CHAPTER

3

What Is a Tiger? Genetics and Phylogeography

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Tyger! Tyger! burning bright
In the forests of the night
What immortal hand or eye
Could frame thy fearful symmetry?
William Blake (1794)

OUTLINE

Of all the big cats, or perhaps of all the endangered species, the tiger may be both the most charismatic and the most feared, as phrased in the timeless poem by William Blake [1]. Indeed, what has been the evolutionary history framing the tiger into the exquisite predator that we admire today? Its ancestral roots and history are depicted in its phylogeography, the genetic patterns of diversification among individuals and populations on both temporal and geographical scales. The rapidly changing field of molecular genetics, particularly advances in genome sequence analyses, has provided new tools to reconstruct what defines a tiger and its origins.

GENETIC ANCESTRY OF MODERN FELIDS AND TIGERS

DNA evidence [2, 3] shows that all of the 37 living cat species trace back to a panther-like predator that lived in Southeast Asia in the late Miocene over 11 million years ago (MYA). The radiation of modern felids began with the divergence of the *Panthera* lineage from the ancestral cat species around 10.8 MYA. A few million years later, this lineage diverged into the ancestral species of two groups, one consisting of two species of clouded leopards [4, 5], and the other encompassing the 'great roaring cats' of the *Panthera* genus: the lion (*P. leo*), jaguar (*P. onca)*, snow leopard (*P. uncia*), leopard (*P. pardus*), and tiger [2]. The split of the *Panthera* lineage was followed by a rapid series of divergence and migration events starting around 3.7 MYA that led to the five extant *Panthera* species. Some of the Asian-derived *Panthera* species subsequently spread into America (jaguar and lion), Africa (lion and leopard), and the others remained in Asia (tiger, snow leopard, and clouded leopard). The details of these events remain a matter of conjecture among paleontologists, morphologists, and geneticists [2, 6].

The earliest tiger fossils, found in northern China and Java (Indonesia), date back to around 2 MYA [6, 7]. By the end of the Pliocene and beginning of the Pleistocene, tigers were widely distributed in eastern Asia [6, 8–10]. Alternating cold (glacial) and warm (inter-glacial) periods resulted in changing sea levels throughout the Pleistocene that probably caused repeated restrictions and expansions of the geographic distribution and abundance of tigers [6, 11, 12]. As has been observed in certain other modern Felidae species [13], the tiger has a relatively low population genetic diversity, a consequence of relatively recent demographic reductions and/or founder events [14].

The most recent common ancestor for tiger matrilineal mitochondrial DNA (mtDNA) has been estimated to have originated 72,000–108,000 years ago, with an overall lower and upper bound of 39,000–157,000 years [14]. This is much more recent than similar estimates derived from mtDNA analyses of modern leopards, which were considered to have originated in Africa between 470,000–825,000 years ago and to have arrived in Asia 170,000–300,000 years ago [15]. Likewise, extant jaguar lineages diverged approximately 280,000–510,000 years ago [16].

The coalescence time of modern tiger mtDNA (i.e., the merging of lineages backwards) occurred around 73,500 years ago during the late Quaternary and coincides with a catastrophic volcanic eruption of Toba in Sumatra, the largest known explosive volcanic event on earth [17]. The associated hemispheric 'volcanic winter' of the Toba super-eruption likely persisted for several years, and was followed by a millennium featuring the coldest, driest climate of the Late Quaternary as well as substantially decreased plant primary productivity. At higher latitudes (30°N to 70°N) the effect of climate cooling would have been amplified

by increased reflectance of solar energy caused by greater snow cover, resulting in a 5° to 15°C decades-long reduction in land temperature [17]. This devastating eruption, which has been linked to a Late Pleistocene bottleneck in human evolution [18] and a major northward dispersal event in Asian elephants [19], perhaps also contributed to a massive prehistoric range reduction in tigers.

REDEFINITION OF SUBSPECIES IN THE TIGER

The subspecies concept provokes both scientific and political controversy because several subspecies are considered to be specific units of conservation, which are protected by international treaties and organizations concerned with the stewardship of wildlife on the species level. The recognition of subspecies has particular relevance here because tiger conservation strategies are inextricably tied to subspecific taxonomic divisions [20–22]. Therefore, the establishment of formal subspecies definition and recognition, and an understanding of the implications of subspecies assignment are critically important.

Historically eight subspecies were recognized [8, 9] (Fig. 3.1): three (*P.t. sondaica*, Javan tiger; *P.t. balica,* Bali tiger; and *P.t. virgata*, Caspian tiger) became extinct in the mid- to

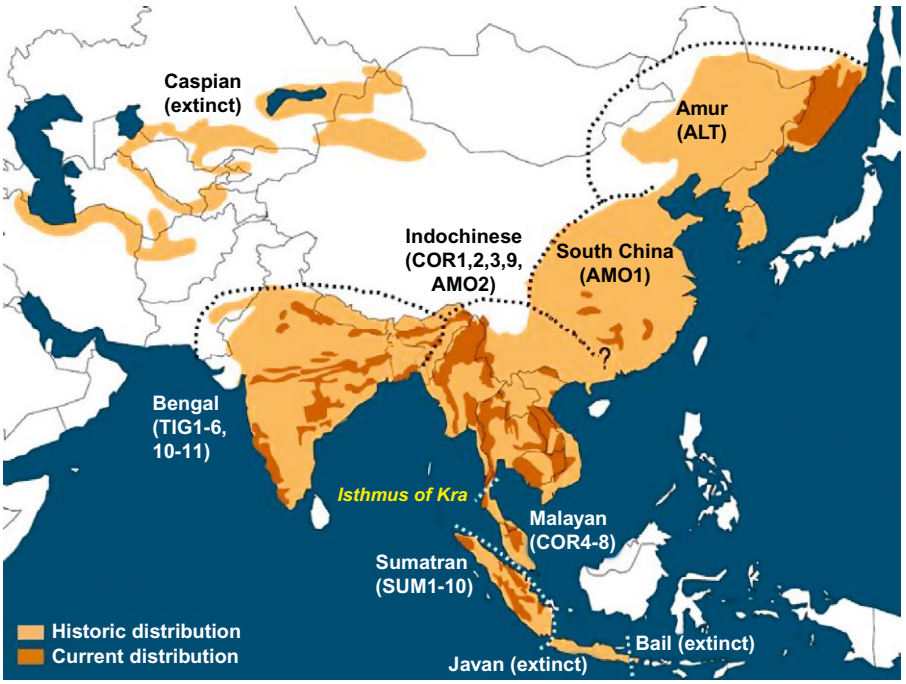

FIGURE 3.1 Historic and current geographic distribution of nine tiger subspecies. Dotted lines are approximate boundaries between subspecies. MtDNA haplotypes codes found for each subspecies are indicated within parenthesis. Note that the Isthmus of Kra divides the traditional Indochinese tigers into the northern Indochinese tiger, *P.t. corbetti* and the Malayan tiger, *P.t. jacksoni*.

late-twentieth century; *P.t. amoyensis,* South China tiger, exists only in captivity [23]; and four (*P.t. altaica,* Amur tiger; *P.t. corbetti,* Indochinese tiger; *P.t. sumatrae,* Sumatran tiger; and *P.t. tigris,* Bengal tiger) survive in a much reduced and fragmented range relative to one century ago [24, 25]. Traditionally, these subspecies were defined by their geographic distribution combined with morphological traits such as body size, skull traits, coat color, and striping patterns [11]. Later, several lines of evidence suggested that the classical subspecies designations were not reliable. First, the application of molecular genetics methods to investigate tiger phylogenetics, initiated two decades ago at the behest of the renowned tiger conservationist Ulysses S. Seal, revealed diminished genetic variation and little evidence of genetically distinct subspecies among the limited number of specimens examined [26, 27]. In addition, a biogeography study of historical tiger habitat also found few physical barriers sufficient for subspecies isolation [12], leading to the suspicion that subspecies designation among modern tigers may require modification. In 2004 we and our collaborators published the conclusions of a 20-year study to characterize differences among living tiger populations and subspecies using molecular genetic approaches [14], based on biological samples from 134 tigers verified as wild-born from a specific geographic locale or descended in captivity directly from parents of known geographic origins, termed 'voucher specimens.' Several technical hurdles that complicated prior efforts to fully describe patterns of genetic variation in tigers were overcome, primarily by developing better and more extensive molecular markers (Box 3.1).

Based on definitions of Avise and Ball [28] in 1990, and O'Brien and Mayr [29] in 1991, recognition and pronouncement of a subspecies requires 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. 'Members of a subspecies share a unique geographical range or habitat, a group of phylogenetically concordant phenotypic characters, and a unique natural history relative to other subdivisions of the species. Because they are below the species level, different subspecies are reproductively compatible. They will normally be allopatric and they will exhibit recognizable phylogenetic difference in the absence of gene flow.' [29] Over time, all subspecies accumulate novel mutations that will distinguish them from each other and which can lead to adaptations to their specific ecological habitat. The accumulation of these differences tends to be more prominent in small populations due to the effects of genetic drift. Most importantly, all subspecies have the potential to eventually evolve into new species, providing a compelling rationale for identifying, conserving, and managing subspecies individually.

Our genetic analysis demonstrated a unique and separate geneologcal history (phylogenetic monophyly) (Figs 3.2 and 3.3A) for the separation and recognition of at least five and possibly six tiger subspecies: (1) *P.t. altaica,* Amur tiger; (2) *P.t. amoyensis,* South China tiger, based on two specimens whose uniqueness is to be affirmed by more extensive sampling; (3) a refined *P.t. corbetti*, Indochinese tiger, in mainland Southeast Asia restricted to the north of the Isthmus of Kra; (4) a new peninsular subspecies *P.t. jacksoni,* Malayan tiger, that is different from the other Indochinese tigers, named for the renowned tiger conservationist Peter Jackson; (5) *P.t. sumatrae*, Sumatran tiger; and (6) *P.t. tigris*, Bengal tiger. These conclusions are based on significant genetic structure among tigers from these different geographic regions with the MHC, mtDNA and microsatellite data, and extremely limited gene flow as

BOX 3.1

METHODS USED TO CHARACTERIZE VOUCHER TIGER SUBSPECIES AND IDENTIFY CAPTIVE TIGERS WITH VERIFIED SUBSPECIES ANCESTRY (VSA)

Several factors have complicated earlier efforts to fully describe patterns of genetic variation in tigers. Foremost among these has been the limited sample size of 'voucher specimens' (defined as individuals that were verified as wild-born from a specific geographic locale or born in captivity from geographically verified wild-born parents). In addition, the presence of 13-kb *Numt*, a nuclear pseudogene insertion of the cytoplasmic mtDNA in tiger autosomes [49, 61, 62], has made it difficult to utilize universal mammalian primer sets for mitochondrial genes since they will co-amplify *Numt*. Furthermore, the paucity of genetic diversity across tigers, especially in mtDNA [27], made it necessary to sequence a large portion of the mtDNA genome and to assess genetic variation in multiple rapidly evolving microsatellite loci.

To overcome these technical hurdles, we first designed cytoplasmic mitochondria (*Cymt*)-specific primers that did not amplify portions of *Numt* [62]. We described phylogeography patterns in a rather large assembly of 134 voucher tigers using three distinct families of genetic markers [14]: 4,078 nucleotides of mitochondrial DNA sequence; a highly variable nuclear DNA sequence *FLA-DRB* (an immune response gene within the tiger's major histocompatibility complex), and a group of 30 short repetitive nuclear elements called microsatellites. The results were interpreted together and converged on a rather illuminating and generally robust (meaning high statistical confidence) picture of the tiger's natural history and subspecies recognition (see text).

We applied the subspecies diagnostic molecular genetic markers verified in the voucher tiger samples to assess genetic ancestry in captive tigers with uncertain origins. First, mitochondrial DNA haplotypes 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 [63] 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., $q = 0.90$) with high confidence interval (0.8–1). 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 test, were classified as admixed tigers. Specimens with only mitochondrial data were considered to have incomplete evidence.

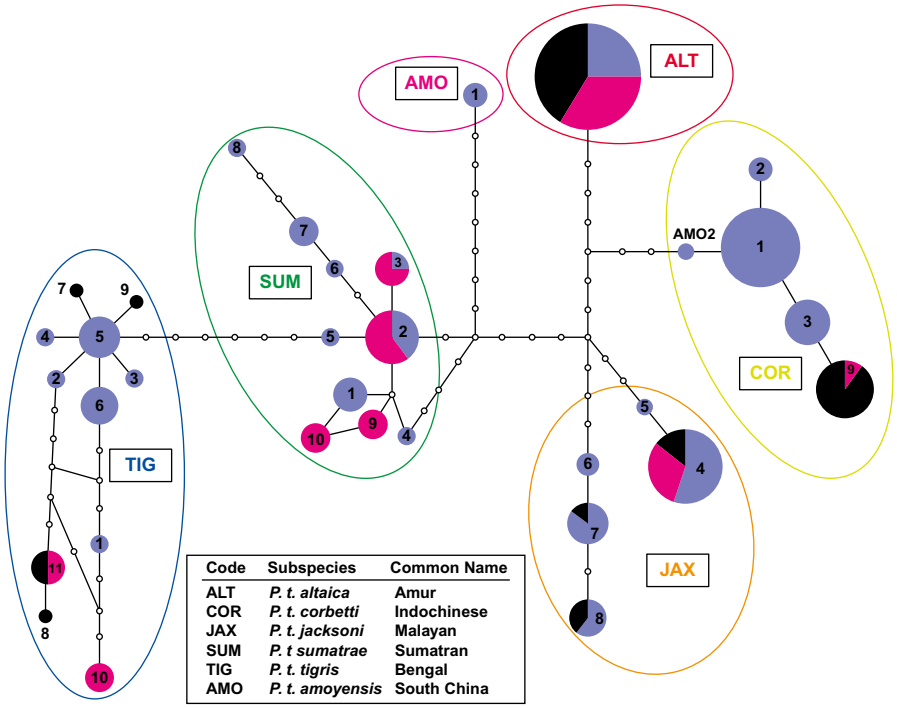

FIGURE 3.2 Statistical parsimony network of 33 mtDNA haplotypes based on 4,078bp of sequences from worldwide voucher and captive tigers (n = 188). The size of each haplotype circle is proportional to the mtDNA haplotype frequency and each is labelled with the subspecies mtDNA haplotype code ('number') defining monophyletic groups for subspecies. Pie chart colors indicate the proportion of tigers that are vouchers (in blue; n = 100), newly identified VSA captive tigers (in pink; n = 45) or newly identified admixed-origin captive tigers (in black, n = 43) based upon both composite microsatellite and mtDNA subspecies assignments.

shown by disjunct distributions of genetic variation (unique mtDNA haplotypes and signature microsatellite alleles) and high inter-population differentiation (mtDNA F_{ST} is 0.838 and microsatellite R_{ST} is 0.314). In addition, each subspecies has an allopatric (geographically isolated) distribution and differential natural history (Table 3.1).

The partition of the traditional Indochinese tiger *P.t. corbetti* subspecies into two groups, each as distinctive from each other as were the other subspecies (e.g., Bengal versus Amur tigers), has significant implication for understanding regional biogeography in Southeast Asia. Our results support the hypothesis that the Isthmus of Kra has been an ecological barrier restricting gene flow between tiger populations in Peninsular Malaya and mainland Southeast Asia (Fig. 3.1). Indeed, the Isthmus of Kra is considered a significant biogeographical transition between Indochina and Sundaic bioregions, which display significant climatic differences and floral transitions [30]. Various studies have suggested assemblages of amphibians [31], reptiles [32], birds [33], mammals [34, 35], freshwater crustaceans [36], and insects [37] were limited to varying degrees by the Isthmus.

Tiger subspecies most likely differentiated through the combined effects of genetic drift in isolated populations and local adaptation to rapidly changing habitats across their range

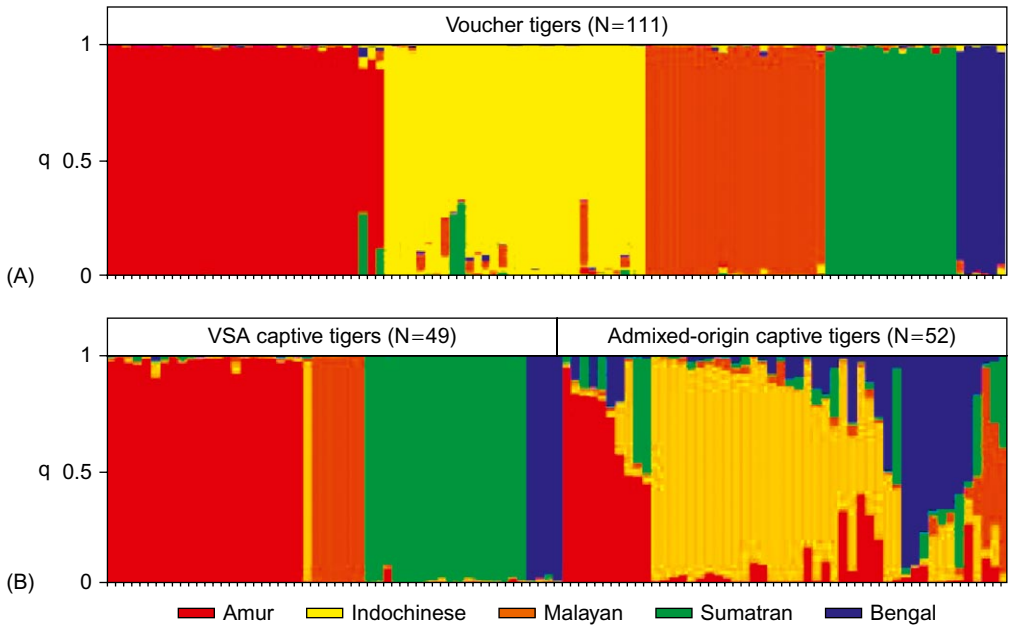

FIGURE 3.3 Bayesian population structure analysis of the worldwide voucher and captive tiger populations based on 30 microsatellite loci using the program STRUCTURE [63]. Each individual is represented by a thin vertical bar, which is partitioned into five colored segments that represent the individual affiliation (q) to each of the five tiger subspecies. South China tigers were not included in the analysis due to limited sample size. (A) Population structure analysis without prior population information clusters voucher tiger samples (n = 111) to distinct subspecies grouping. (B) Using the option of prior population information in voucher tigers, 49 captive tigers with uncertain genetic ancestry are assigned with Verified Subspecies Ancestry (VSA) and 52 with admixed origin.

during the Holocene [38]. The hypothesis that tiger population structure reflects recent (<10,000 years) human-induced population fragmentation and random lineage loss from a single panmictic population is not supported by our genetic data. However, we cannot rule out the possibility that some of the currently observed population subdivisions, particularly in the case of the divergence of *P.t. altaica* and *P.t. amoyensis/P.t. corbetti*, could be related to the recent disruption of regional population structure. This can be tested only when a larger geographic and historical sampling becomes available.

DILEMMA OF TIGERS—DECLINING IN THE WILD, BOOMING IN THE CAGES?

First recognized as endangered back in 1975, the tiger is vanishing rapidly from its natural habitat, with only an estimated 3,000 remaining in the wild as compared with 100,000 a century ago [39]. In contrast to the declining wild tigers, worldwide captive tiger populations are booming. Currently 15,000–20,000 tigers live in captivity, five to seven times more than their wild relatives (see Nyhus et al., Chapter 17). A relatively small portion

TABLE 3.1 Estimated population size and genetic variability of voucher and captive tiger populations

Name	Habitat	World Census No.		Microsatellite						MtDNA				
		Wild	Captive[a]	No. voucher (VSA)[b] tigers	Average observed heterozygosity	No. alleles per locus	Alleles in voucher tigers[c]	No. new alleles in VSA tigers	Subspecies-unique alleles	Sample size voucher (VSA)[b] tigers	No. of MtDNA haplotypes	No. of diagnostic sites	Nucleotide diversity (π)	MtDNA haplotype[d]
Amur *P.t. altaica*	Temperate deciduous forest	450	421	57(21)	0.4765	4.03	104	12	FCA77-160, FCA176-200, FCA441-138	32(21)	1	4	0	ALT
Indochinese *P.t. corbetti*	Mixed moist deciduous	700–1,300	14	33(1)	0.7349	5.97	181	1	FCA005-160, FCA032-190, FCA043-115, 125, FCA044-110, FCA069-97, 99, FCA077-152, FCA091-128, 130, 132, FCA123-140, FCA139-146, FCA212-154, FCA220-208, FCA229-164, FCA290-224, FCA293-208, FCA391-224	33(1)	5	3	1.32×10^{-4}	AMO2, COR1/AMO3, COR2, COR3, COR9
Malayan *P.t. jacksoni*	Evergreen dipterocarp rainforest	500	113	28(6)	0.5516	3.90	117	0	FCA008-132, 148 FCA096-203	28(6)	5	0	1.18×10^{-3}	COR4, COR5, COR6, COR7, COR8

Bengal *P.t. tigris*	Dry tropical forestry/tall grassland	1,300-2,200	210	10(4)	0.5126	4.07	105	18	FCA005-140, 162 FCA096-201, FCA126-128, FCA161-173, 187, FCA212-142, FCA229-174, FCA290-226, FCA304-121, FCA310-133, FCA441-148	19(4)	8	3	3.55×10^{-4}	TIG1, TIG2, TIG3, TIG4, TIG5, TIG6, TIG10, TIG11
Sumatran *P.t. sumatrae*	Moist tropical forest	300	295	36(17)	0.4783	3.77	108	5	FCA032-204, FCA044-126, FCA077-156, FCA129-175, FCA176-218, FCA211-120, FCA229-160, FCA304-125, 139, FCA391-206, 214	31(17)	10	2	7.17×10^{-3}	SUM1, SUM2, SUM3, SUM4, SUM5, SUM6, SUM7, SUM8, SUM9, SUM10
South China *P.t. amoyensis*	Subtropical/temperate forest	extinct	64	2(0)	0.3167	1.53	46	n/a	FCA126-142	2	1	7	0	AMO1
Tigers with purebred origin[e]		3,000–5,000	1,116	166	0.5212	7.57	227	*		145	29		2.48×1^{-3}	
Tigers with unknown origin		n/a	15,000–20,000[f]	52	0.6795	6.33	190	10		43	9		1.97×10^{-3}	ALT, COR4, COR7, COR8, COR9, TIG7*, TIG8*, TIG9*, TIG11
Total		3,000–5,000	6,000–21,000	218(49)	0.5528	7.90	219	46		188(49)	33		2.21×10^{-3}	

[a]Captive tigers registered in regional or international stud books.
[b]Number of verified tiger individuals in as purebred subspecies this study is in parenthesis.
[c]Voucher tigers refer to sample set used previously, See Table 7 in Luo *et al*. [14].
[d]Underlined MtDNA haplotypes represent new haplotypes found from the study in addition to those reported by Luo *et al*. [14].
[*]Indicates MtDNA haplotypes found only in tigers with admixed genetic origins.
[e]Purebred tigers include both the voucher tigers [14] and VSA tigers in captivity identified from Luo et al. [47].
[f]Minimum estimates.

(~1,000 individuals) of the captive tiger population is managed through coordinated breeding programs among zoos with the goal of preserving genetic variability that is representative of geographic and subspecies groupings found in the wild [22]. In 2007, there were 421 Amur, 295 Sumatran, 78 South China, 210 Bengal, 14 Indochinese and 113 Malayan tigers in captivity as recorded in regional and international zoo studbooks [40–45]. However, the vast majority of captive tigers are not part of these managed breeding programs; with most residing in roadside zoos, breeding farms, makeshift breeding facilities, circuses, and as pets (see Nyhus et al., Chapter 17). With few exceptions, these tigers are considered as 'generic' tigers of hybrid or unknown origins, and thus are not included in internationally sanctioned conservation programs [22, 46].

Captive populations of wild animals have been justified based on the principle that they are genetic representations of their natural counterparts and thus insurance against extinction in the wild. However, 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 any 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 in relation to the wild populations.

Based on the subspecies diagnostic genetic markers obtained from the panel of 134 'voucher' tigers [14], we developed a stringent strategy for evaluating the subspecies affiliation of a tiger with unknown genetic origin [47] (Box 3.1). Subspecies genetic ancestries were characterized for 105 captive tigers with various degrees of uncertainty in their origins. The samples had been collected over a 20-year interval (1982–2002) from zoos or private owners in 14 countries or regions: USA, UK, China, Japan, Singapore, Ukraine, Mexico, Germany, Estonia, Indonesia, Taiwan, Cambodia, Thailand, and Malaysia. This sample set represented a fairly good coverage of the world's *ex situ* captive tiger gene pool.

Verified Subspecies Ancestry (VSA; i.e., they belong to the specific subspecies with high probability; see Box 3.1) captive tigers were identified corresponding to a recognized subspecies (21 Amur, 17 Sumatran, 6 Malayan, 1 Indochinese, and 4 Bengal) and 52 had admixed subspecies origins (Fig. 3.3B). Most (80%) of the results matched their suspected origins provided by owners, including 42 named as a specific subspecies and 41 suspected admixed. Nine tigers initially identified as purebred were admixed and VSA origin was confirmed for seven of 48 (~15%) tigers of unknown subspecies ancestry.

Among the verified admixed-origin tigers, 27 clearly had genetic ancestries from more than one subspecies according to the microsatellite assignment tests. Nine tigers were tentatively assigned to a single subspecies, but with lower bounds for confidence levels below 0.80, and 16 tigers had discordant mtDNA haplotype and microsatellite assignments and were classified as admixed. 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.

The newly tested captive tigers harbored novel alleles and genotypes that extend beyond the endemic diversity from the voucher samples (Table 3.1). From the newly tested captive tigers 14 mtDNA haplotypes were identified, including eight new ones (three in VSA tigers, three in admixed ones and two in both), increasing the number of reported mtDNA haplotypes in the

tiger from 25 voucher sample haplotypes to 33. The new haplotypes fell within one of three subspecies groups: Indochinese, Sumatran, and Bengal tigers. The captive tigers also had 46 new microsatellite alleles (36 in VSA and 10 in admixed tigers) not observed in the voucher specimens.

The overall level of genetic variability in the captive Amur tigers is similar to or slightly higher than that observed in the wild Amur tiger population from the Russian Far East (see next section). A previous study also found Sumatran, Amur, and Bengal tigers had comparable levels of MHC variation as their wild counterparts [26]. The large amount of genetic variation retained in the captive population is plausible because tiger captive breeding programs have been ongoing for over a century, with a continual influx of animals from the wild, a large interbreeding population, and a large number of original founders with a broad geographic and genetic background [40–43, 45].

Because captive and wild tigers today are consciously managed to maintain pure subspecies, the discovery of 49 additional purebred VSA- tigers in a sample of 105 captive individuals (~50%) has important conservation implications. Our sampling may overestimate VSA tiger prevalence for all captive tigers because 41 of the tigers that we tested were enrolled in management breeding programs for designated subspecies. Nevertheless, 14 of the 64 unenrolled tigers (22%) show VSA origins, while seven of 48 (15%) tigers of unknown origin were verified as VSA. If 15–22% 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 more than double. Also, an important fraction of captive tigers retain genetic diversity unreported, and perhaps absent, in the wild populations. Consideration of comprehensive identification of captive VSA tigers and their potential inclusion into management plans would help to increase the population size as well as to maintain maximal levels of available genetic variability among managed tiger populations.

WHAT IS A TIGER? — A CLOSER LOOK AT SUBSPECIES

P.t. tigris—Bengal Tiger

Bengal tigers range from Bangladesh, Bhutan, western China, India, western Myanmar, and Nepal [25]. The voucher Bengal tigers are defined by three distinct mitochondrial nucleotide sites and 12 unique microsatellite alleles (Table 3.1). The pattern of genetic variation in the Bengal tiger is consistent with the premise that tigers arrived in India approximately 12,000 years ago [12]. This history of tigers in the Indian subcontinent is coherent with the lack of tiger fossils from India prior to the late Pleistocene, and the absence of tigers from Sri Lanka (except for one record by Manamendra-Arachchi *et al*. [48]), which was separated from the subcontinent by rising sea levels in the early Holocene.

Indian zoos have bred Bengal tigers since 1880 and currently all 210 registered Bengal tigers are maintained within India [45]. Bengal tigers were transported around the world and frequently crossed with other tiger subspecies, as reflected by the large number (33%) of the captive tigers we tested that had admixed genetic heritages derived partially from Bengal tigers. Three newly identified mtDNA haplotypes that are closely related to the voucher Bengal tigers are only found in the admixed-origin tigers. These genetic findings

are in accordance with the notion that tigers from outside India have often been mixed with tigers from India so that many so-called Bengal tigers are of admixed ancestries and therefore inappropriate for conservation breeding purposes.

P.t. sumatrae—Sumatran Tiger

Sumatran tigers range across the island of Sumatra in Indonesia. Captive populations have been managed in North America, Europe, Australia, and Indonesia since 1937 at relatively stable levels, currently with 295 registered animals [40, 43, 45]. The isolation of Sumatran tigers from mainland populations is supported by multiple unique characters, including two diagnostic mtDNA nucleotide sites, ten mtDNA haplotypes, and 11 (out of 108) unique microsatellite alleles (Table 3.1). Cracraft et al. [49] and Hendrickson et al. [26] also described genetic variations distinguishing Sumatran tigers from other tiger subspecies, and Mazak and Groves described morphological differences based on a study of museum specimens [50]. The relatively high genetic variability and the phylogenetic distinctiveness of Sumatran tigers suggest a historically large effective population size, followed by highly restricted gene flow between the island and other populations.

P.t. corbetti—Indochinese Tiger

Our genetic data suggest that the Pleistocene centrum of the modern tiger radiation is northern Indochina/southern China, which currently consists of mixed moist deciduous forest. Modern Indochinese tigers have a large number of mtDNA diagnostic sites (three), the most unique microsatellite alleles (19 out of 130), and the highest overall microsatellite diversity (Table 3.1). In addition, no microsatellite allele at any locus occurred with a frequency higher than 81%. The observed allele size distribution in *P.t. corbetti* was generally continuous for most loci (there were fewer allele size gaps compared to other subspecies), evidence of the fairly stable demographic history, and alleles found in the other subspecies were almost always a subset of those found in *P.t. corbetti*.

One main challenge of the redefined Indochinese tiger is that most of the founders in the captive management programs for the subspecies in Europe and North America (113 individuals), were originally from Peninsular Malaysia [42, 45]. The Malayan tiger is now classified as a separate subspecies, thus leaving the Indochinese tiger the least represented in captivity (14 recognized as of 2007), at facilities in Thailand, Vietnam, and Cambodia, and not part of a coordinated breeding program. In our sample set, we identified only one purebred Indochinese tiger from the Taipei Zoo in Taiwan. Preservation of Indochinese tigers in the wild, which are currently little studied [51, 52], should also be set as a priority in order to maintain the high genetic diversity and structure harbored in the natural tiger populations from the region.

P.t. jacksoni—Malayan Tiger

The Malayan tiger, found only in Peninsular Malaysia, is characterized by three unique microsatellite alleles, five subspecies-specific mtDNA haplotypes, and three MHC *DRB* alleles (Table 3.1) [14]. The genetic difference between *P.t. corbetti* and *P.t. jacksoni* as measured

by the pairwise mtDNA F_{ST} of 0.797 and microsatellite R_{ST} of 0.225 ($p < 0.0001$), is comparable to differences among other recognized and separately managed tiger subspecies. For consistency, the Malayan subspecies should also be managed as a unique subspecies, unless inbreeding depression has become an issue due to declined genetic variability. The Malayan tiger subspecies is designated *P.t. jacksoni* to honor the dedication and career of tiger conservationist Peter Jackson, former head of the IUCN/SSC Cat Specialist Group, who tirelessly labored for 50 years on behalf of tiger conservation (see Jackson, Chapter 12).

P.t. altaica—Amur Tiger

Amur tigers, with an isolated population of fewer than 500 individuals, are confined almost entirely to the Russian Far East and the border to China and North Korea [53]. They display low genetic diversity in comparison to other subspecies, with a single mtDNA haplotype most closely related to a northern Indochinese tiger haplotype (Fig. 3.2). The reduced genetic variability in Amur tigers may have resulted from a post-ice age colonization of the region and population bottleneck less than 10,000 years ago, and/or during the early twentieth century when an estimated 20–30 tigers survived intense human persecution [54].

The Amur tiger captive management program is the largest among all the tiger subspecies, with (~420) animals, a number comparable to that remaining in the wild. All captive Amur tigers that we tested shared a single identical mtDNA haplotype with the wild population and no closely related haplotypes were discovered. There is no significant difference ($R_{ST} = 0.0029$; $p > 0.05$) between captive and wild Amur tigers in terms of microsatellite allele composition and heterozygosity, suggesting that captive Amur tigers adequately represent the genetic diversity surviving in their wild counterparts. In addition, the wild Amur tigers displayed significantly higher relatedness *in situ* than *ex situ* VSA Amur tigers (Fig. 3.4) (i.e., there were more pairs of closely related individuals in the sampled Russian Far East tiger population than in the global captive Amur tiger population). This may reflect the broad genetic heritage of the founders that have entered the captive Amur tiger population intermittently over the last 100 years. Further there is a strong likelihood that the wild Amur tiger population has a smaller effective population size due to a greater influence of unequal sex ratios, unequal numbers of progeny from adults, and more extreme fluctuations in population size, promoting a more-rapid reduction of genetic variation and greater probability of inbreeding [55–57].

In the case of Amur tigers, the captive breeding programs have proved to be successful in maintaining high genetic diversity and low relatedness among captive individuals. This means that captive Amur tigers can serve, at least genetically, as a healthy supplement to *in situ* tiger conservation, if that eventually becomes a necessity.

P.t. amoyensis—South China Tiger

Among all of the subspecies, the South China tiger is the most controversial, as the subspecies is functionally extinct in the wild [23] and is survived in captivity by 78 animals [44] derived from six wild-caught founders of unresolved genetic heritage (see Traylor-Holzer, Chapter 37). Early sampling of *P.t. amoyensis* in the genetic analysis included five animals from two Chinese zoos collected in 1994. These samplings revealed two distinctive lineages

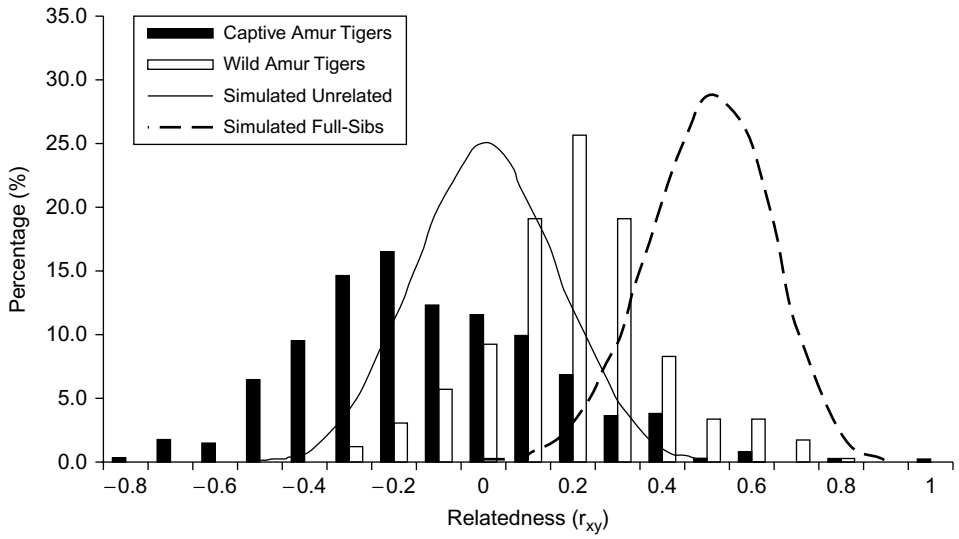

FIGURE 3.4 Distribution of pair-wise relatedness values (rxy) for all pair-wise combinations within the wild (open histogram) and captive (solid histogram) Amur tiger (*P.t. altaica)* populations, as compared to relatedness value distribution of simulated unrelated individuals (solid curve) and full-sibs (dashed curve). The global Amur tiger captive breeding programs consist of less pairs of closely related individuals than the wild population in the Russian Far East, where the world's largest remaining wild Amur tiger population survives (Mann-Whitney U test, $p < 0.0001$).

as supported by both mtDNA and microsatellite evidence [14, 58]: one which is unique and distinct from the other subspecies, possibly the actual *P.t. amoyensis* (the original Chongqing Zoo lineage), and a second which is indistinguishable from mainland *P.t. corbetti* (the original Suzhou Zoo lineage). However, according to the studbook record [44], the two originally separate lines have been cross-bred since 1995 in order to minimize the potential effect of inbreeding. The likelihood of identifying a substantial number of unique South China tigers from the population is thus presumably not possible. An explicit genetic assessment of the captive Chinese tigers using the diagnostic system set here in the context of comparison with other purebred subspecies should be immediately conducted to validate the uniqueness, or non-uniqueness, of South China tigers [59, 60].

SUMMARY

Modern tiger genome diversity is estimated to derive from a founder event that occurred around 72,000 to 108,000 years ago, coinciding with the Toba volcano super-eruption in Sumatra, Indonesia, that had possibly reduced the historical tiger population to a small demographic bottleneck. Since then ecological and biogeographic factors have led to the distinct population differentiation of at least six surviving subspecies. Assessment of verified subspecies ancestry (VSA) based on both mtDNA and microsatellite diagnostic systems offers a powerful tool that, if applied to captive tigers of uncertain background in the world, may increase by thousands the number of purebred tigers suitable for conservation management. A sample

of captive tigers showed that they retain appreciable intrinsic genomic diversity unobserved in their wild counterparts; perhaps a consequence of inclusion of wild-caught founders to the large captive breeding world established for over a century.

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