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Frank Hailer *et al.*
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Response to Comment on "Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage"

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Nakagome et al. reanalyzed some of our data and assert that we cannot refute the mitochondrial DNA–based scenario for polar bear evolution. Their single-locus test statistic is strongly affected by introgression and incomplete lineage sorting, whereas our multilocus approaches are better suited to recover the true species relationships. Indeed, our sister-lineage model receives high support in a Bayesian model comparison.

Studies of mitochondrial DNA (mtDNA) in polar and brown bears show polar bears deeply nested within brown bear diversity [paraphyly; "standard model" in (1)], with an origin of the extant polar bear lineage around 111 to 166 thousand years ago (ka) (2, 3). Recently, we showed that polar bears form a distinct sister lineage to brown bears based on genomic variation at 14 independently inherited nuclear loci (4) [Fig. 1A; "new model" in (1)] and dated their speciation to 338 to 934 ka using multilocus methods.

Nakagome et al. (1) argue that our results cannot reject the standard model based on re-analysis of a subset of our data (see Fig. 1B) for two reasons:

(i) Time to the most recent common ancestor (T_{MRCA}) of polar bears is smaller than T_{MRCA} of brown bears and of brown and polar bears combined.

This observation is entirely compatible with both the standard model and the new model. T_{MRCA} is proportional to effective population size (N_e). Polar bears have a much smaller population size than brown bears and thus have a shorter expected time to coalescence. Intraspecific T_{MRCA} values therefore reflect population size rather than divergence time from another bear species.

(ii) The T_{MRCA} ratios (1) for individual loci do not conform to the multilocus expectations based on the new model (T_{MRCA} ratio = 0.21).

This approach implicitly assumes that if a species tree shows reciprocal monophyly among taxa (Fig. 1A), then individual gene trees will show that same topology. However, gene trees and species trees are not always the same, and coalescence theory predicts that it takes around 4 N_e generations to complete lineage sorting at nuclear loci (5). In bears, this corresp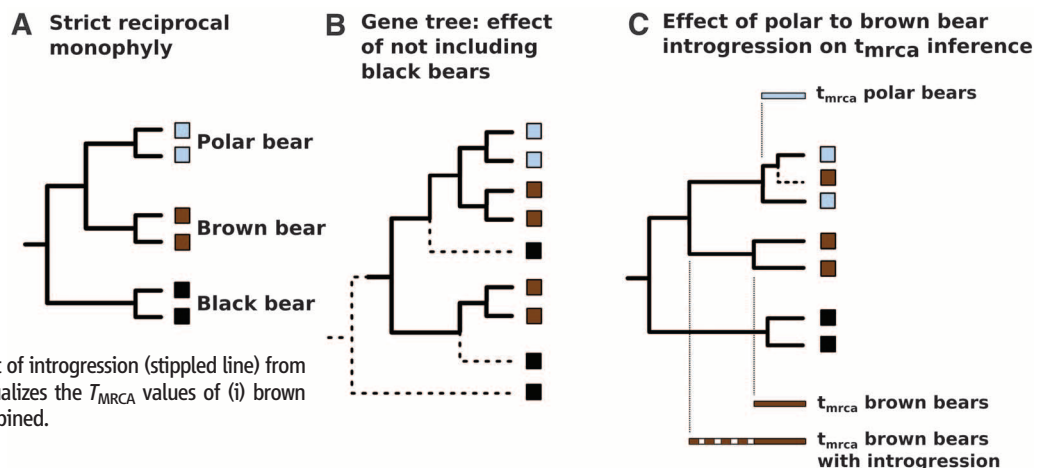onds to 1.12 million years, assuming an effective pop-ulation size of 28,000 (1, 6) and a generation time of 10 years (7, 8). Lineage sorting is thus expected to be incomplete among polar and brown bears, leaving many of their alleles intermingled at gene trees of individual loci [incomplete lineage sorting (ILS)] (Fig. 1B). Similarly, a genomic comparison of human, chimp, and gorilla demonstrated that 30% of bases in their genomes exhibit ILS (9) on time scales of several million years.

Introgression is also known to affect lineage sorting and, thus, T_{MRCA} of DNA sequences (Fig. 1C). Hybridization between polar and brown bears has been confirmed in the wild (10), and clustering of polar bear mtDNA within brown bear diversity likely reflects introgressive mitochondrial replacement in polar bears (3, 4, 6). Gene flow signals from polar into brown bears were present in our data (Fig. 2B), and (6) found 5 to 10% of the genome of some brown bears to be introgressed from polar bears. Hence, due to ILS and introgression, many nuclear loci are not expected to show reciprocal monophyly under either scenario of polar bear evolution. Thus, T_{MRCA} patterns for many individual loci will deviate from the species tree, and $T_{MRCA-uar}$ will approach $T_{MRCA-all}$ (Fig. 1C), as observed in (1). This likely explains why T_{MRCA} ratios of many loci are close to 1 (1), without contradicting the new model.

The analysis of Nakagome et al. did not include the black bear sequences that were part of our original paper (4). Black bears diverged from brown and polar bears approximately 1 to 5 million years ago (4, 6). Nevertheless, five out of 41 (12%) black bear alleles at nuclear introns were shared with brown/polar bears (4), and in many cases they did not cluster ancestrally (Fig. 2). Inclusion of black bear data in the analyses is important, because the results highlight the impact of ILS and introgression in bears on time scales far beyond a few 100 ka (Fig. 1B).

Phylogenetics research has moved toward interpreting gene trees as local optima that show snapshots of the evolutionary history of the studied taxa (11). Recognizing that gene trees do not always reflect species trees, inference of

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Fig. 1. Hypothetical gene trees in bears. (**A**) Idealized phylogeny of black, brown, and polar bear genes; the topology is identical to our species tree (4). This topology does not show introgression and ILS at the single-gene level. (**B**) Inclusion of black bear sequences (stippled lines) highlights the impact of ILS/introgression on gene trees in bears. Without black bears (1), the gene tree appears to have the standard model topology—i.e., polar bears nested within brown bears (see Fig. 2C for real data showing an analogous pattern). (**C**) Impact of introgression (stippled line) from polar into brown bears. Introgression equalizes the T_{MRCA} values of (i) brown bears and (ii) brown and polar bears combined.

Fig. 2. Representative gene trees in bears, based on our data (*4*). (**A** to **D**) In each case except (B) (evidence of polar to brown bear introgression), inclusion of the black bear sequences is crucial for correct interpretation of clades, highlighting the impact of ILS and/or introgression on the gene trees. Gene names and corresponding locus identifications from (*4*) are shown above each tree. The trees and support values (only values above 0.75 are shown) were obtained from Bayesian analyses (*15*) (4 heated chains, 200×10^6 generations each). Black, brown, and blue denote black, brown, and polar bear alleles, respectively. Light brown shows alleles in brown bears from the Alaskan ABC islands.
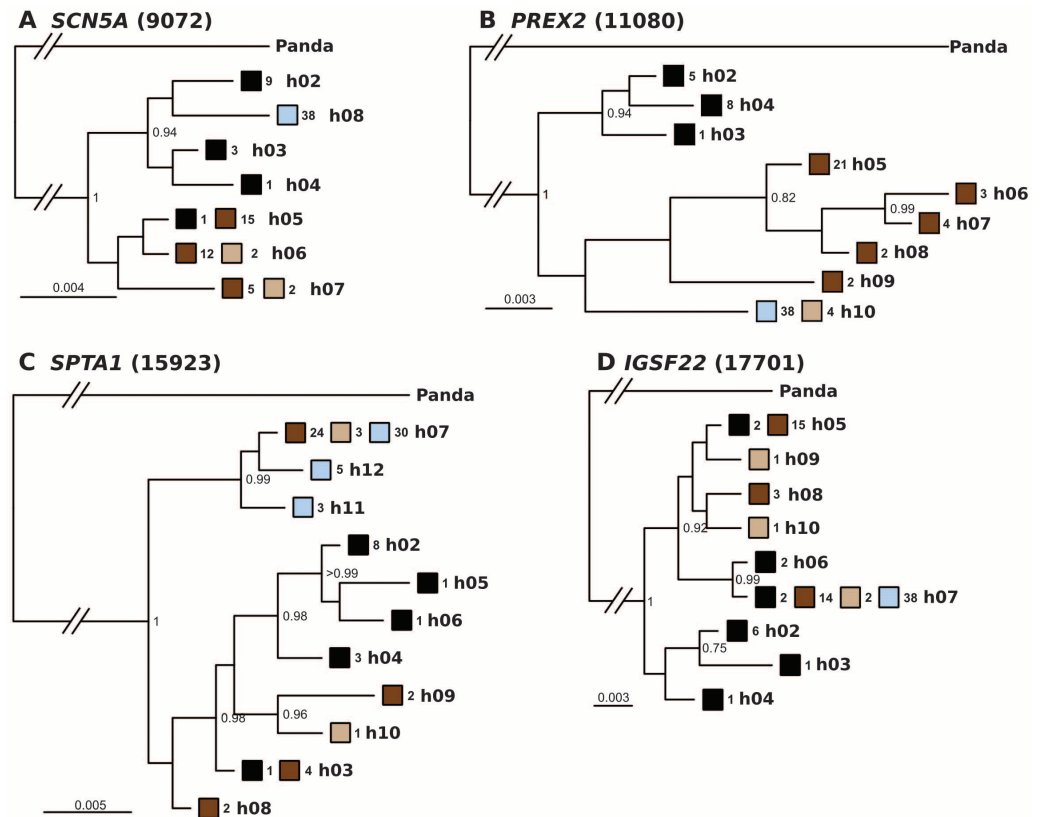
species trees as opposed to single gene trees has become an important goal (*5, 11, 12*). To this end, we jointly analyzed results from individual loci in multilocus analyses (*4*). Results from *BEAST (*13*), concatenation, and the ϕ_{ST} (genetic differentiation)-tree consistently recovered the same topology, with strong support for polar bears as a sister lineage to brown bears ($P > 0.99$), and black bears clustering ancestrally (*4*)—the new model. Inferences in *BEAST can also be affected by introgression. This makes our multilocus estimate of the speciation time conservative (*14*), increasing the contrast between our findings and the standard model. Further, our species-tree based T_{MRCA} of brown bears divided by T_{MRCA} of brown/polar bears combined deviates significantly from expectations for single loci under the standard model ($P = 0.002$) (*1*). This confirms that our nuclear data, analyzed in a multilocus framework, capture a different signal than that predicted by the mtDNA-based standard model.

To explicitly investigate whether our data fit better to the new model [figure 1A in (*4*)] or whether the standard model was better or equally consistent with our data [as suggested by (*1*)], we performed new analyses using *BEAST. We enforced constraints on the species tree based on (i) the standard model, constraining polar bears to

form a monophyletic group with brown bears from the Alaskan Admiralty, Baranof, and Chichagof (ABC) islands (*2, 3*); and (ii) the new model, constraining monophyly of ABC-island brown bears with North American mainland brown bears [as in our unconstrained species tree (*4*)]. The latter was done to confirm that constraining a certain species tree topology per se did not affect the analysis. This confirmation enabled us to perform statistical model comparisons. Consistent with our original interpretation of polar bears being a distinct sister lineage to brown bears, the standard model received significantly lower statistical support than the constrained (ii) and unconstrained (*4*) new models (support for new model: both Bayes factors = 375). Furthermore, recent analyses of genomic data (*6*) have also recovered a sister-lineage relationship among polar and brown bears and interpreted polar bear mtDNA as introgressed from brown bears.

Our data thus strongly support the sister-lineage model, highlighting that the overall evolutionary history of recently evolved taxa is best portrayed by multilocus approaches with appropriate outgroup data, due to the complicating processes of ILS and introgression. "Recently evolved" can span time scales ranging from several hundred thousand to millions of years in

bears, and even longer in species with larger effective population sizes.

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