

Molecular Genetic Analysis Reveals Six Living Subspecies of Tiger, *Panthera tigris*

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Tigers historically inhabited much of Asia and likely numbered near 100,000 as recently as a century ago (Fig. 1). Today's tiger census is much lower, and is estimated by various sources to be around 7000 individuals in the wild (Nowell & Jackson, 1996; Dinerstein *et al* 1997; Kitchener & Dugmore 2000). Tigers have been traditionally classified into eight subspecies (Fig. 1), three of which (*P. t. sondaica*-Javan tiger; *P. t. balica*-Bali tiger and *P. t. virgata*-Caspian tiger) were lost to extinction in the mid to late 20th century. The challenge to preserve the existing tiger populations has become a major goal of conservation efforts throughout their range.

As with many endangered species, tigers have been classified into subspecies - natural geographically separate populations - for purposes of recognition and conservation. The subspecies concept is controversial, but many subspecies including those for tigers are considered as specific units of conservation and are protected by treaties and organizations that are concerned with the management and stewardship of species. For this reason alone, the establishment of a formal subspecies definition, an explicit basis for subspecies recognition, and an understanding of the implications of subspecies assignment become critically important. In 1991, in collaboration with the distinguished evolutionary biologist Ernst Mayr, one of us (SJOB) proposed some working guidelines for subspecies considerations (O'Brien & Mayr 1991).

In that essay we defined subspecies as "geographically defined aggregates of local populations which differ taxonomically from other species subdivisions." To help government regulators recognize subspecies we suggested guidelines. Members of a subspecies share a unique geographical range or habitat, a group of recognizable genetically con-

trolled characteristics, morphological or molecular, and a unique natural history as compared to other subspecies. Since subspecies are not distinct species, they are reproductively compatible and will periodically interbreed with adjacent subspecies. All subspecies have the potential to acquire suitable adaptations to their specific ecological habitat and the longer they are separated the more cumulative adaptation we might expect. All subspecies also have the potential to one day evolve into new species as suggested by Charles Darwin in "On The Origin of Species" in 1886. These two potentials, which are unfortunately not certain for any individual subspecies, nonetheless provide compelling rationale for their conservation management.

Recognition and pronouncement of a subspecies require the description of objective heritable characters that every individual of the subspecies carries, which are in effect diagnostic for the subspecies; that is, they are found only in that subspecies and not in other populations within the same species. Avise and Ball (1991) and we suggested that valid criteria for subspecies include concordant distribution of multiple independent genetic traits. These can be morphological or molecular or both. Traditional morphology-based assessment (body size, skull characters, pelage coloration, and striping patterns) have been applied to tiger subspecies but have been equivocal in adequately describing tiger subspecies (Kitchener, 1999; Herrington, 1987; Mazak, 1981). Previous molecular studies (Wentzel *et al.* 1999; Hendrickson *et al* 2000; Cracraft *et al.* 1998) have also been disappointing in affirming the commonly accepted tiger subspecies designations (Fig. 1).

In December 2004, the culmination of a twenty-year long study to characterize living tiger populations and subspecies differentiation using molecular genetic approaches was published in the new free-online-access journal Pu-

blic Library of Science PLoS-Biology (Luo *et al.* 2004: http://biology.plos-journals.org/archive/1545-7885/2/12/pdf/10.1371_journal.pbio.0020442-S.pdf). In that study, we and twenty additional authors (including members of the IUCN-Cat Specialist Group and pioneers in tiger ecology, behavior, and conservation: Joelle van der Walt, Janice Martenson, Naoya Yuhki, Dale G. Miquelle, Olga Uphyrkina, John M. Goodrich, Howard B. Quigley, Ronald Tilson, Gerald Brady, Paolo Martelli, Vellayan Subramaniam, Charles McDougal, Sun Hean, Shi-Qiang Huang, Wenshi Pan, Ullas K. Karanth, Melvin Sunquist, and James L. D. Smith) examined "voucher specimens" (biological blood and skin materials) from 134 tigers born in the wild at a known location or descended directly from parents of known geographic origins. The paper described the phylogeography patterns using three distinct families of variable genetic markers: 1.) 4000 nucleotide letters of mitochondrial DNA sequence; 2.) a highly variable nuclear DNA sequence FLA-DRB (an immune response gene within the tiger's major histocompatibility complex) and a group of short repetitive nuclear elements called microsatellites. This was a rather large data set analyzed with the most advanced population genetic and phylogenetic computational algorithms available to molecular geneticists. The results were interpreted together and converged on a rather illuminating and in most cases statistically robust (meaning high confidence) picture of the tiger's natural history and subspecies recognition. Here is what the data showed about living tigers.

First, there was strong genetic evidence for the separation and recognition of four of the five traditional subspecies: (1) Amur tiger *P. t. altaica*; (2) Indochinese tiger *P. t. corbetti* (3) Sumatran tiger *P. t. sumatrae* and (4) Bengal tiger *P. t. tigris*. (Fig. 1).

Second, Indochinese tiger *P. t. corbetti*

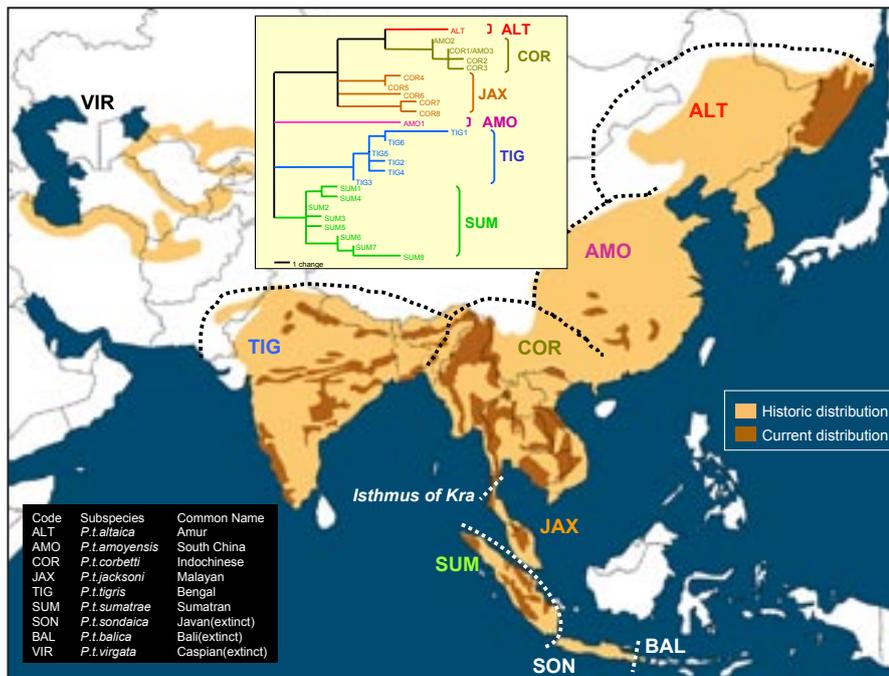


Fig. 1. Historic and current geographic distribution of tigers corresponding to the traditional and the newly defined subspecies designation. Three-letter codes (TIG, ALT, etc.) indicate subspecies abbreviations. Dotted lines are approximate boundaries between tiger subspecies studied here. The inserted diagram depicts phylogenetic relationships among tiger mtDNA haplotypes, a pattern consistent with other molecular markers. Branches of the same color represent haplotypes of the same subspecies. The Isthmus of Kra divides the traditional Indochinese tigers into the northern Indochinese tigers *P. t. corbetti* and the Malayan tigers *P. t. jacksoni* based on the present study. {Modified after Fig. 1 and 3b in Luo *et al.* (2004)}.

showed a distinct partition into two separate groups, each as distinctive from each other as were the other subspecies (e.g. Bengal versus Amur tigers). One Indochinese group was geographically located in the Malayan peninsula, while the second distributed across the traditional range of *P. t. corbetti* in southeast Asia (Fig. 1).

Third, our sampling of South China tiger *P. t. amoyensis* was sparse, including only five captive animals from two Chinese zoos. Nonetheless, these animals defined two very distinct genetic lineages: one which is unique and distinct from the other subspecies (AMO1 in Figure 1) and a second which is indistinguishable from mainland *P. t. corbetti* (AMO2, AMO3 in Fig. 1).

Fourth, tigers overall show population genetic variation that is relatively small (see also Wentzel *et al.* 1999) indicating that all tigers derive from a founder effect of a small number of founders that existed 72-108,000 years ago. It is possible that tiger populations were severely reduced by the catastrophic Toba volcano that erupted in Sumatra 73,500 years ago (Rampino & Self, 1992), as were other large mammals of that time and region.

Fifth, the Amur tigers in the Russian Far East, estimated at approximately 500 individuals show an extreme reduction in genetic variation indicating a more recent population reduction in

the founders of that subspecies. To our knowledge, physiological correlates or measures of inbreeding depression have not been observed in the Amur tigers.

Based on these results, we have recommended the recognition and conservation management of six living tiger subspecies (Fig. 1):

- (1) **Amur tiger** *P. t. altaica*;
- (2) **Sumatran tiger** *P. t. sumatrae*;
- (3) **Bengal tiger** *P. t. tigris*;
- (4) **South China tiger** *P. t. amoyensis*. The South China tiger subspecies is presumed extinct in the wild (Tilson *et al.* 2004) and exists today only in captivity. The proposed South China tiger subspecies lineage is tentative due to limited sampling and should be examined among the present captive population managed by the Chinese Zoo Association.
- (5) **Northern mainland Indochinese tiger** *P. t. corbetti*; and
- (6) **Malayan tiger**. Luo *et al.* (2004)

suggested that the Malayan tiger subspecies be designated *P. t. jacksoni*, to honor the dedication and career of tiger conservationist Peter Jackson, former head of the Cat Specialist Group and Editor of CAT NEWS. An alternative proposal from Mohd Nawayai Yasak, Chairman of the Malaysian Association of Zoos, Parks and Aquaria suggested *P. t. malayensis* to emphasize the geography of the new subspecies. We have compromised by suggesting two names:

common name Malayan tiger and Latin name *P. t. jacksoni*.

The results have important implications for tiger conservation and management. Specifically, they would suggest that the newly designated Malayan subspecies be recognized and managed as a high priority in Malaysia as has occurred in the other host nations of tigers. In addition, an explicit genetic assessment of the captive Chinese tigers should be conducted to validate the uniqueness or non-uniqueness of South China tiger, or indeed the survival of *P. t. amoyensis*. As both *P. t. corbetti* and *P. t. amoyensis* are labeled *P. t. amoyensis* in our samples and perhaps also in zoos, each individual South China tiger in captivity should be identified using the molecular genetic markers that discriminate the two subspecies (Luo *et al.* 2005). We remain available for support, counsel, and encouragement of this important initiative.

There remain important unanswered questions for tiger systematics, classification and conservation. Are there morphological correlates that parallel these molecular subspecies partitions? In molecular terms, are the three recently extinct subspecies equally as distinctive as living subspecies? What is the subspecies structure of admixed zoo-bred generic tigers, numbering in the thousands in captivity? Does the South China tiger exist as a distinct entity in captivity or is

admixture a problem. The tiger community has the needed expertise and technology at its disposal to address each of these questions and we look forward to even more precise characterization of the subspecies that comprises this icon for world conservation programs.

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Figure 2. Malayan tiger (*P. t. jacksoni*). Photo: UF-Malaysia Tiger Project.