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Report

Subspecies Genetic Assignments of Worldwide Captive Tigers Increase Conservation Value of Captive Populations

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Summary

Tigers (*Panthera tigris*) are disappearing rapidly from the wild, from over 100,000 in the 1900s to as few as 3000 [1, 2]. Javan (*P.t. sondaica*), Bali (*P.t. balica*), and Caspian (*P.t. virgata*) subspecies are extinct, whereas the South China tiger (*P.t. amoyensis*) persists only in zoos [1, 3]. By contrast, captive tigers are flourishing, with 15,000–20,000 individuals worldwide, outnumbering their wild relatives five to seven times [4]. We assessed subspecies genetic ancestry of 105 captive tigers from 14 countries and regions by using Bayesian analysis and diagnostic genetic markers defined by a prior analysis of 134 voucher tigers of significant genetic distinctiveness [5]. We assigned 49 tigers to one of five subspecies (Bengal *P.t. tigris*, Sumatran *P.t. sumatrae*, Indochinese *P.t. corbetti*, Amur *P.t. altaica*, and Malayan *P.t. jacksoni* tigers) and determined 52 had admixed subspecies origins. The tested captive tigers retain appreciable genomic diversity unobserved in their wild counterparts, perhaps a consequence of large population size, century-long introduction of new founders, and managed-breeding strategies to retain genetic variability. Assessment of verified subspecies ancestry offers a powerful tool that, if applied to tigers of uncertain background, may considerably increase the number of purebred tigers suitable for conservation management.

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Results and Discussion

Assignment Test

A relatively small portion (~1,000 individuals) of the world's captive tiger population is managed through coordinated breeding programs geared toward preserving genetic variability that is representative of geographic and subspecies groupings found in the wild. As of 2007, there are approximately 421 Amur (*P.t. altaica*), 295 Sumatran (*P.t. sumatrae*), 72 South China (*P.t. amoyensis*), 198 Bengal (*P.t. tigris*), 14 Indochinese (*P.t. corbetti*), and 113 Malayan (*P.t. jacksoni*) tigers in captivity as recorded in regional and international zoo studbooks [6–12]. However, nearly all other captive tigers are of hybrid or unknown origins and are kept in zoos, farms, breeding facilities, circuses, and private homes for entertainment, commerce, pets, and ostensibly for conservation [4]. Debates persist over the role of captive tigers in conservation efforts, whether managed captive populations serve as adequate genetic reservoirs for the natural populations, and whether the presumptive "generic" tigers have conservation value. The most direct way to address the dilemma is through a thorough understanding of the genetic ancestry, the extent of genetic admixture, and the level of genetic diversity of captive tigers relative to wild populations.

Based on the subspecies diagnostic genetic markers obtained from the panel of 134 "voucher" tigers [5], we developed a stringent strategy for evaluating the subspecies affiliation of a tiger with unknown genetic origin. First, mitochondrial DNA haplotypes from 4 kb cytoplasmic mtDNA sequences were constructed to assign maternal lineage-subspecific ancestry based on its phylogenetic relationship to the voucher specimen subspecies group. Second, we used Bayesian clustering-assignment analysis implemented in the program STRUCTURE [13] based on 30 biparentally inherited tiger microsatellite loci to calculate the likelihood (q) that a tiger could be assigned to one of the six extant subspecies or, alternatively, the extent of admixture between subspecies. The reference voucher subspecies clusters were used as prior population information in the analysis. Individuals were considered to have a single verified subspecies ancestry (VSA; i.e., they belong to the specific subspecies with high probability) if they were consistently supported by both mitochondrial lineage and microsatellite genotype assignment results (e.g., $q \geq 0.90$) with a high confidence interval (0.8–1; Table S1). Individuals with a discrepant subspecies ancestry assignment from mitochondrial and microsatellite data or those with affiliations (e.g., $0.2 < q < 0.8$) to two or more subspecies based on microsatellite assignment tests were classified as admixed tigers. Specimens with only mitochondrial data were considered to have incomplete evidence.

Subspecies genetic ancestries were characterized for 105 captive tigers with various degrees of uncertainty in their origins. The samples had been collected over 20 years (1982–2002) from zoos or private owners in 14 countries or regions, including the United States, the United Kingdom, China, Japan, Singapore, Ukraine, Mexico, Germany, Estonia, Indonesia, Taiwan, Cambodia, Thailand, and Malaysia. Based upon the above-mentioned subspecies-identification criteria, 49

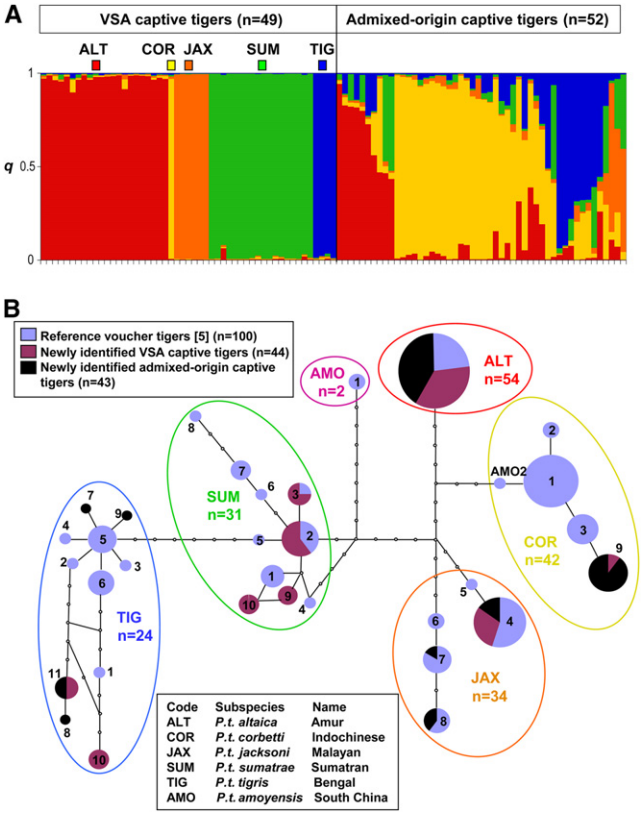

Figure 1. Subspecies Genetic Ancestry of a Worldwide Sample of Captive Tigers

(A) Bayesian population clustering analysis of 105 captive tigers assigned 49 individuals with VSA, 52 with admixed origins, and 4 undetermined (not shown). Genotypes from 30 microsatellite loci were analyzed by using the prior population information option (from voucher tigers [5]) as set in STRUCTURE [13]. Each individual is represented by a thin vertical bar (defined by tick marks under colored regions) partitioned into five colored segments representing individual affiliation (*q*) to five indicated tiger subspecies.

(B) Statistical parsimony network of 33 mtDNA haplotypes (4078 bp) from 100 voucher and 87 captive tigers, each defining monophyletic groups of phylogeographic tiger subspecies [5]. The size of each haplotype circle is proportional to the mtDNA haplotype frequency within a subspecies and each is labeled with the subspecies mtDNA haplotype code [5]. Pie chart colors indicate the proportion that are voucher (n = 100), newly identified VSA (n = 44), or newly identified admixed-origin captive tigers (n = 43), based upon both microsatellite and mtDNA subspecies assignments.

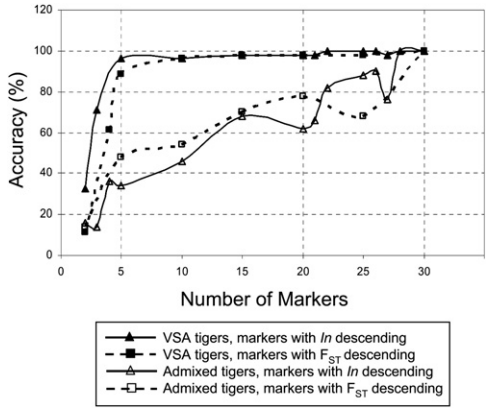

Figure 2. Relative Assignment Accuracy of STRUCTURE in Captive Tigers with Microsatellite Markers

The markers are added incrementally by *In* in descending and ascending order in 49 VSA tigers (closed symbols) and 52 with admixed genetic origin tigers (open symbols) as identified by the full set of markers. Assignment accuracy was determined by comparing the result of assignment tests using a subset of markers to that using the full set of 30 markers.

VSA captive tigers corresponded to a recognized subspecies (21 Amur [*P.t. altaica*], 17 Sumatran [*P.t. sumatrae*], 6 Malayan [*P.t. jacksoni*], 1 Indochinese [*P.t. corbetti*], and 4 Bengal [*P.t. tigris*]), and 52 had admixed subspecies origins (Figure 1A and Table S1). Most (80%) were consistent with the origins provided by owners, including 42 named as a specific subspecies and 41 suspected admixed. Eleven tigers initially identified as purebred were admixed, and VSA origin was confirmed for 7 of 50 (14%) tigers of unknown/admixed subspecies ancestry.

Among the verified admixed-origin tigers, 26 clearly had genetic ancestries from more than one subspecies according to the microsatellite assignment tests. Ten tigers were tentatively assigned to a single subspecies but with confidence levels below 0.80, and 16 tigers had discordant mtDNA haplotype and microsatellite assignments and were classified as admixed. For instance, 11 tigers from Thailand and Cambodia were *P.t. corbetti* with microsatellite data, but the confidence interval was wide (e.g., 0.50–1), and the maternal *P.t. altaica* mtDNA haplotype (ALT) did not support such a designation. Such discordance between maternal and nuclear genealogy may result from asymmetric breeding between two subspecies in captivity. Less likely, this may be from ancient in situ introgression of the ALT haplotype into the *P.t. corbetti* population, which has not been observed to date in wild-born tigers.

Assignment Efficiency of Microsatellite Markers in Identifying Tiger Subspecies

The assignment efficiency of each of the 30 microsatellite loci was assessed by using both F_{ST} and the informativeness-for-assignment index *In* [14], which were derived from allele frequencies of the voucher tiger subspecies samples [5]. The maximal possible value of *In* was 1.79, and *In* ranged from 0.133 in FCA-201 to 1.100 in FCA-5 (Table S2). To determine the minimum number of microsatellite markers necessary to identify the genetic origin of a captive tiger, the relative assignment accuracy for STRUCTURE using a smaller marker set was evaluated by incrementally adding markers in both descending and ascending order of *In* (Figure 2). When only the two highest score markers (FCA-5 and FCA-161) were used, STRUCTURE assignment test was able to identify 17 of 51 or 33% of the captive tigers of VSA genetic origin. The assignment accuracy was >95% using the five most-efficient markers (FCA-5, -161, -91, -211, and -304). In the admixed-origin individuals, 28 markers were needed for >95% consistency with the results from 30 markers. When the five most informative markers were used in the admixed individuals, the accuracy rate was 34% (33 of the 51 were inaccurately assigned). For most inaccurate assignments, an admixed tiger was designated a different admixed origin, although four admixed individuals were mistakenly assigned a single-subspecies origin. With fewer markers, it was more likely to incorrectly assign a purebred individual an admixed origin rather than the opposite. Therefore, extreme caution should be taken when interpreting the admixed origins of a tiger when only a subset of markers is used in the assignment test.

F_{ST} was less informative than *In* as a means of ordering markers by information content, supporting previous

Table 1. Estimated Size and Genetic Variability of Tiger Populations

	Worldwide Census Number		Microsatellite					mtDNA			
Name	Wild	Captive[a]	Sample Size Voucher[b] (VSA Captive)[c] Tigers	Average Observed Hetero-zygosity	Number of Alleles per Locus	Number of Alleles in Voucher Tigers[b]	Number of New Alleles in New Captive Tigers	Sample Size Voucher[b] (VSA Captive)[c] Tigers	Number. of mtDNA Haplotypes	Nucleotide Diversity (π)	MtDNA Haplotype[d,e]
Amur *altaica*	450	421	34 (21)	0.4765	4.03	104	12	13 (18)	1	0	ALT
Indochinese *corbetti*	700–1300	14	33 (1)	0.6349	5.97	181	1	32 (1)	5	1.32×10^{-4}	AMO2, COR1/AMO3, COR2, COR3, COR9
Malayan *jacksoni*	500	113	22 (6)	0.5516	3.90	117	0	22 (6)	5	1.18×10^{-3}	COR4, COR5, COR6, COR7, COR8
Bengal *tigris*	1300–2200	198	6 (4)	0.5126	4.07	105	18	15 (4)	8	3.55×10^{-4}	TIG1, TIG2, TIG3, TIG4, TIG5, TIG6, TIG10, TIG11
Sumatran *sumatrae*	300	295	16 (17)	0.4783	3.77	108	5	16 (15)	10	7.17×10^{-3}	SUM1, SUM2, SUM3, SUM4, SUM5, SUM6, SUM7, SUM8, SUM9, SUM10
South China *amoyensis*	extinct	72	2 (0)	0.3167	1.53	46	n/a	2 (0)	1	0	AMO1
Tigers with purebred origin[f]	3,000–5,000	1116	162	0.5212	7.57	227	–	144	30	2.48×10^{-3}	
Tigers with unknown or undetermined origin	n/a	15,000–20,000[g]	52	0.6795	6.33	190	10	43	9	1.97×10^{-3}	ALT, COR4, COR7, COR8, COR9, TIG7*, TIG8*, TIG9*, TIG11
Total	3000–5000	16,000–21,000	214 (49)	0.5528	7.90	219	46	187 (44)	33	2.21×10^{-3}	33

[a] Approximate numbers of captive tigers registered in regional or international stud books as of 2007 [7–13].
[b] Voucher tigers refer to samples used previously in Tables 6 and 7 in Luo et al. [5], including those wild born from a specific geographic locale or captive born from geographically verified wild-born parents.
[c] Number in parenthesis indicates VSA (Verified Subspecies Ancestry) tigers identified from this study as purebred subspecies.
[d] Underlined mtDNA haplotypes represent new haplotypes found from the study in addition to those reported by Luo et al. [5].
[e] An asterisks (*) indicates mtDNA haplotypes found only in tigers with admixed genetic origins.
[f] Purebred tigers include both the voucher tigers and the newly identified VSA tigers in captivity from this study (samples within parentheses).
[g] Minimum estimates.

simulation studies [14]. Ten markers with the highest F_{ST} values were required to reach >95% assignment accuracy among the purebred subspecies, compared with five markers with the highest *In* (Figure 2). The Spearman rank correlation coefficient between *In* and F_{ST} (Table S2) in the reference voucher tiger subspecies clusters was 0.36 ($p > 0.05$), indicating no correlation.

The genetic differentiation among voucher tiger subspecies, as measured by average pairwise microsatellite R_{ST} and mitochondrial F_{ST}, was highly significant ($R_{ST} = 0.314$, $F_{ST} = 0.838$, $p < 0.0001$) [5]. Therefore, the number of markers required for identifying subspecies ancestry of an individual tiger could be much lower if the individual is known to be purebred [15]. However, this prerequisite is inappropriate because unknown-origin tigers are more likely to have admixed genetic ancestries. We, thus, recommend using the full 30 microsatellite panel combined with mitochondrial haplotype sequencing. Using the voucher tiger dataset as the prior population reference in the STRUCTURE analysis also is critical. Otherwise, related animals might have the tendency to form additional groups (family groups instead of population/subspecies grouping), thus complicating the clustering of the test samples relative to the voucher subspecies. In such cases pedigree and relatedness data also would assist in the evaluation of their conservation value.

Genetic Variation in Captive versus Wild Tigers

The newly tested captive tigers harbored novel alleles and genotypes that extend the endemic diversity observed in voucher samples (Table 1 and Figure 1B). Fourteen mtDNA haplotypes were identified among captive tigers, including eight not found in voucher samples previously (three in VSA tigers, three in admixed ones, and two in both), increasing the reported mtDNA haplotypes in tiger from 25 [5] to 33. The new haplotypes corresponded with either Indochinese (*P.t. corbetti*), Sumatran (*P.t. sumatrae*), and Bengal (*P.t. tigris*) tigers (Figure 1B). There were also 46 new microsatellite alleles (36 in VSA and 10 in admixed tigers) not observed from the

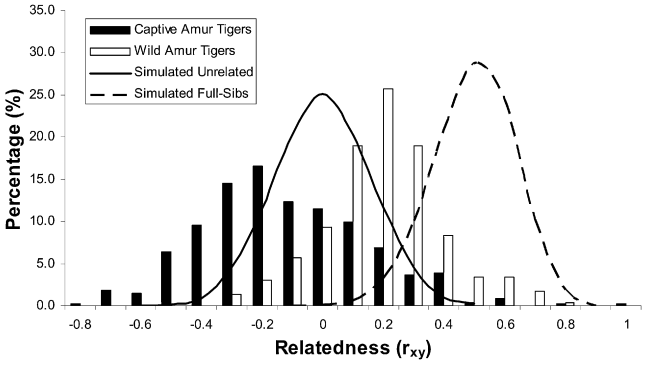

Figure 3. Distribution of Pairwise Relatedness r_{xy} Values for All Pairwise Comparisons within the Wild and Captive Amur Tiger *P.t. altaica* Populations

The global Amur tiger captive breeding programs (solid histogram, n = 32) consist of fewer pairs of closely related individuals than the wild population in Russian Far East (open histogram, n = 25), where the world's largest remaining wild Amur tiger population survives (Mann-Whitney u test, $p < 0.0001$). Distribution of r_{xy} of simulated unrelated individuals and full-sibs are shown in solid and dashed curves, respectively.

voucher specimens. About one-third of the tested captive tigers had evidence of admixed genetic heritages from *P.t. tigris* (Figure 1A). Although possibly a sampling artifact, it might be related to the fact that *P.t. tigris* Bengal tigers have been captive bred since 1880 and widely crossed with other tiger subspecies [7, 8]. Currently admixed tigers are removed from managed-breeding programs when they are identified. However, in the future consideration also may be given to certain hybrid tigers that carry genotypes of value for conservation purposes.

The Amur tiger (*P.t. altaica*) breeding program is the largest among all subspecies with about 420 animals. A similar number of individuals remain in Russian Far East, the largest remaining wild Amur tiger population. All captive Amur tigers we tested had the same mtDNA haplotype as the wild population, and no closely related haplotypes were discovered (Figure 1B). This reduced mtDNA variability may result from a post-ice-age colonization of the region and population bottleneck less than 10,000 years ago and/or the early 20th century when an estimated 20–30 tigers survived intense human persecution [16]. *P.t. altaica* had appreciable microsatellite allele composition and heterozygosity and a nonsignificant difference ($R_{ST} = 0.0029$; $p > 0.05$) between captive and wild populations, suggesting that captive Amur tigers adequately represent the genetic diversity surviving in their wild counterparts.

The distribution of pairwise genetic relatedness values (r_{xy}) suggested that captive Amur tigers (*P.t. altaica*) are less related to each other than their wild counterparts (Mann-Whitney u test, $p < 0.0001$, Figure 3). Wild-born *P.t. altaica* (n = 25) had a mean r_{xy} of 0.194 ± 0.011 (range: −0.344 to 0.786), and captive individuals (n = 32, including both wild-caught and VSA *P.t. altaica* kept in captivity) had a mean of −0.121 ± 0.012 (range: −0.781 to 0.959). Although neither population deviated significantly from the simulated distribution of unrelated individuals (mean of 0.0004 ± 0.001, 95% CI: −0.303 to 0.303, $p > 0.05$), as expected for an outbred population, the captive tiger population was significantly different ($p < 0.01$) from the simulated full-sib pairwise r_{xy} distribution (mean of 0.497 ± 0.001, 95% CI: 0.219 to 0.737), whereas the wild population was moderately different ($0.01 < p < 0.05$).

Previous analyses found captive Sumatran (*P.t. sumatrae*), Amur (*P.t. altaica*), and Bengal (*P.t. tigris*) tigers had equivalent levels of MHC variation as their wild counterparts [5, 17]. Comparable levels of genetic variation probably reflect over a century of captive breeding with a large number of original founders of broad geographic and genetic background, a large, managed interbreeding population, and a continual influx of animals from the wild [6–11]. By contrast, some wild tiger populations may have smaller effective population sizes due to unequal sex ratios, unequal numbers of progeny, and more extreme fluctuations in population size, promoting a more rapid reduction of genetic variation and greater probability of inbreeding [18–20]. The *P.t. altaica* captive breeding program has maintained high genetic diversity and low relatedness among individuals, suggesting the captive population could supplement in situ tiger conservation if necessary.

The captive programs for Malayan tiger *P.t. jacksoni* in North America and Southeast Asia manage about 100 individuals [7, 8, 10]. The 28 Malayan tigers in this study, including both voucher and new VSA tigers, are housed in facilities in Thailand, Malaysia, Singapore, and the United States. The Malayan tiger *P.t. jacksoni* has been classified as a different subspecies from Indochinese tiger *P.t. corbetti* [5], leaving the latter the least represented in captivity, with only 14 recognized as of 2007. Indochinese tigers (*P.t. corbetti*) are mostly kept in Southeast Asian range countries (e.g., Thailand, Vietnam, and Cambodia) and are not part of a coordinated breeding program. We identified one additional purebred Indochinese tiger from the Taipei Zoo in Taiwan. Verification of purebred Indochinese tigers (*P.t. corbetti*), establishment of captive breeding programs, and preservation of remaining populations in the wild should be a priority [21, 22].

Conclusions

Well-managed captive populations of wild animals can assist in public education, research, and fundraising (S. Christie, personal communication) and have been justified as a "genetic reservoir" of their natural counterparts and, thus, insurance against extinction in the wild. Our results demonstrate the power of combining nuclear microsatellite genotyping and mitochondrial genealogy to genetically assign captive tigers of suspected or unknown origin to one or more of the living tiger subspecies.

Because captive and wild tigers today are consciously managed to maintain pure subspecies, the discovery of 49 purebred VSA tigers in a sample of 105 individuals (47%) has potentially important conservation implications. Our sampling likely overestimates VSA tiger prevalence for all captive tigers because 43 of the tigers we tested were enrolled in management breeding programs for designated subspecies. Nevertheless 14 of the 62 unenrolled tigers (23%) show VSA origins, whereas 7 of 50 (14%) tigers of unknown origin were verified as VSA. If 14%–23% of the over 15,000 existing captive tigers would prove to be VSA, the number of tigers with pure subspecies heritage available for conservation consideration would considerably increase. Also, an important fraction of captive tigers retain genetic diversity unreported, and perhaps absent, in the wild populations. A wide-ranging identification of captive VSA tigers to assess their potential for inclusion into comprehensive, integrated in situ and ex situ management plans could significantly increase population sizes and help maintain genetic variability and population viability of this iconoclastic species.

Supplemental Data

Supplemental Experimental Procedures and two tables are available at http://www.current-biology.com/cgi/content/full/18/8/592/DC1/.

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