

# The endangered *Dama dama mesopotamica* Brooke, 1875: genetic variability, allelic loss and hybridization signals

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## Abstract

The Persian fallow deer (*Dama dama mesopotamica*) formerly widespread in the Middle East was described scientifically at the end of the 19<sup>th</sup> century and considered extinct ever since. In 1956 it was rediscovered in south-western Iran. As a result, several countries have undertaken actions to reintroduce this subspecies in its native territory. In 2007 the Christian Oswald Foundation, in close cooperation with Iranian institutions, launched plans of *in situ* and *ex situ* breeding actions, with its centre in the German Von Opel Zoo and with cooperative Mediterranean partner countries as Israel, to support conservation efforts under scientific control. We performed genetic studies to study the suspected hybridization with European fallow deer (*Dama dama dama*) and a commitment to preserve pureblood populations. We used a set of microsatellite loci to examine genetic variation and recent hybridization with the European fallow deer. All microsatellite loci used were polymorphic, but some were monomorphic within subspecies. The allelic richness was similar in both subspecies but the 'private allelic richness' was reduced to a half in the Persian fallow deer, signalling allelic loss due to genetic drift and inbreeding. Moreover, we showed the presence of two discrete groups representing the two subspecies, with no signs of admixture or hybridization. Furthermore, Persian fallow deer studied here belong to two pre-defined genetics groups: the wild and the (more genetically impoverished) captive populations of Persian fallow deer. Finally, the Persian fallow deer deserves a high conservation priority, both in the Iranian stock and in the captive populations, so as to avoid hybridization.

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## Introduction

The fallow deer (*Dama dama* Linnaeus, 1758; Mammalia: Cervidae) belongs to those species of the western Palaearctic mammalian fauna whose history in the early Holocene is still far from understood (Masseti *et al.*, 2008) although it is well known throughout Europe. It was believed that the fallow deer as a deer species had been excluded from most of the European mainland before the last interglacial. Its post-Pleistocene natural occurrence in western Europe has not been proven. However, current knowledge suggests it survived after the Pleistocene in Sicily, southern Balkan Peninsula and southern Anatolia (Masseti and Rustioni, 1988; Masseti, 1996, 1999). Archaeological evidence suggests that during the Holocene the diffusion of the European fallow deer was human-mediated for hunting and embellishing palaces (Chapman and Chapman, 1975, 1980; Putman, 1988; Masseti, 1996, 1999; Croft, 2002) across Europe, but archaeologists and historians knew the existence of another fallow deer inhabiting at least eight countries of the Near East (Iran, Iraq, Israel, Jordan, Lebanon, Palestine, Syria and eastern Turkey, see Hemami and Rabiei, 2002): the Persian fallow deer (Chapman, 2008). The Persian fallow deer (*Dama dama mesopotamica* Brooke, 1875) has a complex conservation history. Historically, its populations underwent some sort of primitive but sustainable game management in the Mediterranean isles during the Neolithic Periods (in Cyprus 10-4.5 thousand years ago) (Croft, 2002). It apparently became extinct in the 16<sup>th</sup> and the 17<sup>th</sup> centuries (Flourentzos, 2002) at Mediterranean sites, but it was rediscovered by the British Vice Consul Roberson in the south of Iran and was first described

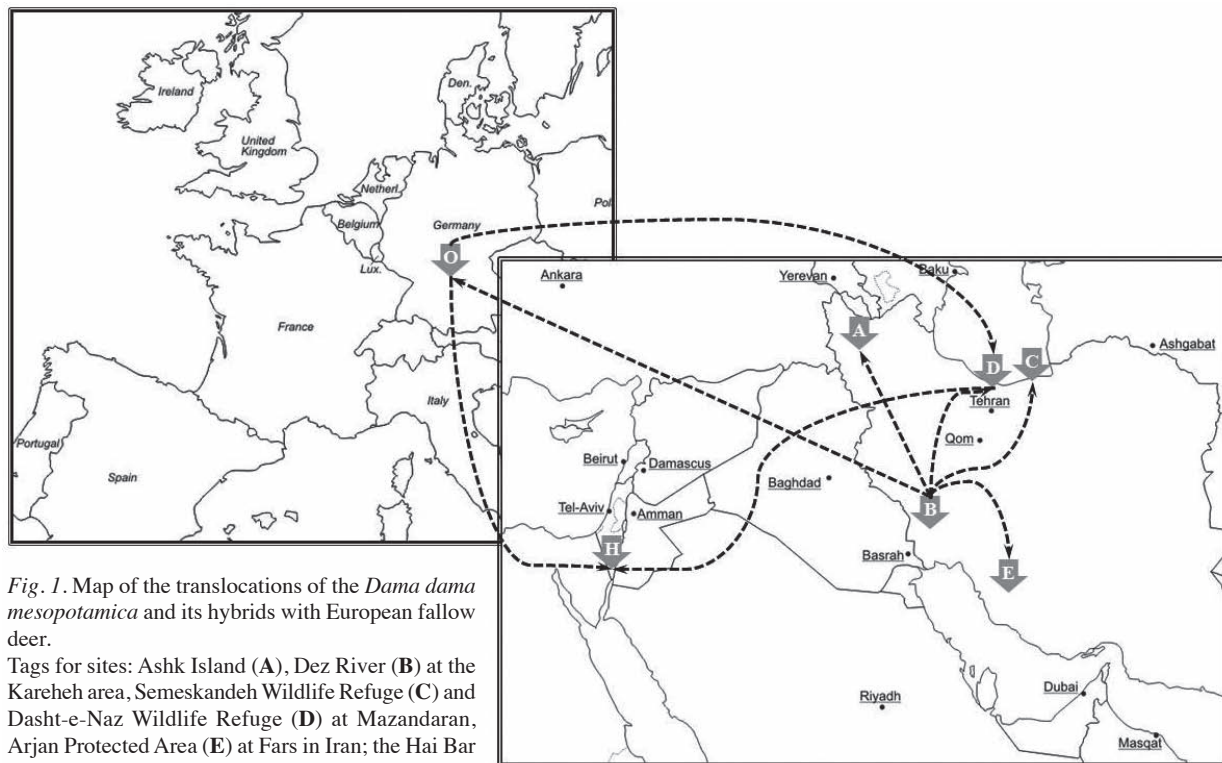


Fig. 1. Map of the translocations of the *Dama dama mesopotamica* and its hybrids with European fallow deer.

Tags for sites: Ashk Island (A), Dez River (B) at the Kareh area, Semeskandeh Wildlife Refuge (C) and Dasht-e-Naz Wildlife Refuge (D) at Mazandaran, Arjan Protected Area (E) at Fars in Iran; the Hai Bar Nature Reserve (H) in Israel and the Von Opel Zoo (O) in Germany. B to O arrow signals the first and the second of the translocations to the captive breeding core in Germany (1957/58 and 1964). B to D/C and A/E arrows to mark those translocations during the first conservation actions (1964/67) and posterior conservation measures (1976/77), respectively, in Iran. To refer to translocations of hybrids (1973) see arrow O to D. Finally, the translocations of pureblood from Germany (1976) and Iran (1979) to Israel is shown by the arrows O to H and D to H, respectively.

As a result of these expeditions, six animals were captured (3 males, 3 females) aimed to initiate the first action for the species' conservation at the Dasht-e-Naz (near the southern shore of the Caspian Sea) and the Kareh Wildlife Refuge. One of the males was sent to Germany in 1964 (to replace the first male which died) as part of the European captivity breeding program (Ch. Oswald pers. comm.; Chapman, 2008). Between 1960 and 1965 some hybrids with the European fallow deer (Ch. Oswald, pers. comm.; Jantschke, 1990; Chapman, 2008) were produced in Germany prior to the replacement of the first male. Seven of the surviving hybrids were sent to Dasht-e-Naz in 1973, but were kept separate from the others pureblood Persian fallow deer (Chapman, 2008). In 1976-77 conservation measures allowed the transfer of Persian fallow deer to different Iranian locations (Ashk Island, Arjan Protected Area (Zagros Mountain), Semeskandeh Wildlife and Kareh Wildlife Refuges). At the same time, and because Persian fallow deer were well-known during biblical times, a reintroduction program in Israel was initiated with

by Sir Victor Brooke in 1875 (Jantschke, 1990). After a few decades, in the 1940s, it was again believed to be extinct worldwide, including a captive herd at Woburn Abbey in the United Kingdom. But one decade later, in the 1950s, it was again rediscovered (Chapman, 2008). This reopened the taxonomic debate about these two fallow deer in the *Dama* genus: the European fallow deer (*Dama dama dama* Linnaeus, 1758) and the Persian fallow deer (*Dama dama mesopotamica*), subspecies or species (Pitra *et al.*, 2004). Plans for its conservation were initiated (Ch. Oswald, pers. comm.). In 1956 a handful of animals was seen in a riverine habitats in south-western Iran (Dez river, Kareh area) and from 1957-58 one wild pair of pureblood fawns were brought from south-west Iran to the Von Opel Zoo in Germany after a first expedition to the Kareh area (Chapman, 2008). The wild female gave birth to its first pureblood female in 1960, the year of the death of the male partner (Jantschke, 1990). From 1964 to 1967, the Iranian Game and Fish Department sent three expeditions to the Kareh area, near the place where it was rediscovered.

As a result of these expeditions, six animals were captured (3 males, 3 females) aimed to initiate the first action for the species' conservation at the Dasht-e-Naz (near the southern shore of the Caspian Sea) and the Kareh Wildlife Refuge. One of the males was sent to Germany in 1964 (to replace the first male which died) as part of the European captivity breeding program (Ch. Oswald pers. comm.; Chapman, 2008). Between 1960 and 1965 some hybrids with the European fallow deer (Ch. Oswald, pers. comm.; Jantschke, 1990; Chapman, 2008) were produced in Germany prior to the replacement of the first male. Seven of the surviving hybrids were sent to Dasht-e-Naz in 1973, but were kept separate from the others pureblood Persian fallow deer (Chapman, 2008). In 1976-77 conservation measures allowed the transfer of Persian fallow deer to different Iranian locations (Ashk Island, Arjan Protected Area (Zagros Mountain), Semeskandeh Wildlife and Kareh Wildlife Refuges). At the same time, and because Persian fallow deer were well-known during biblical times, a reintroduction program in Israel was initiated with

three pureblood animals from the Von Opel Zoo (Bar-David *et al.*, 2005; Chapman, 2008), but another translocation (four animals) from Dasht-e-Naz was made in 1979 under dramatic circumstances (Chapman, 2008) (see Fig. 1 for details).

In spite of these conservation measures, the Persian fallow deer is among the most endangered animals on the CITES list (Appendix I) and qualifies in the Endangered category under criterion D (IUCN 2010) with less than 250 mature individuals and with a sole autochthonous population living in Dez Wildlife Refuge and Karkheh Wildlife Refuge in south-western Iran (Rabiei, 2010). In addition, the possibility that hybrids were sent from the Von Opel Zoo back to Iran and subsequently translocated to Dasht-e-Naz (Chapman, 2008) casts a shadow over the pureblood conservation programme. A thorough knowledge and understanding of the genetic aspects of a species is important for its management, but especially in the fallow deer due to its low genetic variability in different systems: allozymes (Pemberton and Smith, 1985; Randi and Apollonio, 1988; Dratch and Pemberton, 1992), RAPD (Masseti *et al.*, 1997) and microsatellites (Poetsch *et al.*, 2003; Say *et al.*, 2005). This low variability was suggested to be due to a human-mediated bottleneck starting with a limited number of breeding individuals from genetically impoverished sources (Masseti *et al.*, 2008, and references therein). The management of very small and bottlenecked populations has had a pronounced effect on genetic diversity causing a reduction in the number of alleles, low heterozygosity, loss of rare alleles, and potentially fixation of deleterious alleles leading to inbreeding depression (Frankham, 1995; Luikart *et al.*, 1998). However, the latter has not been clearly demonstrated in the fallow deer (Say *et al.*, 2005). Moreover, historical records, often congruent with molecular data, showed these effects in deer species subjected to hunting or relocation (Broders *et al.*, 1999; Williams *et al.*, 2002; Webley *et al.*, 2004). But a population bottleneck does not always involve a reduction of the genetic variability (De Young *et al.*, 2003; Zenger *et al.*, 2003; Doerner *et al.*, 2005). Three main reasons have been signalled to describe this: (1) as part of a process of domestication for tameness and coat colour using small populations (Randi and Apollonio, 1988), (2) as part of foundation of small populations for hunting purposes (Webley *et al.*, 2007; Masseti, 2009) and (3) as part of a historical demographic reduction (Masseti, 2009), but with an overall effect on the genetic diversity of no return to the original variability level (Lowe *et al.*, 2004).

The current conservation plans only considered the reintroduction of pureblood *Dama dama mesopotamica* (Saltz *et al.*, 1994). It has been suggested that before being released in the wild, a very thorough assessment of the genetic diversity as well as a statistical evaluation of the optimal number and ages of specimens to be released should be carried out (Saltz *et al.*, 1998). These measures have brought the subspecies back from the brink of extinction in Iran. However, the truly wild populations remain seriously threatened and need strict protection in order to preserve them (Masseti, 2009).

In the present work, two enclosed and one wild group of *Dama dama mesopotamica* were studied in order to investigate its genetic variability and hybridization status. From a conservationist point of view, this topic is of great interest for several reasons: (1) the wild population of the Persian fallow deer is confined to a few national parks in Iran and has an unknown census size; (2) it is known that in 1973 seven hybrids (two males and five females from the Von Opel Zoo, Germany) were sent to Dasht-e-Naz site (northern Iran), but were later moved a few km away to another enclosure (Semeskandeh) to ensure genetic purity at Dasht-e-Naz and, (3) the lack of a specific conservation policy might put the last wild stocks of Persian fallow deer at risk of extinction (Chapman, 2008).

We used a suite of microsatellite markers from ungulates to evaluate (1) the genetic variability of the Persian fallow deer, particularly in the breeding group from the Von Opel Zoo (Berlin) which can be considered descendants of only one pair of parental individuals, (2) the presence of hybrids in the sample and (3) the magnitude of the allelic loss during the last half century between the wild and the captive Persian fallow deer populations.

## Material and methods

### *Specimens and DNA typing*

Antler bone was collected from European fallow deer (Ddd-Europe, *Dama dama dama*; n=9) and Persian fallow deer (*Dama dama mesopotamica*; n=29). The samples from the European fallow deer came from different sites: Turkey (1), Sweden (1), Hungary (1) and Spain (1) Austria (2) and Germany (3) covering highly unrelated specimens for genotypic comparisons with *Dama dama mesopotamica*. According to sender suggestions, the samples of the Persian fallow deer were grouped as follows: (a) nine samples from the breeding core of the Von Opel Zoo coded as Ddm-

German, dating from 1984 to 2007 and which are distributed in the Von Opel Zoo (4), in the Wilhelmina (2) and the Tierpark Gardens (3), (b) three Persian pure-blood samples (named Ddm-Israel) dating from before 2000 belonging to the Israeli stock (Ch. Oswald, pers. comm.) and (c) seventeen antlers preserved in the Das Cerviden-Museum von Christian Oswald (Germany) that were collected prior to 1960 during the first expedition (named Ddm-Iran) from the original wild Iranian stock (Ch. Oswald, pers. comm.). DNA extraction was performed by the salting-out method (Martínez *et al.*, 2002). Microsatellites were selected based on their amplification success on templates from different ungulates. After that, seven (7) microsatellites were chosen: ILST06; FCB304 (Buchanan *et al.*, 1994), JP15 (Crawford *et al.*, 1995), TGLA53 (Barendse *et al.*, 1994), SPS115 (Hanslik *et al.*, 2000), BM1818 (Bishop *et al.*, 1994), CSSM66 (Moore *et al.*, 1994). Genomic DNA was subjected to PCR and then the amplicons analyzed in an ABI3130 sequencer and typed using GeneMapper software (Life Technologies®).

#### Genetic analysis

The transfer of microsatellite markers among related species is not free of null allele and/or PCR failures due to mutations in the flanking region of the microsatellites (especially at the 3' end of the priming site;

Kwok *et al.*, 1990; Dakin and Avise, 2004). For this reason typing scoring errors such as stutter bands, null alleles, and large allele dropout (small allele excess) were assessed in the program MicroChecker (1,000 Monte Carlo simulations with Bonferroni correction for Confidence Interval; van Oosterhout *et al.*, 2004). ML-NullFreq software was used to estimate the null allele frequency and to evaluate missing data associated to PCR failures (Kalinowski and Taper, 2006).

The program Arlequin ver.3.5 (Excoffier and Lischer, 2010) was used to obtain the basic descriptive genetic parameters, the analysis of molecular variance (AMOVA) and the *F*<sub>st</sub> pairwise distance (based on the number of different alleles; using 1,000 permutations) to quantify the genetic divergence among groups. The allelic richness was estimated using the rarefaction procedures to compensate sampling disparity among regrouped data in both, Fstat 2.9.3.2 (allelic richness averaged over loci based on minimum sample size of two diploid individuals) and HP-Rare software (allelic richness averaged over loci with a standardized sample size baseline of four genes which assumes two diploid individuals) (Goudet, 2002 and Kalinowski, 2005; respectively). Moreover, HP-Rare was used to estimate the private allele richness allowing the evaluation of the uniqueness of each population (Fouley and Ollivier, 2006). The assessment of population structure, admixture and hybridization was carried out

*Table 1.* Basic descriptive genetic parameters: Allele number per locus in the sample groups Ddd-Europe, Ddm-German, Ddm-Israel and Ddm-Iran, total allele number by loci in *Dama dama mesopotamica* (total Ddm) as a whole and the total data set; mean by loci across population ( $\pm$ sd) and averaged across loci by populations ( $\pm$ sd). Averaged Allelic Richness (AR  $\pm$  sd) and Private Allelic Richness (PAR  $\pm$  sd). Overall polymorphic loci the mean observed heterozygosity (*H*<sub>o</sub>) and mean expected heterozygosity (*H*<sub>e</sub>).

Locus	Ddd-Europe	Ddm-German	Ddm-Israel	Ddm-Iran	Total Ddm	Total data set	Mean ( $\pm$ sd)
B1818	3	2	3	3	4	4	2.75 (0.5)
FCB304	1	2	1	6	6	6	2.50 (2.4)
TGLA53	2	1	1	1	1	3	1.25 (0.5)
CSSM66	3	1	1	1	1	4	1.50 (1.0)
CSPS115	2	1	1	2	2	3	1.50 (0.6)
ILST06	2	2	1	1	2	3	1.50 (0.6)
JP15	2	1	1	1	1	2	1.25 (0.5)
Average ( $\pm$ sd)	2.14 (0.69)	1.43 (0.54)	1.29 (0.76)	2.14 (1.86)	2.43 (1.90)	3.57 (1.27)	
AR ( $\pm$ sd)	1.48 (0.40)	1.19 (0.25)	1.26 (0.68)	1.47 (0.61)			
PAR ( $\pm$ sd)	0.65 (0.76)	0.09 (0.16)	0.08 (0.22)	0.32 (0.41)			
<b>Ho and He Overall loci</b>							
Ho ( $\pm$ sd)	0.14 (0.19)	0.04 (0.06)	0.00 (-)	0.06 (0.10)			
He ( $\pm$ sd)	0.27 (0.18)	0.13 (0.12)	0.11 (0.28)	0.30 (0.29)			

using two different approaches by the Bayesian model-based clustering method as implemented in the software Structure v.2.3.3 (Falush *et al.*, 2007). To do this, two approaches have been studied. In the first approach no correction was made to the raw data to account for null alleles and consequently null homozygotes were coded as missing genotypes. In the second approach the null alleles was coded as recessive allele, instructing the software to correspond the missing genotypes to homozygous recessive. In both approaches the information model with admixture and correlated allele frequencies were used (burn-in period of 100,000 steps initiated before 10,000 Markov chain Monte Carlo replications). The results from these two approaches were very similar. Here we present only the first approach. The estimated 'log probability of data' over a single run does not provide the correct estimation of the number of clusters (K). Fifteen runs were performed from K=1 to K=6 (90 total runs) and the number of clusters was accurately inferred using the Structure Harvester software (Earl and von Holdt, 2011) which estimate  $\Delta K$  according with Evanno *et al.* (2005). The graphical visualization (K=2 and K=3) was performed by optimizing 15 runs each by Clump ver.1.1.1 (Jakobsson and Rosenberg, 2007) and redrawn using Distruct (Rosenberg, 2004). As mentioned above in the introduction, since the three Israeli pureblood samples might be of different origin, that is, from the Von Opel Zoo and/or translocation from the Dasht-e-Naz reserve, the prior information model (Falush *et al.*, 2007) was used to obtain the posterior probability of the correct assignment of each individuals to one or more of the pre-defined populations (K=4) in a similar way than in Beaumont *et al.* (2001).

Evidence of a bottleneck was assessed for *D. dama mesopotamica* in Bottleneck v.1.2.02 by performing the Two Phase Model (TMP) because it is powerful and robust when less than 20 polymorphic loci are used (Piry *et al.*, 1999). The variance and the SSM model were adjusted to 12 and 70% as in Webley *et al.* (2007).

## Results

### Genetic variability

All seven loci were used to describe the genetic variability. The Appendix shows the list of alleles observed by locus. The BM1818, FCB304 and CSSP115 loci showed signs of null alleles using MICRO-CHECKER in the

Persian fallow deer and PCR failure might be important in BM1818 and CSSP115 (not shown) for Ddm-Iran and Ddm-German populations, respectively, but the results should be considered with caution due to the small sample size and the limited overall number of alleles (Kalinowsky and Taper, 2006). Moreover, PCR failures continued after repeated touch-down amplifications as recommended by Van Oosterhout *et al.* (2004), suggesting sequence bias between primer and template. All marker loci were polymorphic across the whole sample but some were monomorphic within subspecies. As a result, fixation of alleles occurred at loci TGLA53, CSSM66 and JP15 in the Persian fallow deer, but not regarding the European fallow deer (Appendix). Across loci and subspecies, allele numbers ranged from 3 to 6, but from 1 to 6 in the Persian fallow deer. The mean number of alleles was 3.571 after pooling all the data, only one average allele higher than the Iranian stock but decreasing more than a half for Ddm-German and Ddm-Israel. If we compare the mean allelic richness from the European fallow deer (Ddd-Europe) with the Persian fallow deer from Iran, similar values were obtained, but the 'private allelic richness' was reduced to a half in the Persian stock. However, a greater reduction of this parameter occurred in the Von Opel Zoo (Ddm-German) and the Israeli breeding programme (Ddm-Israel). Also, a very low observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosity was obtained (Table 1).

### Differentiation, structure and bottleneck

The AMOVA revealed that 50.1% of the genetic variation was between Persian and European fallow deer and 3.9% among the Ddm-German, Ddm-Israel and Ddm-Iran. Significant values of  $F_{is}$  (0.659  $p < 0.00001$ ) and  $F_{st}$  (0.540  $p < 0.00001$ ) again suggest strong genetic isolation and inbreeding. The genetic distance estimated by pairwise  $F_{st}$  was only significant between *D. d. dama* and *D. d. mesopotamica* (Table 2).

The most likely number of clusters was reached at K = 2 when the modal value of  $\Delta K$  and even after null genotypes was coded as recessive alleles, therefore, this corresponds to the uppermost partitioning level (Fig. 2). These two well-defined genotypic groups divided all European fallow deer from all Persian fallow deer, signalling two distinct gene pools (Fig. 3a). The next partition (K=3) resulted in three clusters. Now, the Persian fallow deer was split into two different clusters but regarding these two Persian clusters the genotypic proportion in every individual was variable. However, each *D. d. dama* individual was completely assigned to

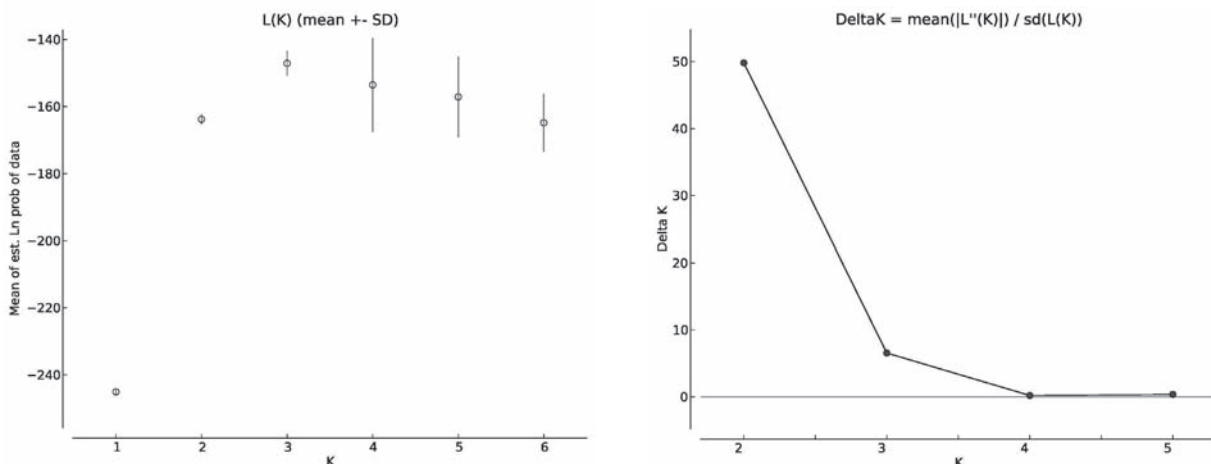


Fig. 2. Detection of the uppermost level of structure from the complete data set assuming nulls as missing data. (left) Mean of estimated Ln probability of data ( $\pm$  sd) averaging fifteen runs from K = 1 to K = 6 and (right) Delta K (DK) estimated following Evanno *et al.* (2005).

a third but different cluster (Fig. 3b). Regarding the European sample of Persian fallow deer, except for two animals belonging to the Von Opel Zoo stock (from Berlin Zoo and Wilhelma Garden, respectively), seven specimens were assigned to only one of these three clusters (Fig. 3b) with  $q > 0.77$  ( $q$ : assigned genotypic proportion; data not shown). Moreover, there was evidence that all Persian fallow deer samples had recent ancestry in one of the two pre-defined populations, in the Von Opel Zoo or in the Iranian stock (Table 3). The recent ancestry of the three samples from Israel was

undoubtedly and correctly assigned, one sample to the Ddm-German population and the other two samples to the Iranian stock. In three generations back, there was no detected parental either from the source population or from different population (data not shown) including the European fallow deer.

Moreover, under this scenario and using the TMP we detected a heterozygosity deficit only under the SSM model and with three of the four statistics: one-tailed Wicoxon’s test ( $p = 0.03125$ ); Sign Tests ( $p = 0.04362$ ) and standardized differences test ( $T2: -4.301$ ;  $p =$

	Ddd-Europe	Ddm-German	Ddm-Israel	Ddm-Iran
Ddd-Europe		+	+	+
Ddm-German	0.67		-	-
Ddm-Israel	0.63	0.27		-
Ddm-Iran	0.71	0.19	-0.09	

Table 2. Pairwise  $F_{st}$  values. Above diagonal, significant (+) and no significant (-) p-value after Bonferroni correction. Codes for groups as in Table 2.

Individuals from	Posterior assignment			
	to Ddd-Europe	to Ddm-German	to Ddm-Israel	to Ddm-Iran
Ddd-Europe	0.53-0.97	<0.10	<0.40	<0.05
Ddm-German	0.0	0.52-0.98	0.0	<0.45
Ddm-Israel	0.0	0.78-0.82 (***) (n=1)	<0.002	0.69-0.98 (***) (n=2)
Ddm-Iran	0.0	<0.006	0.0	0.95 - 0.98

Table 3. Score ranks of the posterior probability ( $q$ ) to correctly assign individuals to pre-defined ancestry populations coded as in Table 1. It is showed the minimum-maximum scores of the individual assignment having into account 10 Structure runs under the prior Information model. Pre-defined populations coded as in Table 1. Asterisk indicates the significance level. n= 1 and n=2 was the number of samples coming from a pre-defined population that was assigned to another as its pre-defined ancestry population.

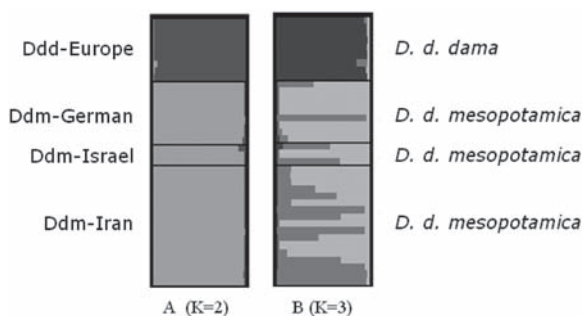


Fig. 3. The proportion of the individual fallow deer genotypes from the four samples (A) K=2 (modal K) and (B) K=3 clusters. Legends indicate the coded groups as in Table 1 (left) and the taxonomic name of subspecies (right).

0.00001), but ‘Mode-Shift’ showed a normal L-shaped distribution of genotypes. This suggested that Persian fallow deer population expanded in size from a small  $N_e$  population characterized by a systematic deficiency in heterozygosity that can be detected with these statistical tests (Cornuet and Luikart, 1996).

## Discussion

The European fallow deer around the world have reduced gene diversity at nuclear loci (Poetsch *et al.*, 2001; Say *et al.*, 2003; Scandura, 2004; Webley *et al.*, 2007 and references therein). However, this is the first time that a low level of genetic variation has also been reported in the Persian fallow deer, both in breeding programmes and in the original wild stock. Moreover, our results show the effects of a lack of gene flow between wild and captive breeding animals, exacerbating both allele loss that affect the loss of ‘private alleles’, typical of a bottlenecked population (Nei *et al.*, 1975).

### *Genetic distinctiveness of the Persian fallow deer*

The Persian fallow deer as a whole was polymorphic in 57% of STRs and therefore a 29% less than for the European subspecies *Dama dama dama*, but reduced from 43% in the original wild stock to 14% by pooling the three samples from Israeli breeding program. Moreover, discrepancies and similarities comparing fallow deer subspecies should be pointed out. On the one hand, Webley *et al.* (2007) showed that JP15 and SPS115 were monomorphic, whereas in here they were polymorphic. On the other hand, FCB304 was monomorphic in the *D.*

*d. dama* as in Webley *et al.* (2007) but highly polymorphic in the *D. d. mesopotamica* and the other way around in TGLA53 and CSSM66 (see Table 1). The allelic richness and  $H_e$  were similarly low in Ddd-Europe and Ddm-Iran which likely suggest historical resemblances due to systematic allele loss leading to lack of genetic diversity (see also Pemberton and Smith, 1985; Randi and Apollonio, 1988). Also, an interesting finding was the fixation of distinct alleles at two of the loci (TGLA53, CSSM66) in each subspecies, representing the 29% of them. Although this result was obtained with a very limited number of loci, it suggests that at least a quarter of microsatellite might reveal private alleles. Thus, the Persian and the European fallow deer differ at microsatellites loci (this study) and at mitochondrial genes (Randi *et al.*, 1998; Gilbert *et al.*, 2006; Masseti *et al.*, 2008). With this last genetic marker its separation was estimated on around 400 thousand years ago (Masetti *et al.*, 2008).

The Bayesian model-based clustering clearly differentiated Persian and European fallow deer genotypes (K=2 following Evanno *et al.*, 2005; Fig. 3a), even assuming nulls as recessive, probably due to ‘private alleles’. K=3 did not result in further partition in the European fallow deer (Fig. 3b) but the Persian fallow deer was split in a similar way as the European fallow deer in Webley *et al.* (2007) suggesting genetic substructure within them. In spite of the low number of markers, the prior information model (Falush *et al.* 2007) defines two genotypic groups one in the Von Opel Zoo (the first captive herd) and the other in the Iranian stock (the original wild stock). Moreover, it is suggested that in its origins the Israeli breeding core, at least the samples analysed here, consisted in a mixture of individuals from the Von Opel Zoo (n=1) and the Iranian wild stock (n=2), respectively. This strongly agrees with a recent report by Saltz *et al.* (2011) which stated that the Israeli permanent breeding core was established in 1976 (Hai-Bar Carmel) with two males and five females, but Chapman (2008) also reported that three animals were from the Von Opel Zoo (translocation year 1976) and four from Dasht-e-Naz (translocation year 1979). So, it is tempting to think that the Ddm-Israel samples might probably belong to three of these first seven animals.

### *Allele loss and level of genetic variability in the Persian fallow deer*

The number of alleles observed by grouping both subspecies was slightly higher than the European fallow deer populations from Tasmania (see Webley *et al.*, 2007) but on averaged was reduced by one third after

regrouping all the Persian fallow deer samples. The loss of allelic richness, ‘private allele richness’ and low heterozygosity may surely be due to systematic inbreeding and measures the severity of the loss of genetic identity and the degree of threat to the population (Foulley and Olliver, 2006). The loss of diversity caused the largest genetic damage in the Persian fallow deer from Europe (Ddm-German) and Israel (Ddm-Israel). Recently, it has been stated that genes drawn from a single deme belonging to an island population may give signals of a recent bottleneck (Nielsen and Beaumont, 2009). Then, the failure to detect bottleneck with the Two Phase Model (TMP) might be caused by, both, the extremely low degree of variation and scarce number of loci used. This is why the loss of private alleles should be considered a relevant genetic parameter (Broders *et al.*, 1999; Williams *et al.*, 2002) in the study of the endangered Persian fallow deer. The magnitude of the variation loss and identity loss on breeding programmes initiated with only one male and a pair of related females at the Von Opel Zoo (Chapman, 2008) may be estimated of the order of 75% for the last forty-seven years. As a consequence, a high genotypic proportion (seven out of nine) in only one of three possible clusters at  $K=3$  happened in the Ddm-German samples (see Fig. 3b). It may probably be explained as the effect of genetic drift under an unavoidable and systematic inbred mating in this captive population, which started with only three parents in 1964 and in 1969 (date of the death of the second male) that produced four to nine offspring (Jantschke, 1990) of full and/or more than half sibling. It is therefore not surprising to obtain greater genetic variability in the wild populations compared with that from the von Opel Zoo.

### *The preservation of pureblood*

The Persian fallow deer from the three sampling sites were genetically similar but distinct to European fallow deer, deserving to be considered as one independent management unit (Lowe *et al.*, 2004). Although genetic-based studies have been subject to discussion in conservation matters (Pääbo, 2000; Waits *et al.*, 2000), it has been shown that both the mean allelic richness and ‘private allele richness’ is a fundamental criterion to assess both the genetic uniqueness (Foulley and Olliver, 2006) and the level of genetic threat of populations subject to conservation. Accordingly, the Iranian stock should be considered of special conservation interest because it has the highest allelic richness.

Boecklen and Howard (1997) stated that eight microsatellites are required to attain a 10% of error rate for the misclassification of a second Back-Cross as pureblood. Although with one marker less, across all the pre-defined populations no parent, grandfather and great-grandfather were detected in three generations back. This fact may support, at least, no recent hybridization origin (F1 and first back-cross) for every sample. Also, there were no TGLA53 and CSSM66 private alleles derived from European fallow deer in the Persian fallow deer. If the reported hybridization between both fallow deer occurred in the Von Opel Zoo between 1961 and 1967 (Chapman, 2008), apparently, no samples of such hybrids were observed in the present study. However, we cannot extrapolate our results obtained from Ddm-Iran to the present Iranian population for two reasons: (1) these Ddm-Iran wild specimens were collected in 1960 and (2) it was in 1973 when ‘two males and five females bred in Germany from a mating with common fallow deer were sent to Dasht-e-Naz’. ‘Subsequently these were moved to another enclosure (Semeskandeh) a few km apart, to be sure to maintain the genetic purity of the Mesopotamian animals’ (Chapman, 2008).

Finally, Rudloff (2010) recorded a list of Persian fallow deer from 20 zoos in the Annual International Stud Book of this fallow deer, with the largest number residing in Israeli zoos. However, six small captive groups from Israel were excluded by the IUCN, because of some doubts as to their genetic purity (Chapman, 2008). There is a worldwide interest in avoiding genetic contamination of pureblood Persian fallow deer, and one must be aware that at least 30 polymorphic markers should be used to accurately detect hybridization (Boecklen and Howard, 1997). Moreover, genetic analysis should be achieved in breeding plans aimed at minimizing the mating between relatives for reducing or delaying the inbreeding effect in the short term (Caballero and Toro, 2002). This way of preserving the genetic variation conforms to modern conservation aims (Pääbo, 2000).

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## Appendix

List of alleles observed at every locus and subspecies (Ddd and Ddm for *Dama dama dama* and *Dama dama mesopotamica*, respectively).

	BM1818	FCB304	TGLA53	CSSM66	CSSP115	ILST06	JP15
Ddd	234	125	163	175	239	287	98
	238		165	179	241	291	100
	242			181			
Ddm	232	125	169	177	241	287	98
	234	127			243	293	
	238	129					
	242	131					
		133					
	137						

