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Genetic Variability and Population Differentiation in Captive Baird's Tapirs (*Tapirus bairdii*)

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The objectives of this study were to assess the level of genetic variability and population differentiation within captive populations of an endangered large mammal, Baird's tapir (*Tapirus bairdii*). We genotyped 37 captive animals from North American (NA) and Central American (CA) zoos and conservation ranches using six polymorphic microsatellite loci. Standard indices of genetic variability (allelic richness and diversity, and heterozygosity) were estimated and compared between captive populations, and between captive and wild population samples. In addition, we evaluated levels of population differentiation using Weir and Cockerham's version of Wright's *F*-statistics. The results indicate that the NA and CA captive populations of Baird's tapirs have retained levels of genetic variability similar to that measured in a wild population. However, inbreeding coefficients estimated from the molecular data indicate that the CA captive population is at increased risk of losing genetic variability due to inbreeding. Despite this, estimated levels of population differentiation indicate limited divergence of the CA captive population from the wild population. Careful management appears to have kept inbreeding coefficients low in the NA captive population; however, population differentiation levels indicate that the NA population has experienced increased divergence from wild populations due to a founder effect and isolation. Based on these results, we conclude that intermittent exchanges of Baird's tapirs between the NA and CA captive populations will benefit both populations by increasing genetic variability and effective population size, while reducing inbreeding and divergence from wild populations. Zoo Biol 23:521–531, 2004. © 2004 Wiley-Liss, Inc.

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INTRODUCTION

The primary management goal of zoos has evolved from one of housing and displaying as diverse a collection of species as possible, to one of creating self-sustaining captive populations that may one day act as a source for restocking wild populations [Ralls and Ballou, 1986; Soule et al., 1986]. One obstacle to achieving a self-sustaining captive population, however, is the limited space available to maintain populations large enough to avoid the negative impacts of inbreeding and loss of genetic variability through genetic drift. Increased levels of inbreeding have been associated with reduced fecundity, offspring viability, and individual survivorship [Brewer et al., 1990; Eldredge et al., 1999; Jimenez et al., 1994; Lacy et al., 1993; Madsen et al., 1996; Reed et al., 2002].

Consequently, avoiding inbreeding and maintaining genetic diversity within captive populations have become a priority [Ralls and Ballou, 1986; Soule et al., 1986]. Most zoos have been carefully managing captive populations to maximize effective population sizes and minimize inbreeding by exchanging animals. However, with the limited space available in zoological parks and conservation ranches, even carefully managed populations have experienced some level of inbreeding [Lacy et al., 1993; Ballou, 1997; Reed et al., 2002].

This is the situation facing the captive population of a large, endangered Central American (CA) forest-dwelling ungulate, Baird's tapir (*Tapirus bairdii*). Furthermore, very little data about wild population size and structure are available, which makes it even more difficult to assess the potential impact of mixing animals descended from different regions of their geographic distribution (which ranges from southern Mexico to northern Colombia and Ecuador).

The extant North American (NA) captive population of Baird's tapir is descended from only eight wild animals that were brought to North America in the 1960s and 1970s (Fig. 1) [Roman, 1999]. As a result of this small number of founders, the population is at risk. In contrast, the CA captive population consists of many wild-caught animals that were brought into captivity as recently as 1994 (Fig. 2) [Roman, 1999]. Analysis of the studbook indicates that the groups of animals in CA zoos tend to be maintained independently, with minimal exchange of animals between institutions. Additionally, with the continued decline of wild Baird's tapirs, the incorporation of wild animals into CA zoos will likely decrease. In the future, cooperative breeding among CA and NA zoos may be necessary to maintain the genetic variability and long-term sustainability of both NA and CA captive Baird's tapir populations. However, facilitating breeding between individuals from populations that would not normally interbreed can result in a reduction in fitness and fecundity known as "outbreeding depression" [Templeton, 1986; Lacy et al., 1993]. Outbreeding depression is believed to be a result of the disruption of coadapted gene complexes that confer characteristics that provide an advantage in the local environment [Lacy et al., 1993]. Although no clear means of estimating the likelihood or severity of outbreeding depression from genetic parameters has been established [Hedrick and Miller, 1992; Frankham, 1995], it is more advisable to link or exchange individuals between populations whose level of population structure

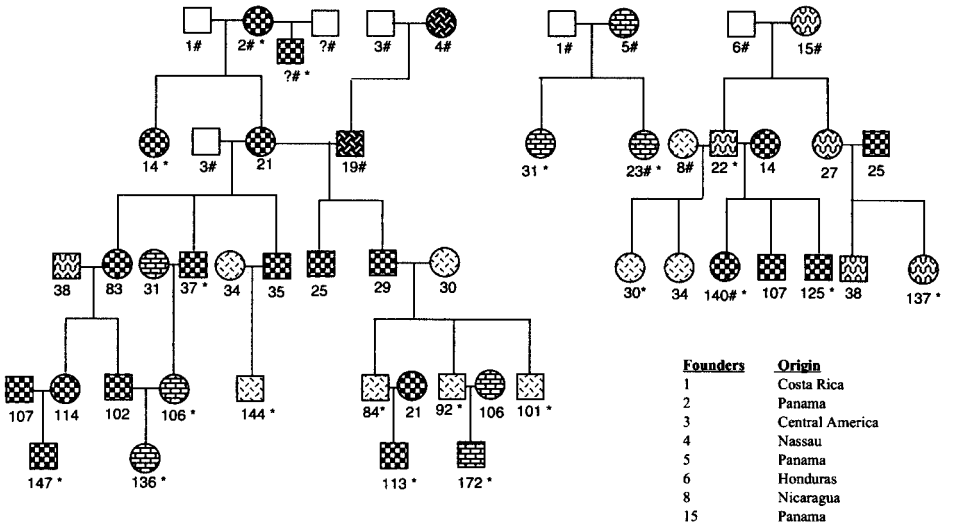

Fig. 1. Pedigree of captive Baird's tapirs in NA zoos, including individuals in this study (indicated by *). The numbers correspond to studbook numbers in the *1998 International Studbook, Central American tapir* (Tapirus bairdii) [Roman, 1999]. Patterns indicate maternal lineage back to the founders, and deceased animals are indicated by "#".

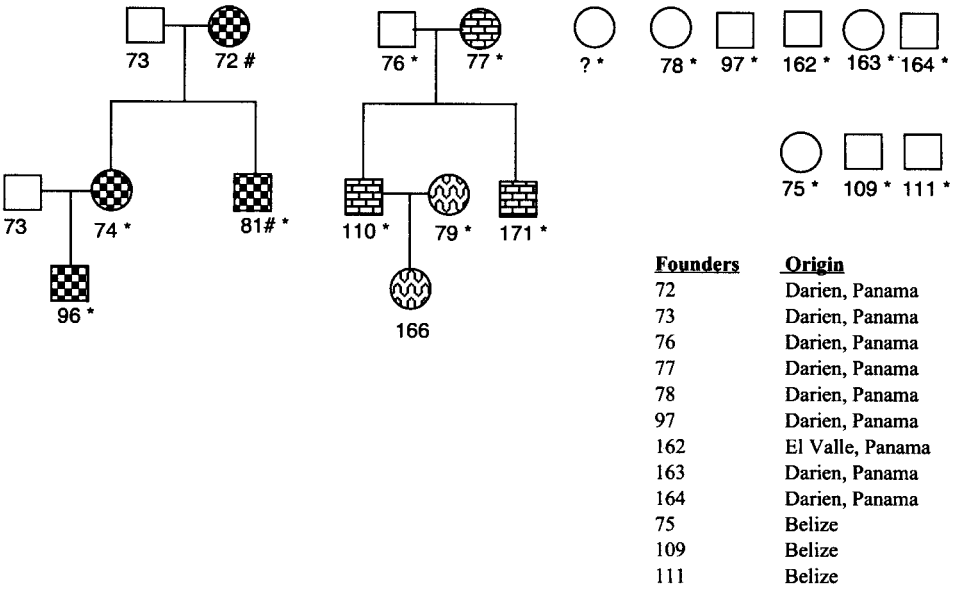

Fig. 2. Pedigree of captive Baird's tapirs in CA zoos, including individuals in this study (indicated by *). The numbers correspond to individual studbook numbers in the *1998 International Studbook, Central American tapir* (Tapirus bairdii) [Roman, 1999]. Patterns indicate maternal lineage back to the founders, and deceased animals are indicated by "#".

indicates at least minimal levels of historic gene flow ($F_{ST} < 0.2$) [Forbes and Hogg, 1999]. Therefore, the level of differentiation or divergence between two populations can provide a qualitative means of assessing the risk of outbreeding depression due to the mixing of two populations.

The objectives of our study were to characterize levels of genetic variability within captive populations of Baird's tapirs, assess how well captive populations represent the genetic variability in wild populations, and measure the level of genetic divergence that has taken place as a result of founder effect and isolation in captivity. To accomplish this, we genotyped 37 captive Baird's tapir samples from NA and CA zoos and conservation ranches, using six polymorphic microsatellite genetic markers. We then estimated standard indices of genetic variability (heterozygosity, allelic richness, and allelic diversity) in each population sample, and compared them with levels measured in a wild population [Norton and Ashley, 2004]. Finally, we estimated the extent of genetic divergence that has occurred in each population by calculating Weir and Cockerham's versions of Wright's F statistics between the captive populations, and between each captive population and a wild population sample.

MATERIALS AND METHODS

Sample Collection and Genotyping

Tissue, blood, or hair samples were requested from institutions listed in the *1998 International Studbook, Central American tapir* (Tapirus bairdii) [Roman, 1999] as having Baird's tapirs in their collection. This resulted in the collection of 37 samples from NA ($n = 20$) and CA ($n = 17$) zoos and conservation ranches, representing 34 (40%) of the 84 living animals listed in the *1998 International Studbook, Central American tapir* (Tapirus bairdii) [Roman, 1999] (Figs. 1 and 2). Descendents of all eight founders of the NA captive population are represented in the NA sample, including a founder and five first-generation offspring. Although the founder and two of the first-generation samples are from deceased animals, each animal produced offspring that are still alive in the captive population, some of which are included in the NA sample assessed in this study (Fig. 1). The CA captive population sample is comprised of animals from Belize and Panama (Fig. 2). Charles Foerster and Dr. Sonia Hernandez-Divers provided blood or tissue samples from a wild population of Baird's tapirs ($n = 15$) in Corcovado National Park, Costa Rica, that they collected during a radiotelemetry study. DNA was extracted from the tissue and blood samples by a standard protocol of proteinase-*K* digestion and phenol: chloroform: isoamyl alcohol washes [Sambrook et al., 1989]. DNA was isolated from hair root bulbs with the use of the PureGene DNA Extraction Kit (Gentra Systems, Inc., Minneapolis, MN).

DNA samples were PCR amplified at six microsatellite loci (Table 1) with fluorescently labeled primers. PCR reactions were completed with 5–50 ng of DNA template added to 1.5–2.0 mM of $MgCl_2$, 1.0 μM of each primer, 0.5 U of Promega (Madison, WI) Taq, 2.0 μM of bovine serum albumin, and 1.0 μl of 10 × Promega PCR buffer, 0.25 μM of dNTP mix, and sterile ddH20 to a total volume of 10 μl. PCR amplification was completed on MJ Research PT-100 (Watertown, MA) and Eppendorf (Westbury, NY) gradient thermocyclers. Allele sizes were scored on an

TABLE 1. Microsatellite alleles and frequencies*

Locus	Alleles	North American captive pop. ($N = 20$)	Central American captive pop. ($N = 17$)	Captive Bred ($N = 24$)	Wild population ($N = 15$)
Tte 1	144	–	*0.13*	–	–
	146	0.82	0.87	0.87	0.97
	148	**0.03**	–	0.02	–
	150	**0.12**	–	0.11	–
	154		–	–	**0.03**
	168	*0.03*	–	–	–
Tte 5	144	0.85	0.56	0.79	0.87
	148	–	*0.03*	–	–
	150	–	*0.03*	–	–
	154	0.15	0.38	0.21	0.13
Tte 9	114	0.26	0.10	0.22	0.20
	118	**0.03**	–	0.02	–
	124	0.71	0.90	0.76	0.80
Tte 12	162	0.08	0.37	0.15	0.28
	168	0.55	0.25	0.46	0.44
	170	0.37	0.38	0.39	0.28
Tba 15	218	0.05	0.25	0.17	0.53
	222	0.45	0.22	0.30	0.235
	224	0.05	0.07	0.03	–
	226	–	**0.07**	0.10	–
	228	0.45	0.39	0.40	0.20
	238	–	–	–	**0.035**
Tba 23	218	0.54	0.22	0.46	0.20
	234	0.23	0.09	0.19	–
	236	0.23	0.66	0.33	0.80
	238	–	**0.03**	0.02	–

*Bold indicates private alleles within the population. Italics indicates alleles not detected in the captive bred population sample. Primers sequences and annealing temperatures in Norton and Ashley [2004].

ABI 373a automated sequencer (Applied Biosystems Inc., Foster City, CA), and genotypes were assigned with ABI's GeneScan software.

Genetic Variability

An unweighted measure of alleles per locus ($\bar{A}$) was calculated for each Baird's tapir population. We also used the computer program FSTAT [Goudet, 1995] to calculate levels of allelic richness (R_s), which takes into account variation in sample size by standardizing the estimate of alleles per locus to the smallest sample size. We then tested for significant differences in allelic richness between populations using a Wilcoxon signed-ranks test. We calculated observed (H_o) and expected (H_e) levels of heterozygosity from the microsatellite genotype data using the computer program Genetic Data Analysis (GDA) [Lewis and Zaykin, 2001]. We also calculated standard deviations for expected heterozygosity estimates within each population

using the square root of Weir's calculation of total variance [Weir, 1996]. Conformation of microsatellite genotype data to Hardy-Weinberg (HWE) and linkage equilibrium was tested with the GenePop analysis package [Raymond and Rousset, 1995]. We calculated probabilities of HWE and genotypic disequilibrium by Fisher's exact test using GenePop default analysis settings (dememorization: 1,000; batches: 100; iterations per batch: 1,000). HWE was tested for each locus and population, and over all loci and populations, while genotypic disequilibrium was tested between all locus pairs within each population sample and pooled samples of all populations. We applied a sequential Bonferonni correction to the data to compensate for the increased chance of a type I error occurring during the performance of multiple tests [Rice, 1989].

Population Differentiation

F statistics provide a means of assessing genetic differences among populations using the differences in sampled allele frequencies [Hartl, 1988]. In a theoretical ideal population with no mutation, migration, or selection, changes in allele frequencies and subsequent changes in $\theta(F_{ST})$ values can be attributed to independent genetic drift as a result of isolation or lack of gene flow between populations. Although these ideal conditions never occur in natural or captive populations, $\theta(F_{ST})$ values can still provide a qualitative index of population genetic differentiation [Hartl, 1988].

Weir and Cockerham's version of Wright's *F*-statistics were used in this study and calculated with the use of GDA [Lewis and Zaykin, 2001]. We interpreted the resultant $\theta(F_{ST})$ values based on Wright's [1978] suggested qualitative guidelines of $\theta(F_{ST})$ values ($\theta(F_{ST}) = 0$–0.05 indicates little population differentiation, 0.05–0.15 indicates moderate differentiation, 0.15–0.25 indicates great differentiation, and >0.25 indicates very great differentiation).

RESULTS

Only 17 (7.7%) of the 222 (37 samples $\times$ 6 loci) PCR reactions failed to produce sufficient product for genotyping. Failed PCR reactions were recorded as missing data and were taken into account in all analyses.

Each population sample was found to contain private alleles or alleles that are present only in a single population (Table 1). However, these were present at low frequencies, and it is possible that they existed in the other populations but were not sampled. Captive-bred animals were missing four alleles that were detected in the sample of all captive animals, indicating a potential loss of founder alleles in captive-bred generations. The average allelic diversity ($\bar{A}$) within population samples ranged from 2.5 to 3.33 alleles/locus (Table 2), while allelic richness (R_s) at each locus ranged from 1.0 to 5.30 alleles (Table 3). When allelic richness measures were compared across all loci between NA, CA, captive-bred, and wild samples, only the NA captive and captive-bred samples had a significantly higher level of allelic richness than the wild sample ($P<0.05$) (Table 4).

Similarly, the observed and expected heterozygosities in the captive populations were low relative to those observed in other large mammals [Norton and Ashley, 2004], but were not significantly different than those observed in a wild Baird's tapir population (Table 2). Fisher exact tests for deviations from HWE showed that none of the loci demonstrated significant levels of heterozygote excess at

TABLE 2. Microsatellite genetic variability in Baird's tapirs*

Population (N)	(Ā)	H_e (±2SD)	H_o	P^a	f(F_{IS})
NA captive (20)	3.1	0.47 (±0.11)	0.48	0.39	−0.021
CA captive (17)	3.33	0.49 (±0.03)	0.39	**0.0012**	0.138
Captive bred (24)	3.33	0.50 (±0.14)	0.47	0.19	0.052
Wild population (15)	2.5	0.37 (±0.08)	0.39	0.69	−0.058

*Ā, average alleles/locus; H_e/H_o, expected and observed heterozygosity; Global Fisher exact test heterozygote deficiency; f(F_{IS}), inbreeding coefficient; bold indicates private allele.
[a]Fisher exact test *P* values for heterozygote deficiency.

TABLE 3. Allelic richness (R_S) per locus and population based on minimum sample size of 10 diploid individuals

Population	NA	CA	Captive bred	Costa Rica
Locus				
Tte 1	3.447	1.986	2.778	1.667
Tte 5	1.990	3.176	1.998	1.992
Tte 9	2.562	1.970	2.433	2.000
Tte 12	3.430	3.000	3.422	2.998
Tba 15	4.000	4.793	4.635	3.666
Tba 23	2.999	3.776	3.413	2.000
Mean R_S across all loci (SD)	3.071 (0.428)	3.117 (0.976)	3.113 (0.720)	2.387 (0.497)

TABLE 4. Wilcoxon signed ranks test of allelic richness data*

	NA	CA	Captive bred
NA	–		
CA	0.753	–	
Captive bred	0.917	0.753	–
Wild	0.046	0.075	0.028

*Two sided probabilities using normal approximation.

any locus before Bonferonni correction ($P > 0.05$), while global tests for a heterozygote deficit across all loci detected deviations from HWE only in the CA captive population ($P = 0.0012$). When exact tests were conducted on each locus in the CA captive population, only locus *Tte*1 was found to show heterozygote deficiency after sequential Bonferroni correction ($P < (\alpha = 0.008)$). However, global tests for heterozygote deficiency completed without locus *Tte*1 continued to indicate a significant level of heterozygote deficiency ($P < 0.05$) in the CA captive population. Fisher exact test results for the NA captive population did not reveal any significant deviations from HWE across all loci ($P > 0.05$) or within each independent locus after Bonferonni correction ($P > (\alpha = 0.008)$). Tests for linkage disequilibrium revealed that only one loci pair (*Tte*12 and *Tba*23 ($P < 0.0005$)) may be linked or segregating together. However, independent calculations of *F* statistics excluding these loci did not greatly alter the results, and qualitative conclusions remained the same. Consequently, all loci data were retained and used to estimate population differentiation.

TABLE 5. Hierarchical F statistics, Θ (F_{ST}) (above diagonal) & F (F_{IT}) (below diagonal)*

	CR	NA	CA
CR	–	0.142 (0.020–0.237)	0.047 (0.013–0.088)
NA	0.121 (−0.092–0.270)	–	0.096 (0.040–0.281)
CA	0.145 (0.059–0.293)	0.197 (0.118–0.297)	–

*Values are Weir and Cockerham's version of Wright's F statistics calculated by Genetic Data Analysis Software. 95% Bootstrap Confidence Intervals are in parenthesis.
CR, Costa Rican wild population; NA, North American captive population, CA, Central American captive population.

The θ(F_{ST}) values generated for the captive population samples (Table 2) indicate that population differentiation between the NA and CA captive populations is similar to that observed between Costa Rican and Panamanian wild population samples [Norton and Ashley, 2004]. The θ(F_{ST}) values between the NA and CA captive populations and the wild population indicate that the NA population has experienced a greater level of divergence from the wild population compared to the CA captive population (Table 5). However, the 95% confidence intervals for θ(F_{ST}) values between the NA captive population and the wild population sample are large, and differentiation may actually be anywhere from minimal to great (Table 5).

The 95% confidence intervals for the NA captive population F (F_{IT}), which reflect both population substructure and non-random mating within a subpopulation (individual inbreeding), were not significantly greater than zero. However, the F value for the CA population was greater than zero, and is consistent with the substructure among the CA zoo populations (Table 5). The individual inbreeding coefficients (f) (Table 2) also indicate increased levels of homozygosity in the CA captive population, which is most likely the significant factor in the level of differentiation observed in the F value. The inbreeding coefficients (F and f) in the NA population seem to indicate that differentiation between the NA and wild populations is more a factor of founder effect than of inbreeding (Tables 2 and 5). However, despite the lack of gene flow between the NA and CA populations, θ(F_{ST}) and F values are comparable to those observed between the captive and wild population samples (Table 5), as well as between two wild population samples [Norton and Ashley, 2004].

DISCUSSION

The establishment of a population from a small number of individuals has been shown both theoretically [Nei et al., 1975; Fuerst and Maruyama; 1986] and experimentally [Maudet et al., 2002; Broders et al., 1999; Houlden et al., 1996] to result in a significant loss of both allelic diversity and heterozygosity. However, even though the NA captive population of Baird's tapirs was founded with only eight animals, it has retained levels of genetic variability and diversity that are equal to or greater than those found in the sampled extant wild Costa Rican population (Tables 1–3). The high level of allelic richness in the NA captive population is most likely the

result of high genetic diversity in the eight original founders, which were collected from across the range of Baird's tapirs (Honduras (n = 1), Nicaragua (n = 1), Costa Rica (n = 1), Panama (n = 3), and unknown wild origin (n = 2)). Furthermore, the relatively high level of observed heterozygosity is consistent with the integration of animals from different source populations with different allelic frequencies (the Wahlund effect) [Forbes and Boyd, 1997]. Moreover, it is likely that careful captive breeding to maximize the retention of genetic variability by minimizing genetic drift and inbreeding also contributed to the higher level of allelic richness observed.

Even though the levels of genetic variability in the CA captive population appear to be similar to those in a wild population, the estimates of individual inbreeding coefficients (f) were significantly greater than zero (Table 2). The high inbreeding coefficient may be the result of the founding event, as well as the limited translocations between zoos and recent occurrence of inbreeding. The animals captured from the wild may by chance have a high proportion of loci that are homozygous for different alleles. Accordingly, the presence of several alleles at low frequencies would result in an expected heterozygosity that is larger than the observed heterozygosity, and thus produce an apparent high level of inbreeding.

Despite the greater levels of inbreeding detected within the CA captive population, the relatively small $\theta(F_{ST})$ values between the CA captive and the wild Costa Rican population sample indicate that the CA captive population probably has not experienced a significant level of differentiation from wild animals. This is most likely a factor of the large proportion of wild-caught animals still alive in the CA captive population. The larger $\theta(F_{ST})$ values between the NA and wild population sample indicate that the NA population has experienced a greater level of divergence from the wild population than the CA captive population. This is most likely the result of a founder effect, as well as a longer period of isolation from the wild population compared to the CA captive population. However, despite the lack of gene flow between the CA and NA captive populations, the level of differentiation between them appears to be comparable to that found between wild population samples from Panama and Costa Rica ($\theta(F_{ST}) = 0.059$–0.18) [Norton and Ashley, 2004]. The high F values between the CA and NA populations further reveal the impact of a founder effect as a result of the independent allele frequencies established in each captive population.

Based on the levels of population differentiation and inbreeding coefficients observed within the captive population, the potential negative effects of inbreeding appear to be far more likely than any possible outbreeding depression that may result from the exchange of individuals between the two populations. The results of this analysis indicate that NA and CA captive populations have not experienced an increased level of population differentiation as compared to that observed between two wild population samples, and can still be considered part of the same management unit. However, without increased effort to maximize the effective population sizes of the captive population by exchanging breeding individuals between NA and CA populations, drift and inbreeding will reduce genetic variability and increase the level of population differentiation between the two captive populations, as well as between the captive population and the wild populations that they are intended to represent.

Because of the limited sample set we were able to compile, and the low number of microsatellites (n = 6), the conclusions we draw here should be considered

preliminary. We hope these initial efforts will stimulate additional studies that will expand the sample size (in both number and distribution) as well as the number and type of genetic markers used (e.g., additional microsatellite loci and mitochondrial sequences) to verify our conclusions.

CONCLUSIONS

1. This sample of captive Baird's tapir populations contains levels of microsatellite genetic variability that are representative of a sampled wild Costa Rican population of Baird's tapirs.

2. Although breeding management of the NA captive population has limited genetic drift and inbreeding, and helped maintain genetic variability, comparison with a wild population indicates that a founder effect and isolation may have resulted in divergence from wild Baird's tapir populations.

3. The combination of a founder effect and a high level of substructure within the CA population of Baird's tapirs has resulted in increased levels of homozygosity, but has not significantly increased divergence from a wild population sample.

4. The exchange of breeding individuals between NA and CA captive populations and among CA zoos would benefit both populations by increasing genetic variability and effective population size, and reducing inbreeding and population differentiation between the two captive populations, as well as between captive and wild populations.

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