A "*Cervus*" genotyping kit based on automated fluorescent multiplex PCR for rapid characterisation of genetic diversity in several deer populations: a tool for wildlife management.

Thévenon, S.[1,3], Le Thi Thuy[2], Le Viet Ly[2], F. Maudet[1]; A. Bonnet[3] and J.C. Maillard[1].

[1]: CIRAD-EMVT (France), [2]: NIAH (Vietnam), [3]: MNHN (France).

Abstract

Recent techniques in molecular biology, especially the use of microsatellites markers, and advances in statistics are very useful to characterize the genetic structure of populations and to deduce adapted rules for wildlife management and farming. It becomes possible i) to infer demographic changes, to assess the level of gene flow between populations and the impact of human activities on wild populations; ii) to estimate the impact of farming system on genetic diversity of captive populations, to help the farmers on parentage control in their herds and to set up efficient breeding schemes. In the tropical countries, deer production, including farming and hunting, is economically very important but most often empirical. In this context, we set up a genotyping tool for several tropical deer species using microsatellites markers derived from bovine. Twelve polymorphic microsatellites, labelled with three different fluorochromes, are multiplexed in three PCRs, and analyzed using an ABI 377 sequencer. This genotyping tool was used to characterize populations of two economically important tropical deer species, the Vietnamese sika deer and the rusa deer.

Introduction

Animal diversity has been essential for food and agriculture for 12,000 years. Nowadays, wildlife is often forgotten but constitutes an essential resource for human populations, as a source of food, income and employment. The breeding of local wild and domestic species is very important, especially in developing countries. In fact, these animals are locally adapted to their environment, they do not require important inputs quantity or investments contrary to selected breeds of developed countries, which usually fail to breed or to survive[1]. The monitoring and management of wild and captive populations, especially threatened ones, become the first step for a sustainable valorisation. Particularly, genetic management is essential in order to maintain population viability, preserve the adaptive potential and future production options[2] and set up selection schemes. In our study, two tropical species have been chosen for genetic analysis: the Vietnamese sika deer (*Cervus nippon pseudaxis*) and the rusa deer (*C. timorensis rusa*). The Vietnamese sika deer, considered extinct in the wild, is a perfect model of the link between wildlife conservation and valorisation. It is bred in Vietnamese traditional farms (8,000 – 10,000 individuals) for velvet production (yield: 200-300 US dollar per year per male). The rusa deer is an Indonesian species, used also for meat and velvet production. It has been introduced in various islands, like Mauritius and New Caledonia, where populations spread from a few founders. In the framework of genetic resources management, the purpose of this study is to

characterize the genetic structure of populations of these two taxa, using the same microsatellites markers.

Materials and methods

Sampling and DNA extraction

The Vietnamese sika deer were sampled in Nghe An (176 samples) and Ha Tinh (24 samples) provinces in central Vietnam in October 1999 and 2000. Ear tissue was taken from each individual and stored in ethanol 70% until DNA extraction with DNeasy tissue kit from Qiagen. Blood samples on EDTA (DNA extracted with Wizard kit from Promega) were taken from 86 rusa deer in Mauritius (mainly from farms) in 1998 and from 130 animals in New Caledonia (only from farms) in 1999.

DNA amplification and microsatellites analysis

Twelve microsatellites markers, derived from bovine, were amplified in 3 PCR multiplex[3] (group *1*: TGLA57, with INRA121, IDVGA55 and BMC1009; group *2*: VH110, with BM757, BL42 and BM848; group *3*: TGLA126, TGLA53, BM203 and CSSM43) with AmpliTaq® Gold in a Perkin Elmer Amp PCR system 9700. One primer from each pair was synthesized with a fluorescent dye group, FAM (group *1*), JOE (group *2*), NED (group *3*), on the 5'end. The 3 multiplex PCR products were mixed together for each sample and loaded in a 6% Long Ranger™ gel (FMC) using an ABI PRISM™ 377 DNA Sequencer (Perkin Elmer). Gels were analyzed using GENESCAN™ Analysis 2.1 and GENOTYPER™ 2.5 software.

Statistical analysis

Genetic diversity was measured as the observed (*Ho*) and expected (*He*) heterozygosities, and the mean number of alleles per locus. Wright's *F*-statistics (F_{IT}: departure from Hardy-Weinberg proportions at the level of the whole population, F_{IS}: deviation from Hardy-Weinberg proportions at the sub-population level, F_{ST}: proportion of the genetic variance due to differentiation between sub-populations) were used to analyze within and between population structures at different scales with GENEPOP 3.03.

Results

See table 1 and 2. From 12 microsatellites markers, nine were actually used for statistical analysis in the sika deer and eight in the rusa deer. The other markers were either monomorphic, badly amplified or showing high heterozygote deficiency certainly due to null alleles.

Discussion and conclusion

The same markers, amplified in three multiplex PCRs, have been used with success to characterize these two different deer species. All markers were not able to work on each species, but their numbers were sufficient to assess genetic structure. The genetic variability determined by the microsatellite markers in Vietnamese sika deer and Rusa deer corresponds to the one usually observed in wild mammals populations. The microsatellites allowed to show that, in the sika deer, structuring between the villages is significant but small in absolute value: the exchange level is quite large to prevent local inbreeding in general (most villages do not present any heterozygote deficit, not shown). Concerning the rusa deer, a strong differentiation between and also a strong

structuring within the two islands are highlighted. Finally, the global level of genetic variability would indicate that genetic variability is not impoverished in neutral markers and selection schemes would be susceptible to be set up. In conclusion, the tool, based on markers derived from bovine, was used on two different species, but it works also on numerous other deer species. It could be susceptible to be applied, at least partially, on a wider range of taxa. Such a tool is useful for set up management and conservation programs (assessment of bottlenecks, inbreeding, gene flow, hybridization), and to carry out paternity exclusion. Concerning animal health, such a tool could also be used in epidemiology, with the study of animals migration, especially vector populations.

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Table 1: Genetic variability in sika and rusa deer.

	Cervus nippon pseudaxis Sika deer			*Cervus timorensis rusa* Rusa deer		
Locus	Allelic size range	Expected heterozy-gositie	Number of alleles	Allelic size range	Expected heterozy-gositie	Number of alleles
Tgla57	83			83-91	0.63	5
Inra121	134-166	0.81	7	132-156	0.78	13
Idvga55	196-210	0.68	5	200-220	0.68	9
Bmc1009	281-303	0.61	5	273-303	0.69	8
Vh110	96-130	0.62	5	116-136	0.63	9
Bm757	163-187	0.46	5	179-193	0.65	6
Bl42	247-277	0.51	9	237-263	0.66	9
Bm848	150-172			356-398		
Tgla126	107			107		
Tgla53	150-172	0.66	4	130-158		
Bm203	218-234	0.57	4	208-236	0.80	14
Cssm43	277-333	0.51	7	349-369		
MEAN		**0.60**	**5.7**		**0.69**	**9.1**

Table 2. Wright *F*-statistics in populations from sika and rusa deer.

	Population	*Fis*	*Fst*	*Fit*
Rusa	New-Caledonia	0.07***		
	Mauritius	0.08***		
	Global	0.08***	0.25***	0.30***
Sika	Global	0.02*	0.01***	0.03***

P-value: *<0.05, **<0.005, ***<0.001