented compelling evidence in favor of protection using genetic data that had become available since the original listing.¹⁷⁹ Nevertheless, FWS opted to delist the owl, issuing a notice of proposed rulemaking in 2005.¹⁸⁰

1. *The Available Data*

In January 2001, more than two years before *National Ass’n of Home Builders*, Pima County received the first genetic study of the pygmy owl, conducted by the “leading pygmy-owl biologist in the United States,” Glenn Proudfoot, with coauthor R. Douglas Slack.¹⁸¹ The county

173. Determination of Endangered Status for the Cactus Ferruginous Pygmy-owl in Arizona, 62 Fed. Reg. 10,730, 10,737 (Mar. 10, 1997) (to be codified at 50 C.F.R. pt. 17).

174. *Nat’l Ass’n of Home Builders v. Norton*, 340 F.3d 835, 852 (9th Cir. 2003).

175. *See id.*

176. *See id.* at 847.

177. *See id.* at 852.

178. *See* ARIZ. ECOLOGICAL SERVS. OFFICE, U.S. FISH & WILDLIFE SERV., WHITE PAPER: SIGNIFICANCE OF THE WESTERN POPULATION(S) OF THE CACTUS FERRUGINOUS PYGMY-OWL 318 (2003).

179. *See id.* at 321–28.

180. *See* Proposed Rule to Remove the Arizona Distinct Population Segment of the Cactus Ferruginous Pygmy-owl from the Federal List of Endangered and Threatened Wildlife, 70 Fed. Reg. 44,547 (Aug. 3, 2005) (to be codified at 50 C.F.R. pt. 17) [hereinafter Proposed Arizona DPS Removal Rule]. The delisting became final a year later. *See* Final Arizona DPS Removal Rule, *supra* note 171.

181. *See* Memorandum from C.H. Huckelberry, County Administrator, to Pima County Board of Supervisors 1 (Jan. 22, 2001) (on file with author).

had commissioned the study to aid with management of the species, particularly in the context of the Sonoran Desert Conservation Plan.¹⁸²

The Proudfoot and Slack study sampled 899 bp of mtDNA in birds from a variety of locations in Texas, Arizona, and Mexico, as well as an individual from Argentina for comparison.¹⁸³ Using a total of ninety-five birds, the authors calculated summary statistics and estimated relationships among individuals using tree-building methods, but did no distributional or frequency analyses, as might have been instructive.¹⁸⁴ Broadly, they found the Arizona and Texas populations were isolated from one another, and both had low genetic diversity relative to the Mexican populations.¹⁸⁵ Crucially, Proudfoot and Slack found “the low haplotypic diversity and distinct clade occurring in NW Tucson suggests current separation between populations in NW Tucson and populations in the Altar Valley [Arizona], Sonora, and Sinaloa.”¹⁸⁶ Hence, though the signal was subtle, the only existing genetic data provided some evidence for genetic isolation between the northwestern Arizona and Mexican populations.¹⁸⁷

While other language in the report gives mixed messages concerning its management implications, and the level of data analysis could have been more thorough, Proudfoot and Slack had added to FWS’s earlier suspicions that the Arizona owl population was genetically unique. Nevertheless, neither party in *National Ass’n of Home Builders* addressed these data, presumably because the data had not been available at the time of the original 1997 listing decision and thus could not be used to defend that decision.

Subsequent data provide stronger evidence for demographic separation between Arizona and Mexico. A paper that Proudfoot et al. published in 2006 demonstrates that the Arizona populations are more inbred than those in Mexico, have a higher proportion of unique alleles, and have statistically significant values of F_{ST} and related statistics,

182. *Id.* The present versions of the *Plan* are available at SONORAN DESERT CONSERVATION PLAN, <http://www.pima.gov/cmo/sdcp> (last visited Oct. 31, 2010).

183. See GLENN A. PROUDFOOT & R. DOUGLAS SLACK, COMPARISONS OF FERRUGINOUS PYGMY-OWL MTDNA AT LOCAL AND INTERNATIONAL SCALES 5 (2001).

184. See *id.* at 4–5.

185. See *id.* at 5–6.

186. *Id.* at 6.

187. The authors did not calculate the F_{ST} between Arizonan and Mexican (Sonora) populations, but provided a table of pairwise genetic distance between each sampled individual. See *id.* at 20–27 tbls.1–2. Using these values, I calculated $F_{ST} = 0.166$ using the formula $F_{ST} = ((\text{average distance between populations}) - (\text{average distance within populations})) / (\text{average distance between populations})$. This is a rough measure, but agrees well with the authors’ subsequent finding of $R_{ST} = 0.186$ using microsatellites. *Id.* R_{ST} is an analog of F_{ST} . See Proudfoot et al., *infra* note 188. These calculated values for population subdivision suggest an extremely attenuated level of connectivity between Arizonan and Mexican owl populations.

suggesting greatly limited gene flow between the populations.¹⁸⁸ This much larger dataset, employing microsatellites and many of the population genetics analyses described above, provides a thorough look at the owl species and fully supports FWS's inferences in the original 1997 listing decision.

2. Agency Actions and Treatment of the Data

FWS issued its proposed rule to delist the owl on August 3, 2005, with comments to be accepted until October 3, 2005.¹⁸⁹ The agency first noted that it expected more genetic data from the biologists and that it would review such data when available:

With regard to genetic variability . . . we did not have evidence of genetic differences between pygmy-owls in Arizona and northwestern Mexico. . . . Glenn Proudfoot, Texas A&M University, will shortly complete some additional pygmy-owl genetic analysis using a different methodology. These analyses are expected to be available very soon and may be relevant to our final decision. *We will review this information when it becomes available.*¹⁹⁰

The agency then summarized the earlier work by Proudfoot, coming to inconsistent conclusions. The proposed rule notes both that the northwest Tucson population is genetically unique, forming a clade, while concluding the same paragraph by disavowing a marked genetic difference between owls in Arizona and Mexico:

Recent pygmy-owl genetic work, done by Proudfoot at Texas A&M, presents evidence that genetic divergence occurs in both Arizona and Sonora, Mexico. *A distinct genetic clade exists in northwest Tucson* and genetic separation exists between Sonora and Sinaloa indicating that separate groups of pygmy-owls, including Arizona, contribute to the overall genetic diversity of this subspecies (Proudfoot and Slack 2001). Genetic divergence tends to occur at the periphery of a species' range. The peripheral nature of the Arizona pygmy-owls may increase the potential for the population to diverge from populations in Sonora and Sinaloa, Mexico. Because peripheral populations may be isolated to some extent from core populations, peripheral populations may become genetically distinct because of genetic drift (random gene frequency changes in a small population due to chance alone)

188. See Glenn A. Proudfoot, Rodney L. Honeycutt, R. Douglas Slack & Michael F. Ingraldi, *Variation in DNA Microsatellites of the Ferruginous Pygmy-owl (Glaucidium brasilianum)*, 7 CONSERVATION GENETICS 945 (2006). Though the authors report statistically significant levels of population genetic subdivision in the form of F_{ST} and related statistics, I should note that Proudfoot does not consider these values to represent "markedly different" populations in Arizona and Mexico. See E-mail from Glenn A. Proudfoot, Research Associate, Vassar College, to author (Nov. 24, 2009, 06:05 PST) (on file with author).

189. See Proposed Arizona DPS Removal Rule, *supra* note 180, at 44,547.

190. *Id.* at 44,550 (emphasis added) (citation omitted).

and divergent natural selection (the natural process by which organisms leave differentially more or fewer descendants than other individuals because they possess certain inherited advantages or disadvantages). *However, we have no evidence to suggest a marked genetic difference between the Arizona pygmy-owls and the rest of the western pygmy-owls.*"¹⁹¹

The only way to read this paragraph as internally consistent is that the inclusion of the Altar Valley, Arizona population¹⁹² disqualified the northwest Tucson population (which FWS acknowledged as genetically unique) from being protected as a DPS. The Altar Valley population, south of Tucson, is more closely related to those populations in Mexico than it is to the northwest Tucson samples.¹⁹³ As a result, FWS could rightly say that the Arizona owls, *taken as a whole*, are not markedly genetically different from those in Mexico.

It is not clear that this was the logic of the FWS in proposing to delist the owl; the Altar Valley population was not mentioned in either the proposed or final rule to delist. Had the agency considered the new data available in mid-2005, it could not have come to the same conclusion: all Arizona populations, considered together, are substantially distinct from those in Mexico according to extensive sampling with microsatellites,¹⁹⁴ perhaps the ideal data type for assessing the kind of subtle genetic structure the owls seem to exhibit. Despite their earlier indication to the contrary,¹⁹⁵ FWS did not consider Proudfoot's new genetic work in the interim between issuing the proposed and final rules, saying the work contained "no new information."¹⁹⁶

Thus the final rule removed the owl DPS from the endangered species list,¹⁹⁷ despite FWS's acknowledgment that the northwest Tucson population, which seems to have comprised the bulk of the DPS, was unique from all other populations.

191. *Id.* (emphasis added) (citations omitted).

192. See reference to this population in PROUDFOOT & SLACK, *supra* note 183.

193. See PROUDFOOT & SLACK, *supra* note 183.

194. See Proudfoot et al., *supra* note 188.

195. See *supra* note 189 and accompanying text.

196. See Final Arizona DPS Removal Rule, *supra* note 171, at 19,454 ("No new information related to the Arizona DPS is presented that is not already found in [PROUDFOOT & SLACK, *supra* note 183], which is available to the public and cited in our proposed rule. We did not rely on any of the work within Dr. Proudfoot's unpublished papers in making our determination."). Proudfoot et al. had submitted the microsatellite data paper to CONSERVATION GENETICS on July 25, 2005, before FWS issued its Proposed Rule. The dataset eventually contained in their 2006 CONSERVATION GENETICS article, *supra* note 188, was thus available to FWS during the comment period, though the above quote from the Final Rule makes it clear that the agency did not consider the new data to be of any value. Proudfoot has refused to speculate on why the FWS did not take the microsatellite data into account. See E-mail from Glenn A. Proudfoot, Research Associate, Vassar College, to author (Nov. 24, 2009) (on file with author).

197. See Final Arizona DPS Removal Rule, *supra* note 171, at 19,454.

3. *Analysis*

The FWS has the discretion to list species as endangered or threatened, subject only to the statutory requirement that the agency use the “best scientific and commercial data available.”¹⁹⁸ In the case of the cactus ferruginous pygmy owl, the only available genetic data demonstrated that the Arizona populations of the owl were distinct from those in Mexico based on two different genetic data types: Proudfoot and Slack’s 2001 work using mtDNA showed that the northwest Tucson owls were in a distinct evolutionary clade, and the later 2006 work (available in 2005, though not yet published) showed statistically significant, and high, values of F_{ST} and related statistics (suggesting an acute lack of gene flow between Arizona and Mexico).¹⁹⁹

Even without considering the more powerful microsatellite work, the 2001 mtDNA data arguably indicate that Arizona populations of the owl have marked genetic differences from those in Mexico. If the agency determined that the Altar Valley birds should have been delisted for lack of genetic differentiation from their Mexican counterparts, it made no such finding, and the best available data supported the discreteness of the northwestern Tucson populations. Furthermore, “marked” genetic differences²⁰⁰ were not necessary to remedy the Ninth Circuit’s objection to the DPS listing. A finding that the loss of the Arizona owl population would have created a gap in the species’ range, and that the attendant loss of genetic diversity would make that gap significant, likely would have been sufficient.²⁰¹ Thus FWS moved its own goalposts in opting to delist the owl rather than begin another listing process to remedy the earlier listing’s shortcomings with newly available data.²⁰²

The agency has since issued a ninety-day finding that a petition to re-list the owl is warranted, though now on the basis of its being a subspecies rather than a DPS.²⁰³ This finding prominently cites the genetic work that was available at the time of the proposed delisting in 2005.

198. 16 U.S.C. § 1533(b)(1)(A) (2006).

199. See PROUDFOOT & SLACK, *supra* note 183, at 12; Proudfoot et al., *supra* note 188, at 951.

200. See *supra* note 188.

201. See *Nat’l Ass’n of Home Builders v. Norton*, 340 F.3d 835, 847 (9th Cir. 2003) (indicating the loss-of-genetic-variability criterion for making the creation of a “gap” in the range of a species significant was speculative absent actual genetic data).

202. Defenders of Wildlife and allied groups subsequently challenged FWS’s decision to delist the owl, in part arguing that the available genetic data favored a finding of significance for the Arizona population under the DPS Policy. In an unpublished memorandum opinion, the Ninth Circuit upheld the FWS delisting decision without discussing the treatment of genetic data. See *Nat’l Ass’n of Home Builders v. Norton*, 2009 WL 226048, at *1 (9th Cir. Jan. 30, 2009).

203. See *Ninety-Day Finding on a Petition to List the Cactus Ferruginous Pygmy-Owl as Threatened or Endangered with Critical Habitat*, 73 Fed. Reg. 31,418, 31,420. (June 2, 2008).

C. *Lessons from the Two Listing Decisions*

The beluga listing, while an example of responsible agency use of available genetic data, also illustrates the narrow focus of an agency faced with managing diminishing natural resources under pressure to minimize regulation and maximize economic development. Nevertheless, the beluga's genetic patterns were clear-cut, strongly indicating a lack of interchange among populations and easily supporting the agency's conclusion that the Cook Inlet population was both discrete and significant to the taxon as a whole.

The pygmy owl is a counterexample, an illustration of the agency's discretion to parse more ambiguous genetic data in such a way as to mute its significance, or perhaps to ignore the data altogether. As I noted above, no dataset is ideal, and reasonable scientists can disagree on the implications of a genetic dataset. But a good-faith effort on the part of FWS to incorporate the known genetics of the owl might well have led to the opposite conclusion than the one the agency ultimately reached. The Service's own internal white paper had underscored the Arizona population's genetic differences, and the subsequently available microsatellite dataset more powerfully did the same.²⁰⁴ FWS delisted the Arizona DPS of the pygmy owl for lack of significance to the taxon as a whole,²⁰⁵ in the face of information that strongly suggested otherwise. This decision points out the slipperiness of the significance prong of the DPS Policy: while a population can be significant for any number of reasons,²⁰⁶ whether to protect a particular population segment is ultimately a discretionary policy judgment on the part of the agency.²⁰⁷

The cases of the beluga whale and the pygmy owl demonstrate dramatically different degrees of data supporting agency DPS decisions, yet these differences are largely obscured in the rulemaking documents and status reviews the ESA requires. Simplifying the source data is, of course, necessary to some degree; the listing agencies apply their own expertise in interpreting the science and molding it into policy, publishing their reasoning in intelligible form. However, a transparent listing process requires that the data driving agency reasoning be available for interested parties to evaluate independently. Frustratingly, in neither case did the

204. See *supra* note 188.

205. See Final Arizona DPS Removal Rule, *supra* note 171. For a discussion of the significance prong of the DPS test, see DPS Policy, *supra* note 39, at 4725.

206. See DPS Policy, *supra* note 39, at 4725 (indicating that the significance of a DPS is not limited to the listed factors, and that "it is not possible to describe prospectively all the classes of information that might bear on the biological and ecological importance of a discrete population segment").

207. *But see* Ctr. for Biological Diversity v. Lohn, 296 F. Supp. 2d. 1223 (2003) (remanding agency decision for a failure to use the best available science in considering petition to list the "Southern Resident" population of orca as a DPS), *vacated as moot*, 511 F.3d 960 (9th Cir. 2007).

listing agency make directly available the references or datasets the agencies used to come to their conclusions.

The *Federal Register* entry²⁰⁸ for the beluga listing offers no substantive discussion of the data underlying its claims, and nor perhaps should it, as the publication is enormously long and unwieldy as it is. But the cited references for the relevant genetic data do not appear, either. Instead, the following appears under the “References Cited” section of the entry: “A complete list of all references cited in this rulemaking can be found on our website at <http://www.fakr.noaa.gov/> and is available upon request from the NMFS office in Juneau, Alaska.”²⁰⁹ However, searching on the referenced website by browsing, text-searching, and using the site’s search box failed to locate the relevant references. As a result, the final rule provides no direct access to the information on which the agency based its decision.

Identifying and obtaining the relevant data that influenced the agency’s owl decisions was even more difficult. As in the case of the beluga, the *Federal Register* cited to documents that had no corresponding references. However, in the owl’s case, the final rule specified that a list of the references was available by mail from the Arizona Ecological Services Field Office.²¹⁰ No status reviews are available online for the owl, and the identity of the document the proposed and final rules cite prominently²¹¹ became clear only when I contacted the first author. I obtained the document only after requesting (by phone) the Arizona Ecological Services Field Office to find and digitize it.²¹²

The agencies’ failure to provide the referenced primary work creates a significant barrier to external review of agency processes. The availability of such information is critical for maintaining agency transparency, important not least because it forms the basis of a record of reasoned decision making that can withstand judicial review.

208. See *Endangered Status for the Cook Inlet Beluga Whale*, 73 Fed. Reg. 62,919 (Oct. 22, 2008) (to be codified at 50 C.F.R. pt. 224).

209. *Id.* at 62,930. This is typical of ESA rules and proposed rules in the *Federal Register*.

210. See *Final Arizona DPS Removal Rule*, *supra* note 59, at 19,458. Making references available by mail is nearly ubiquitous in FWS listing decisions; NOAA/NMFS routinely references the agency’s website.

211. PROUDFOOT & SLACK, *supra* note 183.

212. I am grateful to FWS’s Nick Carillo for his help in finding, scanning, and emailing the 2001 report. In this case, the process took several weeks, but given less enthusiastic help from an agency office, one imagines that obtaining documents might take longer than the comment period on a proposed rule.

CONCLUSION

Genetic data increasingly inform ESA listing decisions, providing high-resolution information about imperiled species. The drawback of genetics, however, is that its technical nature has the potential to exclude those without specialized training from participating in the decision-making process. Because science alone cannot determine the “solutions” to resource management questions, a degree of judgment inheres in any policy decision.²¹³ But judgment calls must not be cloaked in the seemingly objective language of science. While reasonable people can disagree over a policy decision, reasonable people cannot pretend they made no decision at all. Decisions of the kind made in the case of the pygmy owl would be much more consistent with the underlying science if the data in hand were weighed in a public and transparent way.

Each type of data and analysis has its strengths and weaknesses, with discretion in the hands of the researcher as to which methods to apply to what problem. Datasets for rare species are often fragmentary, with small sample sizes, compounding the uncertainty facing agency decision makers. Finally, the ESA itself and its associated regulations build in enormous discretion as to which species deserve protection, and why.²¹⁴ Making each of these judgments as transparent as possible helps to ensure that agency actions are consistent with the aims of the ESA and its regulatory framework. This transparency, in turn, requires that interested parties see and understand the same data that NMFS and FWS do in making their decisions.

I have used two examples of ESA listing determinations to illustrate some practical impacts of genetics in agency decision making. Clearly, these case studies cannot accurately represent the entire scope of agency actions that make use of genetic data. But the beluga whale and pygmy owl cases point out the tremendous variation in the persuasiveness of the data underlying ESA decisions—in one case, fairly conclusive genetic patterns presented clearly, in the other, more ambiguous data almost marginalized—without those differences being apparent in the proposed or final rules. The cases also illustrate the agencies’ shortcomings in making accessible the primary literature on which the final rules depend. If the primary data are not easily available to the public, public participation in agency decision making is necessarily limited. Lawyers in particular must have this kind of information in hand in order to function effectively as guards against arbitrary agency action. And for their part,

213. See, e.g., Doremus & Tarlock, *supra* note 8, at 36.

214. See, e.g., 16 U.S.C. § 1533(a)(1)(E) (“other natural or manmade factors”); DPS Policy, *supra* note 39, at 4725.

the lawyers should be able to understand what they are looking at once they do have the data in hand.

I will close with some normative suggestions for increasing the availability of the relevant data, thereby encouraging transparent agency decision making.

First, at the very least, agencies must make the full reference lists available on their websites for documents to which proposed and final rules refer. NMFS already refers to its website²¹⁵ in the *Federal Register* as the repository of this reference information,²¹⁶ but if the bibliographic information is there, it is not easy to find. FWS still, in 2010, generally requires that interested parties write to the relevant field office to request the references cited in the *Federal Register* by mail. Making the list of references available online (and it seems very likely the list is in digital form already) would benefit FWS by reducing the time required for staff to fill such requests by mail and would provide critical transparency.

Second, agencies could make available not just the list of references, but also the actual documents to which the rules refer. These documents are generally discoverable in litigation since they form part of the record of the agency's decision,²¹⁷ and they are likely subject to FOIA requests.²¹⁸ Simply making them available would save time and money both for the agencies and the requesting parties, and would serve the strong public interest in participatory agency decision making. This is particularly important for internal agency studies, which are often unavailable even if one has the relevant bibliographic information.

Third, the listing agencies should make the datasets themselves available. Already, genetic datasets in the published literature are often publicly accessible via Genbank, the online public database of genetic information,²¹⁹ but rarely can the critical information on habitat and geographic location be reconstructed. Data from internal agency studies often is altogether inaccessible, though this represents publicly funded work from a federal agency. Simply submitting agency studies' data routinely to Genbank would be a large step forward, and would benefit

215. See ALASKA REG'L OFFICE, NOAA FISHERIES, <http://www.fakr.noaa.gov> (last visited Oct. 20, 2010).

216. See Endangered Status for the Cook Inlet Beluga Whale, 73 Fed. Reg. 62,919 (Oct. 22, 2008) (to be codified at 50 C.F.R. pt. 224).

217. The whole administrative record in an agency rulemaking is subject to judicial review. See 5 U.S.C. § 706 ("[T]he court shall review the whole record or those parts of it cited by a party.")

218. The Freedom of Information Act has a broad presumption of availability of records. See 5 U.S.C. § 552(a)(3) (2006) (requiring agency disclosure of records upon any request reasonably describing such records).

219. See *GenBank Overview*, NAT'L CTR. FOR BIOTECHNOLOGY INFO., <http://www.ncbi.nlm.nih.gov/genbank> (last visited Oct. 3, 2010).

the agencies by serving as a backup in case an agency's office loses the data.

Agencies could institute these few relatively minor changes easily and cheaply, enhancing the credibility, and perhaps the durability, of their ESA determinations. These reforms, along with an increased basic level of genetics comprehension among interested parties, would help to identify agencies' policy judgments for what they should be: reasonable exercises of discretion based on the best available data.

Parties interested in participating in the ESA listing process, such as private individuals and nonprofit or trade organizations, have a responsibility to understand the basics of the relevant science even as it grows increasingly technical. By providing a reference for a basic understanding of genetic data, I have tried to make more accessible the kinds of data and analyses that agencies use. My hope is that this work facilitates external participation in the ESA listing process by making the science more accessible to non-scientists who wish to comment or otherwise meaningfully contribute to an ESA rulemaking. Such participation will help ensure transparency and promote an explicit assessment of the judgment calls that inevitably underlie genetics-based decisions under the ESA.

We welcome responses to this Comment. If you are interested in submitting a response for our online companion journal, *Ecology Law Currents*, please contact ecologylawcurrents@boalt.org. Responses to articles may be viewed at our website, <http://www.boalt.org/elq>.