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Genetic variability in relocated Père David's deer (*Elaphurus davidianus*) populations—Implications to reintroduction program

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Abstract Since 1985, China has established three breeding herds of Père David's deer: the Beijing Père David's Deer Park (39°07'N, 116°03'E), the Dafeng Père David's Deer Nature Reserve (33°05'N, 120°49'E) and Shishou (Tianezhou) Père David's Deer Nature Reserve (29°49'N, 112°33'E), through reintroductions of about 30-40 founders. Since establishment. all three populations have grown steadily. However, genetic backgrounds in those populations are still unknown. We studied the genetic diversity in Père David's deer and genetic consequences of population relocations in China. We revealed that genetic diversity was extremely low in Père David's deer populations in China. Only a single mtDNA D-loop haplotype was found in the deer, furthermore, only five polymorphic microsatellite loci were screened out from 84 pairs of species-transferred primers. Genetic makeup in the three Père David's deer populations were significantly different (P < 0.01). H_E and allelic richness in the Tianezhou population were the highest (0.54, 2.60, n = 31), Beijing population (0.52, 2.4, n = 125) showed the second highest measures, while the Dafeng population (0.46, 2.39, n = 39) measured lowest. Our results suggest that effective management of a species of low genetic diversity like the Père David's deer should consider the genetic background of each founder to

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Y. Zeng · Z. Jiang Graduated School of Chinese Academy of Sciences, Beijing 100049, China make sure genetic variations are preserved in both source population and relocated population. Now, the Tianezhou population is the most appropriate source population in China when establishing new Père David deer populations in the wild.

Keywords Reintroduction · Founder effect · Genetic management · Microsatellite · *Elaphurus* davidianus

Introduction

It is predicted that 2,000-3,000 species of terrestrial vertebrates will require captive breeding over the next 200 years in order to maintain self-sustaining populations and to avoid extinction (Soulé et al. 1986; Frankham et al. 2002). Captive breeding populations will be sources for reintroducing new populations to the wild. Reintroduction programs have grown in popularity as means of restoring populations of threatened species (Snyder et al. 1996; Seddon and Soorae 1999; Williams et al. 2002; Vernesi et al. 2003; Mock et al. 2004). Conservation management of captive populations as well as reintroduced populations should maximize genetic heterozygosities through proper genetic management. Loss of genetic heterozygosity has a deleterious effect on population fitness (Frankham 2005; Kraaijeveld-Smit et al. 2006) and is therefore important to the viability of populations, especially for successful conservation of small and reintroduced populations (Reed and Frankham 2003; Hedrick et al. 2001; Schmitt et al. 2005).

To create populations with high levels of genetic variation, the design of reintroduction programs should

be species-specific and should include an assessment of genetic history and makeup (Miller et al. 1999; Frankham et al. 2002). Many programs have been conducted without genetic assessments (Leberg 1993). Empirical data will shed light on how to maintain genetic variations in relocating endangered species populations (Cross 2000; Williams et al. 2002; Vernesi et al. 2003; Mock et al. 2004; Palkovacs et al. 2004; Kraaijeveld-Smit et al. 2006).

According to fossil records, Père David's deer (Elaphurus davidianus) was once widely distributed in the region south of 43°N and east of 110°E in East Asia. The deer was extinct in the wild in the late 19th century (Ohtaishi and Gao 1990; Cao 1993). Before the demise of the royal herd of Père David's deer in the Nanyuang Royal Hunting Garden, Beijing in 1900, the Père David's deer had been introduced into Europe. During the last decade of the 19th century, the 11th Duke of Bedford collected the last 18 Père David's deer from Berlin, Paris and Antwerp to form a breeding herd at the Woburn Abbey, England. Only 11 of those deer were capable of reproducing, and it was suspected that all offspring were sired by a single male after the population was established (Jones and Manton 1983; Sowerby 1949). At the end of World War II, the size of the Woburn Abbey herd reached 250. Since then, Père David's deer have been transferred to zoos in Britain, and to other places all around the world. Now, Père David's deer appear recovered from the brink of extinction and have become an example of successful conservation of a critically endangered species (Jiang et al. 2000).

The Reintroduction procedure and populations in China

The first conservation reintroduction of the Père David's deer to China included two groups of 20 and 18 in 1985 and 1987, respectively. All 38 deer were donated by the Marques of Tavistock from Woburn Abbey and sponsored by the World Wildlife Fund (WWF, now the World Wild Fund for Nature). The deer were transported to the centre of the original Nanyuang Royal Hunting Garden in the south suburb of Beijing and the Beijing Père David's Deer Park (39°07'N, 116°03'E) of 60 ha was created there. The Dafeng population was established by the second reintroduction of a group of 39 Père David's deer from seven British zoos in August 1986, with 18 deer from the Whipsnad Wild Animal Park. The herd was released into everglade on the coast of the Yellow Sea in eastern China and the Dafeng Père David's Deer Natural Reserve (33°05'N, 120°49'E) of about 1,000 ha was established there. A total of 94 Père David's deer were trans-located from Beijing to a peninsula in the middle range of Yangtze River in three batches in 1993, 1994 and 2002, respectively. The Shishou Père David's Deer Nature Reserve (29°49'N, 112°33'E) of 1,560 ha was established after the first relocation in 1993.

Prior to 2001 there were three major Père David's deer populations: the Dafeng population of 516 individuals, the Beijing population of 122 deer and the Tianezhou population of 290 deer, as well as approximately 50 small herds with sizes under 25 deer in the zoos and farms (Yang et al. 2003). From 2001 to 2005, the size of the Dafeng population increased to 819, and the Tianezhou population grew to 640. Size of the Beijing population reached 202 in 1993. To prevent the over-grazing of vegetation, the Beijing population size has been maintained at about 120 deer since 1994. Beijing Père David's Deer Park exported excess Père David's deer to zoos and safari parks in China. Dafeng and Tianezhou populations had no other immigration and few emigrations since their establishment.

Genetic diversity in Père David's deer was low. Blood samples from Père David's deer were analyzed with plasma protein electrophoresis, and no polymorphism was found (Ryder et al. 1981). Sternicki et al. (2003) extracted recorded data from the International Species Information System (ISIS) and calculated the coefficient of inbreeding in Père David's deer ranged from 0.2422 to 0.2812, which is highly inbred. During the long process of captive breeding and relocation, historical records were either lost or not completed. To record pedigree was impossible in a group breeding population. Consequently, we don't know the genetic background of Père David's deer in China, though such information is of vital importance for the management of the deer. So far no study has addressed the question of how genetic makeup in Père David's deer populations change during regrouping and relocation, or the conservation implications.

The aims of this study were to assess the genetic diversity of the Père David's deer populations in China with the mitochondrial (mtDNA) control region sequences and microsatellites polymorphisms, to compare genetic consequences of Beijing and Dafeng populations of different founder sources, in order to trace the change of genetic making up after reintroduction to China in the three major breeding groups: the Beijing, the Dafeng and the Tianezhou populations, and to determine their implications for current and future reintroduction programs as well as endangered species conservation.

Methods

Samples and DNA isolation

Père David's deer hinds normally give birth to one calf. During the first week after birth, newborn calves hide in grasses most of the time and stay away from the adult deer group (Jiang et al. 1999). A total of 125 new born Père David's deer calves were sampled from 1999 to 2005. Newborn calves were routinely caught and marked with four-position ear notches by deer keepers (Carnio and Killmar 1983) (Fig. 1). All handling lasted less than ten minutes, and ensured that the calves were led away by their mothers after the manipulation. The protocol of handling animal was approved by the Chinese Wildlife Management Authority and the Experimental Animal Ethnic Committee of the Institute of Zoology, Chinese Academy of Sciences.

We randomly sampled fresh faeces dropping from free-raging adults of Dafeng population and Tianezhou population during rut season in 2004. Each stag was distinguished by the shape and size of antler. Because hinds in estrus form harems, we only collected faeces of hinds in each harem once during the rut, in order to avoid duplicating sampling. Altogether, 64 and 37 faecal samples were collected in the Dafeng population and the Tianezhou population respectively. Ear tissue and faeces samples were kept in ethanol (Fig 2).

Total genomic DNA was isolated from ear tissue using standard organic extraction procedures and was of good quality in PCR (Sambrook et al. 1996.). DNA



Fig. 1 Positions of ear notches. Using four positions in each ear, 99 number combinations are possible. Then a small hole in left or right ear represented one or two hundred number respectively

from ethanol-saved faeces was isolated according to Zhang et al. (2004). Due to the limitation of method of DNA-extraction from herbivore faeces, we finally successfully extracted total DNA of good quality from 39 and 31 samples of the Dafeng and Tianezhou populations, respectively.

MtDNA control region and micro-satellites

Twenty-five ear tissue samples were taken from the Beijing population, and 20 fecal samples from the Dafeng population. DNA were successfully extracted and randomly chosen to produce sequence data. We amplify 590bp segments from the HVR-1 of the mtDNA control regions using primers L15926 and H16498 (Zhang and Ryder 1993). Amplification included about 50 ng total DNA in $1 \times$ amplification buffer (10 mM Tris-HCl, pH 8.4, 50 mM KCl) in a 50 μ l reaction volume, with 3.0 mM MgCl₂, 0.1 mM dNTPs, 0.4 µM each primer, 0.1 mg/ml of BSA and 1.25 units of Taq polymerase (MBI-Fermentas). The amplification cycle consisted of an initial denaturing at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. Cycling culminated with 7 min extension at 72°C and then held at 4°C. Segments were sequenced using the BigDyeTM Terminator kit and ABI Model 377 Automated Sequencer. DNA sequences were aligned using ClustalX v.1.81 (Thompson et al. 1997) and visually checked.

Sixty-two pairs of species-transferred (including ox, sheep, goat, red deer and reindeer) microsatellite primers from the autosomes (Slate et al. 1998; Slate et al. 2000; Slate et al. 2002) and 20 pairs of Y-specific microsatellite primers, as well as two pairs on X-specific primers of bovine were involved in this study (Liu et al. 2002)¹. Amplifications included about 25 ng total DNA in 1 × amplification buffer in 10µl volumes, with 2.0–3.0 mM MgCl₂, 0.1 mM dNTPs, 0.4 µM of each

¹ ABS12, AF5, AFR227, AGLA226, AGLA232, BL4, BM1225, BM1329, BM1706, BM203, BM2320, BM2934, BM4107, BM4440, BM4513, BM5004, BM757, BM888, BR3510, C217, CSSM26, CSSM26, CSSM29, CSSM39, CSSM41, CSSM43, HUJ175, IDVGA29, IDVGA3, IDVGA37, IDVGA39, ID-VGA55, IDVGA8, ILSTS6, ILSTS93, INRA107, INRA121, INRA131, INRA169, JAB8, MGTG4B, NRAMP1, RBP3, RM12, RM178, RM188, RM95, RT1, RT23, RT5, T156, T172, T193, TEXAN15, TGLA10, TGLA127, TGLA226, TGLA337, TGLA378, TGLA40, TGLA431, and TGLA86 are from autosomes. BL22, and XBM31 are X-autosome-specific primers. BOV97M(DYS2), BRY.1(DYZ7), INRA008(DYS3), TSPY, UMN0103, UMN0301, UMN0304, UMN0307, UMN0311, UMN0504, UMN0705(TSPY-MS), UMN0920, UMN1201, UMN1203, UMN1307, UMN1514, UMN0406. UMN1113, UMN2303, and UMN3008 are Y-autosome-specific primers.

Fig. 2 Allele frequencies for each Père David's deer population in China by locus. Allele frequencies of each locus were illustrated following a population sequence as Dafeng, Beijing and Tianezhou from left to right. *Genic and genotype differentiation are both significant for each population pair on this locus (P < 0.05)



primer, 0.1 mg/ml of BSA and 0.5 units of Tag polymerase (MBI-Fermentas). An annealing temperature of 55°C and 35 cycles was used for all loci. PCR procedures of 49 markers on autosome, and 16 markers on sexual chromosome were succeed and stabilized. Polymorphism was detected by denatured-PAGE gels (Sambrook et al. 1996). However, only five microsatellite markers on autosomes were polymorphic, which are T172, TGLA10, T193, BM1225, and BM757 (Table 1).

Genotyping and data analysis

Multiplex PCR were carried out with each forward primer fluorescently labelled with TAMRA, 6-FAM, or HEX. To avoid drop-out, multiplex PCR and genotype detection of each sample were independently repeated three times. Genotypes were scored on an ABI PRISM 377 DNA Sequencer (Applied Biosystems/Perkin Elmer). The fluorescently labelled DNA fragments were analyzed and genotyped using Gene-Scan v.3.7 (Applied Biosystems/Perkin).

The MS EXCEL add-in MS TOOLS v.3 toolkit (Park 2001) was used to format microsatellite data setting and to create input files for succeeding analysis. FSTAT version 2.9.3.2 (updated from Goudet 1995) was used to generate summary statistics such as the allele frequency, allelic richness based on the minimal sample, the observed heterozygosity, as well as the expected heterozygosity (Nei 1978) of each population, and to test the loci for a genotypic disequilibrium. Hardy-Weinberg equilibrium exact tests with complete enumeration at each population, Genic differentiation which was the allelic statistic, and genotype differentiation of genotypic goodness of fit statistic (Raymond and Rousset 1995) for each population pair were carried out by the GENEPOP on the Web using Fisher's exact test method.

 $F_{\rm ST}$ estimators which were traditionally used to measure population differentiation, and the inbreeding coefficient which is the allele frequency based correlation (F_{IS}) (Weir and Cockerham 1984) were calculated using the program GENETIX v. 4.03 (Belkhir et al. 2001) by a permutation test with 1000 permutations. We also used GENETIX v 4.03 to calculate the popularly used genetic distance measure, the standard genetic distance of $N_{\rm EI}$ (1972) between population pairs which is defined as $D = -\ln(I_{xy})$, and the I_{xy} is

Table 1 Overall allelefrequencies of Père David's		T172	TGLA10	T193	BM1225	BM757
deer in China for all loci	Allele A Allele B Allele C Allele D	0.0179 (222bp) 0.3256 (226bp) 0.0641 (234bp) 0.5923 (238bp)	0.5923 (145bp) 0.4077 (157bp)	0.4462 (155bp) 0.2205 (157bp) 0.3333 (161bp)	0.4513 (240bp) 0.5487 (252bp)	0.4103 (185bp) 0.5897 (191bp)
	$N_{\rm A}$ $H_{\rm E}$	4 0.5401	2 0.4842	3 0.6429	2 0.4965	2 0.4851

the genetic identity between population x and population y. The significance was based on 95% confidence.

Results

Within-population genetic diversity and departure from random mating

Only one haplotype (GenBank DQ295069) was found in 45 samples which indicated lack of genetic variation in Père David's deer mtDNA. Five microsatellite loci were polymorphic screened out from 84 pairs and number of alleles per polymorphic locus ranged from 2 to 4 (Table 1). The test of genotypic disequilibrium based on 300000 permutations for each pair of the five microsatellite loci over all populations gave two significant values for 10 comparisons.

Frequencies and numbers of alleles in each population were diverse (Fig. 1). There are four alleles on locus T172 in the Tianezhou population, but one allele of 222bp was missing from the Beijing population, while another of 234bp was missing from the Dafeng population. Tianezhou population had the highest heterozygosity whereas Dafeng had the lowest (Table 2). The negative $F_{\rm IS}$ value of all samples ($F_{\rm IS} = -0.075$, P < 0.05) and each population indicated deviation from Hardy-Weinberg equilibrium, and excess of heterozygosity.

The probability test by the Markov chain method showed four to five loci significant deviations from the Hardy–Weinberg equilibrium in the Dafeng and Tianezhou populations. The probability test of all loci revealed departure from Hardy-Weinberg equilibrium in the Beijing population. Exact tests at all five loci with the Fisher's method showed significant deviations from Hardy–Weinberg equilibrium in each population (Table 2).

Table 2 Summary of genetic statistics of Père David's deer populations in China

	Dafeng	Beijing	Tianezhou
Sample size	39	125	31
Allelic richness \pm SD	2.39 ± 0.54	2.40 ± 0.55	2.60 ± 0.89
$H_{\rm O} \pm {\rm SD}$	0.48 ± 0.10	0.54 ± 0.04	0.68 ± 0.12
$H_{\rm E} \pm {\rm SD}$	0.46 ± 0.10	0.52 ± 0.06	0.54 ± 0.05
F _{IS}	-0.043	-0.037	-0.255
Probability of	0.324	0.555	0.053
Hardy-Weinberg equilibrium test			

 $H_{\rm O}$: observed heterozygosity $H_{\rm E}$: expected heterozygosity

 Table 3 Differentiation between all pairs of Père David's deer population in China

Population pair	Genic diff	ferentiation	Genotype differentiation	
	Chi	P-value	Chi	P-value
Dafeng and Beijing Dafeng and Tianezhou Beijing and Tianezhou	Infinity Infinity 23.298	<0.001 <0.001 0.00970	Infinity Infinity 25.167	<0.001 <0.001 0.00504

Analyses of population differentiation

The exact test for genic and genotypic differentiation revealed significant differentiations between the studied populations at all five loci at 0.05 levels (Table 3). The most pronounced distinction was observed at three loci (T172, T193, and BM757) which had high significant level of genic and genotypic differentiations.

The mean F_{ST} value, which estimates the level of interpopulation differentiation, was 0.067 (P < 0.05). Differentiation between Tianezhou and Beijing population was less strong than any other population pairs, which is not significant in *P*-value of F_{ST} estimator test, and N_{EI}'s genetic distance between them was likewise closer than other pairs (Table 4).

Discussion

Genetic variability

The level of polymorphism detected in Père David's deer was very low. Indeed, no polymorphism was observed for the 590bp of the control region of mtDNA in over 45 individuals from two main populations, and only 5 of 62 microsatellite markers in the autosomes were polymorphic, with only one locus having four alleles among all populations. Lack of genetic variation is not unusual in early studies with molecular markers such as allozyme electrophoresis and RFLP of endangered species. The same situation was found in Père David's deer by Ryder et al. (1981). However, the

Table 4 Original measures of Nei's genetic distance and F_{ST} between Père David's deer populations in China

Dafeng	Beijing	Tianezhou
	0.128	0.142
$0.104^{\rm a}$		0.013
0.111 ^a	0.002	
	Dafeng 0.104 ^a 0.111 ^a	Dafeng Beijing 0.128 0.128 0.104 ^a 0.002

^a Indicates *F*-statistic are significant in this pair (P < 0.05). Original measures of Nei's genetic distance are above diagonal, and F_{ST} are below diagonal absence of high variable genetic loci is uncommon in some populations due to demographic factors (the Black-footed rock-wallaby Petrogale lateralis, Eldridge et al. 1999; the Morro Bay kangaroo rat Dipodomys heermanni morroensis, Matocq and Villablanca 2001; the New Zealand snapper Pagrus auratus, Hauser et al. 2002; the Killer Whales Orcinus orca, Hoelzel et al. 1998; and the Hainan Eld's deer Cervus eldi hainanus, Pang et al. 2003) or peculiar life history (the Indian queenless ants Diacamma indicum, Viginier et al. 2004). By applying five microsatellites, we detected the heterozygosity values $(H_{\rm E})$ of Père David's deer were in the low range of average heterozygosity of microsatellites markers compared to wild healthy cervids popoulations and most other mammal populations that were reviewed (Garner et al. 2005). Microsatellite variability of Père David's deer population was also lower than restored populations of other cervids (White-tailed deer Odocoileus virginianus, DeYoung et al. 2003; Red deer Cervus elaphus, Hmwe et al. 2006; Kuehn et al. 2003; and Vietnamese sika deer Cervus nippon pseudaxis, Thévenon et al. 2004).

The 12th Duke of Bedford even suspected that all Père David's deer were the offspring of a single male (Sowerby 1949). Of the 84 loci, there were 62 microsatellite loci located in the autosomes, and others were in sexual chromosomes. We sought to authenticate the suspicion of the 12th Duke of Bedford about single forefather of the Père David's deer herd by using Y-chronmosomal microsatellites, and our results do not refute it. Considering the lacks of genetic variation in now-alive population, as well as the ascertainment bias of species-transferred primers (Garner et al. 2005), we still cannot confirm the verdict.

Given the different sources of founders in the Beijing and the Dafeng populations, low genetic variability should be a characteristic of all Père David's deer populations around the world. Reasons for the loss of genetic diversity in captive or relocated population may be mainly attributed to population decline during the past 50 years, and limited founder size (Cross 2000; Earnhardt et al. 2001; Hedrick et al. 2001; Kraaijeveld-Smit, et al. 2006). The Père David's deer was extirpated in the wild before scientific documentation of its characteristics. Besides unknown history of the Qing dynasty royal herd, Père David's deer experienced several bottlenecks, such as when Père David's deer was transported from China to Europe, when the Père David's deer were collected to found the first breeding population at the Woburn Abbey, and when Père David's deer was reintroduced in China in the 1980s. As a result of these bottlenecks, genetic variation decreased, as has been observed in other captive animal populations (Briscoe et al. 1992). The harem mating system and matriarchal social structure in Père David's deer may compound the inbreeding effect (Jiang et al. 1999; Jiang et al. 2004).

Genetic differentiation

The moderate (Wright 1973) but significant mean F_{ST} value indicated sub-structuring in Chinese Père David's deer populations. The Tianezhou and Dafeng populations showed the highest levels of differentiation. Considering Tianezhou population was derived from the Beijing population, the differentiation between the Dafeng and Beijing populations was comparable to the differentiation between the Dafeng and Tianezhou populations.

The Beijing population and Dafeng population were established by two methods with measurably different consequences. The founder group size of both populations was about 38 individuals, but the former was introduced from the Woburn Abbey, and the latter was created with founders from seven zoo populations. Population sizes of the overall-Beijing population, which is that of Beijing population plus that of Tianezhou population and the Dafeng population have both rapidly grown to more than 500 during a short period after establishment. However, genetic diversity of the overall-Beijing population is higher than that of Dafeng population, and there was one allele in the T172 loci lost in Dafeng population but presented in the Tianezhou population. Consequently the genetic dissimilarity between those two populations may attribute to genetic differentiation of the founders rather than genetic drifts which may need few more time to take place.

Although exact tests of population differentiation were significant in Tianezhou and Beijing populations, $F_{\rm ST}$ value between them was small and not significant. Usually the F_{ST} estimator tests are more powerful than the allelic goodness of fit tests and the genotypic goodness of fit tests, and the exact tests need to be based on the hypothesis of independent sampling of genotypes when the random sampling of alleles is inappropriate (Goudet et al. 1996). However, since disequilibrium is observed in all Père David's deer populations in China, allelic and genotypic goodness of fit statistics tests are less powerful than F_{ST} . The genetic differentiation between Tianezhou and Beijing population is not significant, despite the loss of one allele on locus T172 of Beijing population compared to the Tianezhou population. Loss of one allele on locus T172 in the Beijing population is most likely due to artificial grouping during relocation of deer to establish Tianezhou population, for the two populations only have been separated for a maximum of 13 years, about two to three generations. Loss of genetic variation in small population as Beijing, probability of random sampling individual with rare allele is high when choosing deer for new reintroduction or even to the zoo.

Conservation implications

Loss of genetic variation in the Père David's deer resulted from ignoring genetic factors in population management, and should be of particular concern. A major factor influencing the success of reintroduction programs is the number of individuals released (Tenhumberg et al. 2004). To establish a viable population, it is recommended that a minimum of 20–30 genetically effective founders to be used (Frankham et al. 2002). For invertebrates, 50 female individuals are sufficient to transplant almost the entire genetic variation when establishing a new viable and quickly expanding population. However, to establish a viable vertebrate population, a large number of founders is needed (Schmitt et al. 2005).

Beside the number of founders, design of reintroduction programs to maintain genetic variation in populations has been a subject of much discussion. Some people suggested that one should select founders from a population with highest heterozygosity while others argue to establish a population with individuals from different genetic stocks (Leberg 1993; Frankham et al. 2002). Based on our results, in managing such endangered species as Père David's deer which is of too limited genetic variability, it is advisable to relocate adequate number of individuals from one well documented population with the highest heterozygosity, instead of choosing individuals from populations which were not distinct.

Furthermore, before introductions are conducted, it is important to evaluate the genetic backgrounds of possible replacement populations (Russell et al. 1994; Palkovacs et al. 2004). The reason for reintroduction failure could be successive bottlenecks (Thévenon and Couvet 2002). However, the effects on source population are seldom mentioned. Negative effects of relocation should be considered if the founders are relocated from small and genetically impoverished resource populations. If captive breeding of Père David's deer is impossible because the population is too small animal group to be self-sustaining, the sole function of such populations will be for exhibition. When carrying out such relocating programs from a limited source population to form small zoo groups, genetic background of individual should be checked to ensure that rare alleles will not be random sampled. It is also advisable to check genetic background of each founder to make sure genetic variations are preserved in both source and a new relocated population.

Our results indicate that Tianezhou Père David's deer population would be the most appropriate source population in China when establishing new Père David's deer population to the wild. However, although the Beijing Père David's deer population and the Dafeng Père David's deer population are significantly genetically different, translocation costs and risks of disease transfer should be considered in planning exchange of individuals between the two populations. (Margan et al. 1998).

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