Paternity Determination And Body Weight Analysis In Fallow Deer (*Dama dama*)

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Introduction

The interest on game meat increased strongly during last decade. So far the major amount of game meat has been harvested from wild populations but today most wild populations are decreasing rapidly caused by loss of habitat, overhunting or poaching. Depending on the great economic value of game meat, enclosures come more and more into the focus of producers. Besides the advantage of a permanent production independent from hunting seasons, enclosures provide also the opportunity for intentional breeding of these game species. Although the animals are kept as "farm animals", sometimes over generations, they are not domesticated species. Their flight distance is still large and they have no tame behaviour overall, for example. However, modern DNA-based methods and tagging techniques enable the use of breeding programs for these populations. Pedigree analyses as well as individual trait performances are major prerequisite for each selective breeding program. Here we use both in fallow deer for the first time.

Material and methods

Animals. A herd of fallow deer was investigated from 2005 till 2008. This herd contains 2 bucks (L born in 1997, H 2002) and 53 hinds in fall 2005 as well as 4 bucks (L, H, buck G 2004, M 2004) and 58 hinds in 2006. After female selection, 41 fawns were born in summer 2006 and 42 in summer 2007 respectively. Birth weight was detected. All fawns were tagged using microchips. Body weight was measured a second time at an age of 18 months after harvesting. All animals were reared in one herd in an enclosure with an area of 6.5 ha.

Molecular methods. Tissue samples for DNA extraction were taken from 4 bucks and 83 fawns. Fallow deer specific microsatellites are rare. Therefore, we used cross-species amplifications which is a common procedure in wildlife research today (Ede et al. (1995); Wilson et al. (1997); Røed and Midthjell, (1998); Zsolnai et al. (2009)). A set of 32 microsatellites was used for paternity determination. *MAF70* (Buchanan and Crawford (1992)), *BM1329* and *BM143* (Bishop et al. (1994)), *ILSTS072* and *ILSTS097* (Guérin et al. (1994)), and *NVHRT46* (Røed and Midthjell (1998)) failed to amplify. All successful markers are listed in table 1.

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Table 1: Details of microsatellites, number of alleles, observed heterozygosity, and PIC

Locus	Species	Na	Ho	PIC	Reference
AF5	cattle	3	0.65	0.57	Konfortov et al. (1996)
BM4107	cattle	1	-	-	Bishop et al. (1994)
BM4208	cattle	1	-	-	Bishop et al. (1994)
BM6438	cattle	2	0.22	0.19	Bishop et al. (1994)
BTJAB1	cattle	7	0.71	0.95	Williams et al. (1995)
CDU92064	fallow deer	2	0.17	0.15	Li et al. (2001)
DAM17a	fallow deer	2	0.05	0.04	Ludwig et al. (2010)
DAM2	fallow deer	1	-	-	Ludwig et al. (2010)
DIK082	cattle	1	-	-	Hirano et al. (1996)
HAUT27	cattle	1	-	-	Ihara et al. (2004)
IDVGA59	cattle	1	-	-	Mezzelani et al. (1995)
ILSTS030	cattle	2	0.48	0.36	Guérin et al. (1994)
McM58	sheep	3	0.51	0.46	Hulme et al. (1994)
NVHRT21	reindeer	1	-	-	Røed and Midhjell (1998)
NVHRT22	reindeer	2	0.44	0.34	Røed and Midhjell (1998)
NVHRT31	reindeer	1	-	-	Røed and Midhjell (1998)
NVHRT48	reindeer	1	-	-	Røed and Midhjell (1998)
NVHRT63	reindeer	1	-	-	Røed and Midhjell (1998)
NVHRT73	reindeer	1	-	-	Røed and Midhjell (1998)
RT1	reindeer	1	-	-	Wilson et al. (1997)
RT6	reindeer	2	0.25	0.22	Wilson et al. (1997)
RT9	reindeer	1	-	-	Wilson et al. (1997)
RT23	reindeer	1	-	-	Wilson et al. (1997)
RT27	reindeer	3	0.45	0.38	Wilson et al. (1997)
RT30	reindeer	2	0.41	0.32	Wilson et al. (1997)
VH110	sheep	3	0.64	0.56	Hanrahan et al. 1993

Na = number of alleles, Ho = observed heterozygosity, PIC = polymorphic information content

Standard PCR was carried out in a total volume of 10 µl with 0.4 U FirePol (Solis BioDyne) and 20 ng DNA. Following initial denaturation at 94 °C for 1 min, 35 cycles at 94 °C for 1 min, 50 °C for 30 sec and 72 °C for 40 sec were performed. Allelic patterns were detected using IR dye primer labelling at a LI-COR 4300 DNA analyser (LI-COR).

Statistics. The observed heterozygosity (Ho) for each microsatellite was calculated as [1 minus sum of all (allele frequencies)²]. The polymorphic information content (PIC) is estimated after Botstein et al. (1980). A t-test was done to examine the significance between the weight means ($p < 0.05$).

Results and discussion

In our investigation, twelve of the 26 primers were informative (table 1). However, they are often producing only a few alleles. Low genetic variation in fallow deer is not new. It is well known since decades (Pemberton and Smith (1985)) and was confirmed by several studies (Hartl et al. (1886); Randi and Apollonio (1988)). Nevertheless it is notable, that our

enclosure population is characterized by an extremely low level of genetic variance compared with other studies of fallow deer (Poetsch et al. (2001); Say et al. (2003)). However, here we do paternity analysis using microsatellites for a herd of fallow deer reared in an enclosure for the first time. In 2006, 35 fawns were sired by the dominant old buck L (eight years) and only five by the young H (three years). It was not possible detecting paternity in one case (out of 41). In 2007, the reproductive success of buck L decreased. L sired only 17 fawns whereas H (now four years old) sired 16 fawns. Interestingly, G and M (both only two years) reproduced also successfully. G reproduced in two cases, whereas M sired one fawn. We could not detect the father six times for the 2007 offspring (out of 42). However, L lost his dominant role. The reproductive success of the other bucks increased strongly. Taken together, we detected successfully the paternity in 97.6% in offspring from 2005 (two bucks involved) and in 85.7% for the offspring from 2006 (four bucks involved). A larger panel of fallow deer specific polymorphic microsatellites can help to improve that success.

It can be calculated that the most successful buck L mate about 45 hinds in 2005 and 23 hinds in 2006. Despite the large number of hinds, the mating success was nearly 100% however. During both years, only one hind did not calve. Remarkably, the two young bucks G and M sired also successfully at an age of two years only. Their reproductive success was unexpected. Because no visual observation was done we have no information about mating behaviour, mate choice of females or clustering of male territories as previously described for wild fallow deer (Clutton-Brock et al. (1989); Say et al. (2003)). Likely, there are behavioural differences between wild deer and their counterparts reared in enclosures. Our findings can help managing the reproduction of semi-wild herds. For example, it can be advantageously separating younger bucks during the mating season when only the dominant male should be mating.

Additional to pedigree analysis, we measured the body weight of fallow deer fawns overall and with respect to their different paternal lineages. The fawns reach an average birth weight of 4.4 kg (table 2). They were harvested with a weight of 20.9 kg at an age of 18 months. We observed neither a significant difference in the mean of birth nor in the weight at harvesting among the different paternal lineages. However, we are at a starting point collecting these data. More years/data are necessary to calculate selection coefficients, and also more cohorts should be included.

Table 2: Body weight at birth and at harvesting of the different paternities

Buck	Birth weight (kg)		Hunt weight (kg)	
	mean ± SD	n	mean ± SD	n
L	4.4±0.579	52	22.0±3.612	32
H	4.4±0.464	21	21.8±2.996	16
G	4.8±0.778	2	22.2±6.718	2
M	3.7	1	-	0
all	4.4±0.540	83	20.9±5.080	60

Conclusions

We detected successfully the paternities of 91.6% offspring using microsatellites. We also demonstrate that lower ranked bucks did sire also offspring although only in small numbers. Therefore, we conclude that for selective breeding of such semi-wild populations, all other bucks have to be separated from the herd during mating season avoiding unintended mating. Finally, molecular genetics open the gate for selective breeding plans in these semi-wild populations of game species.

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