



American
Genetic
Association

Journal of Heredity, 2015, 1–3
doi:10.1093/jhered/esv026
Letter to the Editor

OXFORD

Letter to the Editor

Genetic Distinctiveness of Alexander Archipelago Wolves (*Canis lupus ligoni*): Reply to Cronin et al. (2015)

Byron V. Weckworth, Natalie G. Dawson, Sandra L. Talbot, and Joseph A. Cook

From the Panthera, New York, NY 10018 (Weckworth); the College of Forestry and Conservation, University of Montana, Missoula, MT 59812 (Dawson); the U.S. Geological Survey, Alaska Science Center, Anchorage, AK 99508 (Talbot); and the Biology Department and Museum of Southwestern Biology, MSC03 2020, University of New Mexico Albuquerque, NM 87131-0001 (Cook).

Address correspondence to Joseph A. Cook at the address above, or e-mail: cookjose@unm.edu.

Corresponding Editor: Scott Baker

Cronin et al. (2015) examined genetic variability in North American canids, with a primary focus on wolf (*Canis lupus*) populations in southeast Alaska. After exploring broad patterns of variation for single nucleotide polymorphisms (SNPs) in wolves across North America, the authors addressed the taxonomy of selected subspecies of wolves. Their conclusions have implications for conservation of this species in southeast Alaska and elsewhere, as well as management of high-volume old growth forests of the Tongass National Forest. Cronin et al. (2015) discovered significant genetic differentiation between coastal wolves of southeast Alaska and wolves interior to the Pacific coastal mountain ranges of North America. They acknowledge their data are consistent with earlier studies of coastal wolves, including the Alexander Archipelago subspecies (*C. l. ligoni*), which supported their distinctiveness (mitochondrial DNA sequences and microsatellite loci; Muñoz-Fuentes et al. 2009, 2010; Weckworth et al. 2005, 2010, 2011). Surprisingly, however, they conclude their SNP data do not support a subspecies designation of wolves in southeast Alaska. We disagree that the data and analyses presented in Cronin et al. (2015) logically lead to that conclusion.

Cronin et al.'s (2015) demonstration that coastal wolves are distinctive, and that a substantial portion of the genetic variability for this species across North America resides only in these coastal populations, is indicative of a largely independent historical trajectory for these populations. Nevertheless, Cronin et al. (2015) assert that wolves occupying southeast Alaskan habitats are not a subspecies. First, they note that criteria used for subspecies designations are subjective (a criticism of taxonomy generally, and not based on their data). Second, the authors contend that subspecies are expected to be genetically homogenous; that is, the level of population genetic structure within southeastern Alaskan wolves is too high to represent a single subspecies. Finally, although not actually measuring levels of gene flow, Cronin et al. (2015) state that there is gene flow between wolves in one segment (Game Management Unit 1 [GMU1]) of the range of the subspecies *C. l. ligoni*, and wolves within the range

of other proposed subspecies. Thus, despite assertions that subspecies designations are subjective, Cronin et al. (2015) have adopted complete isolation between, and lack of population structure within, proposed subspecies as requisite criteria for subspecies designation. Given that systematists (to our knowledge) require neither of these criteria for subspecies recognition, there exist significant inconsistencies in Cronin et al.'s (2015) taxonomic conclusions. The cumulative scientific evidence demonstrating the distinction of coastal (including island) wolves of southeast Alaska from other wolf populations of North America now includes Cronin et al. (2015); their data are consistent with the early hypothesis (Goldman 1937, 1944) that these coastal wolves were distinctive. Their data also identify considerable genetic structure in wolves within this coastal region that may be consistent with distinctive populations, not surprising given the fragmented nature of the archipelago and studies of other organisms in the region (Cook and MacDonald 2013).

In the *Taxonomy and Management* section (Cronin et al. 2015), the authors suggest *C. l. ligoni* is invalid due to taxonomic revision by Nowak (1995). Nowak's (1995) work (later extended and summarized in 2002) used discriminant function analyses of 10 skull measurements to create a simplified taxonomic framework (many fewer subspecies) for North American wolves. However, Nowak's (1995) characters differed from those shown to be diagnostic in the original description of *C. l. ligoni* (Goldman 1937, 1944). Among the many taxonomic changes suggested for *C. lupus*, Nowak (2002) placed coastal southeast Alaska wolves into a single wide-ranging subspecies, *C. l. nubilus*, which hypothetically extends from the Atlantic to the Pacific coasts. This conclusion is surprising as Nowak's own analyses show the southeast coastal wolves to be morphologically intermediate between *C. l. occidentalis* and *C. l. nubilus*. We note that across a large number of subsequent studies, minimal empirical support exists for the hypothesis that coastal wolves are synonymous with subspecies found east of the coastal cordillera, contrary to Chambers et al. (2012). If a goal of infraspecific classification is to recognize substantive geographic variation (e.g., O'Brien and Mayr

1991; Avise 2004), then genetic, behavioral, and ecological data from coastal wolves (Weckworth et al. 2005, 2010, 2011; Muñoz-Fuentes et al. 2009, 2010; Stronen et al. 2014) refute Nowak's (1995, 2002) proposition that *C. l. ligoni* be subsumed in *C. l. nubilus*.

Later, in *Taxonomy and Management*, Cronin et al. (2015) imply that we should reject the subspecies concept not only for wolves, but for all species. The authors assert that "it is important to acknowledge that subspecies designations, including those of wolves, are generally subjective" (p. 34). It is one thing to assert that *C. l. ligoni* is invalid because one does not subscribe to the subspecies concept, and yet another to demonstrate that this subspecies is invalid based on the consensus from a range of empirical data that transcend morphological and molecular attributes. Our purpose here is not to debate the validity of the subspecies concept, but simply to point out that the conclusions presented in Cronin et al. (2015) are inconsistent with their data. Nevertheless, given their acknowledgment of criteria for subspecies designation and reference to taxonomic treatises that address subspecies taxonomy of wolves, and despite their protests regarding subjectivity, we proceed under the assumption that Cronin et al. (2015) have accepted that the taxonomic unit of interest here is the subspecies.

Although the definition of subspecies has become an increasingly controversial issue, in part due to the use of subspecies as a unit of conservation (Haig et al. 2006), many evolutionary biologists define subspecies as *groups of populations* that are distinguishable and restricted to a geographic region, where characters could overlap to some small degree, and that (as conspecifics) could have the ability to, or may, interbreed with adjoining subspecies (Mayr and Ashlock 1991). By this definition, Cronin et al.'s (2015) finding of population structure among wolves within the Alexander Archipelago does not falsify a subspecies designation; neither does the reported lack of monophyly across SNPs in samples from GMU1 with other southeast Alaska GMUs. In fact, monophyly at nuclear alleles is not always found between species (e.g., vonHoldt et al. 2011), much less subspecies. Limited gene flow (or, alternatively, lack of lineage sorting) between a few northern coastal and continental wolves was surmised in mitochondrial DNA analyses (Weckworth et al. 2010, 2011), although levels of gene flow were hypothesized to be low and insufficient to homogenize nuclear microsatellite alleles in these wolf populations (Weckworth et al. 2005). We submit that few systematists would agree with Cronin et al. (2015) that subspecies should be defined on the basis of complete reproductive isolation (Crandall et al. 2000), and fewer would suggest that subspecies cannot comprise more than one population.

Based on traditional population pairwise F_{ST} values and Bayesian clustering analyses, Cronin et al. (2015) demonstrate that populations within southeast Alaska show high levels of intra-regional differentiation that are similar to levels found between southeast Alaska and other North American wolves, and greater than inter-regional differentiation among interior wolves. Cronin et al. (2015) interpret this to indicate lack of isolation of southeast Alaska, but failed to rigorously test whether the distribution of genetic variation in wolves of the Alexander Archipelago support or reject the subspecies hypotheses called *C. l. ligoni*. Cronin et al. (2015) did not conduct multiple hierarchical-level significance testing, such as hierarchical analysis of molecular variance (Excoffier et al. 1992) that would have identified significant hierarchical partitions in the dataset, or fixed K-clustering analyses that would reveal partitions deeper than the population level. Their analyses that most specifically test for differentiation between regional (subspecies) levels were PCoA (Figure 2, Cronin et al. 2015) and neighbor joining analyses (Figure 4, Cronin et al. 2015), although the latter is of limited value due to failure to report bootstrap values to assess robust nodes. We note, however, PCoA did show two separate

regional-level clusters, one comprised solely of wolves of southeast Alaska (with a single exception of a BC wolf, likely from Vancouver Island), and one comprised almost exclusively of wolves from outside southeast Alaska except for a few mainland coastal individuals. This is not surprising as the coastal mainland, especially near major river drainages that bisect the Coast Mountains, is where multiple distinctive mammal lineages (Cook et al. 2006; MacDonald and Cook 2007) and species (Runck et al. 2009) come into contact. Our concerns with Cronin et al. (2015) also are related to sampling strata (e.g., combining the single Vancouver Island (coastal) sample with 34 samples from interior BC) and repeatability of analyses due to lack of geo-references or archived specimens.

Whether recognizing the coastal wolves as a subspecies or simply a set of distinct populations, 2 points are key: 1) A large set of characters (morphological, behavioral, and ecological), including a series of independent genetic analyses, consistently demonstrates that coastal southeast Alaska wolves are distinctive from continental wolves (those populations found interior of Pacific coastal mountain ranges); and 2) these populations harbor a disproportionately large amount of unique genetic variation of this carnivore in North America. Both points represent scientific evidence of discreteness and significance of the coastal Alaskan wolves within the criteria necessary for agency protection under the Endangered Species Act (Federal Register 1996). These biological findings are not surprising as this region has a dynamic geologic history characterized by isolation of organisms from the continent throughout the late Quaternary. Isolation, which continues today due to high coastal mountains and Holocene fragmentation of the Alexander Archipelago, has produced considerable faunal complexity and a disproportionately large number of endemic lineages (e.g., *Mustela erminea*; Cook and MacDonald 2001; Dawson et al. 2014). Endemics for a number of taxonomic groups are only now being discovered and described due to newly available specimens and novel molecular approaches (e.g., Barry and Tallmon 2010; Sikes and Stockbridge 2013).

Acknowledgements

Sean Farley, Eric Hoberg, Dirk Derksen, Keith Crandall, Bryan McLean, and 2 anonymous reviewers provided helpful reviews. Any use of trade names is for descriptive purposes only and does not imply endorsement by the US Government.

References

- Avise JC. 2004. *Molecular markers, natural history, and evolution*. 2nd ed. Sunderland (MA): Sinauer.
- Barry P, Tallmon DA. 2010. Genetic differentiation of a subspecies of spruce grouse (*Falcapennis canadensis isleibi*) in an endemism hotspot. *Auk*. 127:617–625.
- Chambers SM, Fain SR, Fazio B, Amaral M. 2012. An account of the taxonomy of North American wolves from morphological and genetic analyses. *North Am Fauna*. 77:1–67.
- Cook JA, Dawson NG, MacDonald SO. 2006. Conservation of highly fragmented systems: the north temperate Alexander Archipelago. *Biol Conserv*. 133:1–15.
- Cook JA, MacDonald SO. 2001. Should endemism be a focus of conservation efforts along the North Pacific Coast of North America? *Biol Conserv*. 97:207–213.
- Cook JA, MacDonald SO. 2013. Island life: coming to grips with the insular nature of North Pacific coastal forests. In: Orians GH, Schoen, JW, editors. *Conservation of North Pacific coastal forests*. Seattle (WA): University of Washington Press. p. 19–42.
- Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends Ecol Evol*. 15:290–295.
- Cronin MA, Cánovas A, Bannasch DL, Oberbauer AM, Medrano JF. 2015. Single nucleotide polymorphism (SNP) variation of wolves (*Canis lupus*)

- in southeast Alaska and comparison with wolves, dogs, and coyotes in North America. *J Hered.* 106:26–36.
- Dawson NG, Hope AG, Talbot SL, Cook JA. 2014. A multi-locus evaluation of ermine (*Mustela erminea*) across the Holarctic, testing hypotheses of Pleistocene diversification in response to climate change. *J Biogeogr.* 41:464–475.
- Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics.* 131:479–491.
- Federal Register. 1996. Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. *Federal Register*, 61:4722–4725.
- Goldman EA. 1937. The wolves of North America. *J Mammal.* 18:37–45.
- Goldman EA. 1944. Classification of wolves: part II. In: Young SP, Goldman EA, editors. *The wolves of North America*. Washington (DC): The American Wildlife Institute. p 389–636.
- Haig SM, Beever EA, Chambers SM, Draheim HM, Dugger BD, Dunham S, Elliott-Smith E, Fontaine JB, Kesler DC, Knaus BJ, et al. 2006. Taxonomic considerations in listing subspecies under the U.S. Endangered Species Act. *Conserv Biol.* 20:1584–1594.
- MacDonald SO, Cook JA. 2007. *The mammals and amphibians of Southeast Alaska*. Museum of Southwestern Biology. Special Publication 8, p 191.
- Mayr E, Ashlock PD. 1991. *Principles of systematic zoology*. 2nd ed. New York (NY): McGraw-Hill.
- Muñoz-Fuentes V, Darimont CT, Wayne RK, Paquet PC, Leonard JA. 2009. Ecological factors drive differentiation in wolves from British Columbia. *J Biogeogr.* 36:1516–1531.
- Muñoz-Fuentes V, Darimont CT, Paquet PC, Leonard JA. 2010. The genetic legacy of extirpation and re-colonization in Vancouver Island Wolves. *Conserv Genet.* 11:547–556.
- Nowak RM. 1995. Another look at wolf taxonomy. In Carbyn LN, Fritts SH, Seip DR, editors. *Ecology and conservation of wolves in a changing world*. Edmonton (Alberta): Canadian Circumpolar Institute, Occasional Publication. 35. p. 375–398.
- Nowak RM. 2002. The original status of wolves in eastern North America. *Southeastern Nat.* 1:95–130.
- O'Brien SJ, Mayr E. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. *Science* 251:1187–1190.
- Runck A, Matocq M, Cook JA. 2009. Historic hybridization and persistence of a novel mito-nuclear combination in red-backed voles (genus *Myodes*). *BMC Evol Biol.* 9:114.
- Sikes DS, Stockbridge J. 2013. Description of *Caurinus tlagu*, new species, from Prince of Wales Island, Alaska (Mecoptera, Boreidae, Caurininae). *ZooKeys.* 316:35–53.
- Stronen AV, Navid EL, Quinn MS, Paquet PC, Bryan HM, Darimont CT. 2014. Population genetic structure of gray wolves (*Canis lupus*) in a marine archipelago suggests island-mainland differentiation consistent with dietary niche. *BMC Ecol.* 14:11.
- vonHoldt BM, Pollinger JP, Earl DA, Knowles JC, Boyko AR, Parker H, Gefen E, Pilot M, Jedrzejewski W, Jedrzejewska B, et al. 2011. A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Res.* 21:1294–1305.
- Weckworth BV, Dawson NG, Talbot SL, Flamme MJ, Cook JA. 2011. Going coastal: evolutionary history between coastal British Columbia and southeast Alaska wolves (*Canis lupus*). *PLoS One.* 6:e19582.
- Weckworth BV, Talbot SL, Cook JA. 2010. Phylogeography of wolves (*Canis lupus*) in the Pacific Northwest. *J Mammal.* 91:363–375.
- Weckworth BV, Talbot S, Sage GK, Person DK, Cook J. 2005. A signal for independent coastal and continental histories among North American wolves. *Mol Ecol.* 14:917–931.