KATJA SILBERMAYR, PAMELA BURGER

Hybridization: A Threat to the Genetic Distinctiveness of the Last Wild Old World Camel Species

Natural hybridization has played an important role in the evolution of many animal (Mallet 2005) and plant taxa (Baack/Rieseberg 2007). However, hybridization between wild species and their domestic congeners often threatens the gene pool of the wild species, especially if the species has become rare (Allendorf et al. 2001, Randi 2008). This is the case with the wild camel as we will show in this article.

NATURAL AND ANTHROPOGENIC HYBRIDIZATION

Introgressive hybridization between translocated or invasive organisms and local wild populations adds to a loss of genetic diversity and biodiversity. With the ever increasing encroachment of humans, their livestock and pets on the remaining natural habitats, introgressive hybridization can be observed between wild animals and domestic stock, e.g. between the domestic dog (*Canis familaris*) and the wolf (*Canis lupus*; Randi 2008, Anderson et al. 2009), the domestic cat (*Felis catus*) and the European wildcat (*Felis sylvestris*; Beaumont et al. 2001, Randi et al. 2001) and domestic cattle (*Bos taurus*) and bison (*Bison bison*; Halbert/Derr 2007). Furthermore, massive releases of captive-reproduced game stocks have altered the gene pool of natural red deer (*Cervus elaphus*), sika deer (*Cervus nippon*; Goodman et al. 1999) and galliform species (Barilani et al. 2007).

Hence anthropogenic hybridization threatens natural populations or may even lead to their complete admixture (Allendorf et al. 2001). On the other side, depending on the influence of environmental heterogeneity, hybridization can also rescue local populations from extinction. Intentional hybridization has saved populations from inbreeding depression as observed in the Scandinavian wolf (*Canis lupus*; Edmands 2007) or the Florida panther (*Puma concolor coryi*; Johnson et al. 2010). Moreover, hybridization events can lead to an increase in species numbers (Mallet 2007) and thus, as mentioned above, plays a significant role in the process of evolution. Keeping these dual roles of hybridization on biodiversity dynamics in mind, natural populations and especially the wild relatives of our livestock species should be conserved as valuable resources of genetic diversity.

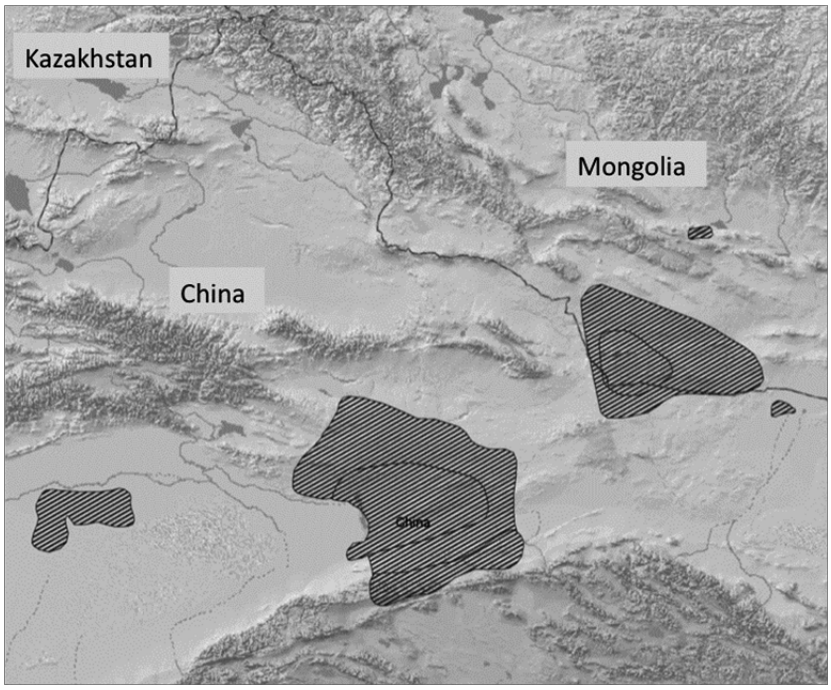
HYBRIDIZATION BETWEEN WILD AND DOMESTIC BACTRIAN CAMELS

The two-humped wild camel (*Camelus ferus*) is the only surviving wild species within the Old World camelids (*Camelini*) and is considered as "critically endangered" in the Red List of species threatened with extinction (IUCN, 2011). It is also protected under local legislation in China and Mongolia. The opinions on the genetic relationship between the wild and the domestic Bactrian camel (*Camelus bactrianus*) have been widely divergent ranging between assumptions that the wild camels are not truly wild but feral and that wild camels are considered to be the ancestors of the living domestic two-humped camel. Recent studies based on molecular clock analysis[1] of complete mitochondrial DNA sequences indicate that the separation of the two line-

[1] The molecular clock is a method of estimating the divergence time between two species/lineages based on changes in the DNA.

ages began in the early Pleistocene, about 0.7 million years ago. The archeological dating of Bactrian camel domestication is estimated at 5000–6000 years before present, thus excluding the extant wild camel populations as direct progenitors of the current domestic Bactrian camels (Ji et al. 2009). In addition to morphologic differences between wild and domestic camels in body size, hair color and skull structure (see picture 2 in the article by P. Burger in this volume), recent genetic analysis (Silbermayr 2009 and 2010a, Ji et al. 2009, Jianlin et al. 1999) identified the two-humped camel discovered by Przewalski (1878) as an original wild form, *Camelus ferus*.[2]

The wild Bactrian camel population appears to be in a steady decline. Population estimates are highly variable and inconsistent (Hare 1997, Luzhang et al. 2005) and the latest surveys have revealed that the population has become severely reduced to only 900–1600 animals worldwide. In contrast, there are over 2,000,000 domestic Bactrian camels worldwide. Once distributed throughout Central Asia the wild camel populations, today, have become restricted to four areas: Gashun Gobi, Lop Nur and Taklamakan desert in China and Great Gobi desert in Mongolia (see graph 11).[3]



Graph 11: Distribution range of the remaining wild camel populations in Mongolia and China (IUCN 2011; http://www.edgeofexistence.org/mammals/species_info.php?id=8#distribution; consulted 3 March, 2011).

The extensive livestock-wildlife interface around the Great Gobi Special Protected Area "A" (GGSPAA) is of particular concern for the conservation of the Mongolian wild camel population (Walzer 2006). The remaining population is surrounded by an estimated 10,000 domestic camels in addition to 50 to 60 already existing fertile hybrid camels in the circumjacent settlements. In some cases, the hybridization of domestic females with wild bulls is initiated to enhance the fitness of domestic camels (Enkhbileg et al. 2006). In addition, the movement of domesticated animals into the habitat of the wild population leads to the transfer of potential pathogens across this domestic-wildlife interface (Walzer 2006). The detection of hybridization between wild populations and their domestic relatives is an essential tool for the preservation and future sustainability of endangered wild populations (Hammer et al. 2008, Oliveira et al.

[2] For more information about this topic see the article by Pamela Burger in this volume.
[3] For a detailed discussion on the situation of wild camels in China and Mongolia see the articles by Yuan Lei et al. and Adiya Yadamsuren et al. in this volume.

2008). The identification of such hybrids is difficult based purely on morphological traits. However, the analysis of the DNA genotypes reveals the relationship of individual animals to different populations.

MOLECULAR MARKERS TO DETECT HYBRIDIZATION

The principal tools of population genetics are the measurement of highly variable molecular markers and the estimation of the genetic variation and distances within and between populations. Genetic variation is measured in terms of polymorphisms and heterozygosity,[4] which is calculated from the different allele frequencies of genes. The genetic distance is a measure of the genetic diversity between different individuals or populations (Schlötterer 1998). For the detection of hybridization and population structure hyper-variable DNA markers such as restriction fragment length polymorphism (RFLP), microsatellites (short tandem repeats, e.g. ACACAC), and single nucleotide polymorphisms (SNPs) in nuclear and mitochondrial genomes are used (e.g. Randi 2008). Therefore, by applying molecular markers on samples of wild and domestic Bactrian camels their evolutionary relatedness can be determined.

So far, polymorphic microsatellites in *Camelidae* have been developed for the alpaca (*Lama pacos*), the llama (*Lama glama*) (Lang et al. 1996, Obreque et al. 1998 and 1999, Penedo et al. 2001) and the dromedary (*Camelus dromedarius*) (Mariasegaram et al. 2002). Cross-species amplification[5] of these markers in the Bactrian camel (*Camelus bactrianus*) has been successful, but species-specific markers, which might better reflect the genetic diversity of Bactrian camels, are still few (Edvotchenko et al. 2003, Silbermayr 2010b).

To investigate hybridization – a key issue in the conservation of wild camels – we analyzed mitochondrial differentiation levels between wild and domestic Bactrian camels and developed a diagnostic polymerase-chain-reaction restriction fragment length polymorphism (PCR-RFLP) assay to identify maternal domestic hybrids in the wild population (Silbermayr et al. 2010a). This method uses a particular enzyme to cut the targeted mtDNA fragment of domestic Bactrian camels into two pieces, but leaves the mtDNA of wild camels uncut. To further investigate population genetic structure and levels of genetic diversity in Bactrian camels and to detect hybridization between Mongolian wild and domestic Bactrian camels, microsatellite markers were isolated and characterized in addition to previously published microsatellites (Silbermayr et al. 2010b).

MATERIAL AND METHODS TO DETECT HYBRIDIZATION IN WILD CAMELS

A total of 103 specimens originating from wild and domestic Bactrian camels as well as reference species were analyzed. For the mitochondrial DNA (mtDNA) analysis we used 81 samples from 27 wild, 41 domestic and five hybrid (captive born) camels, as well as eight individuals with unknown maternal ancestry originating from Mongolia, China and Austria. As references we retrieved nine sequences from GenBank.[6] The specimens originated from 59 blood, five fecal and 14 hair samples, one muscle and one skin biopsy, and one museum skin. They were collected either non-invasively or during routine veterinary treatment. For the determination of genetic diversity within and between the wild and domestic Bactrian camel populations, we sequenced an 804-nucleotide mitochondrial fragment comprising parts of the cytochrome b and the control region in 59 animals. An additional 22 samples were subjected to the PCR-RFLP

[4] A gene locus is heterozygous when it contains two different alleles (forms of a gene), which are inherited from both parents.

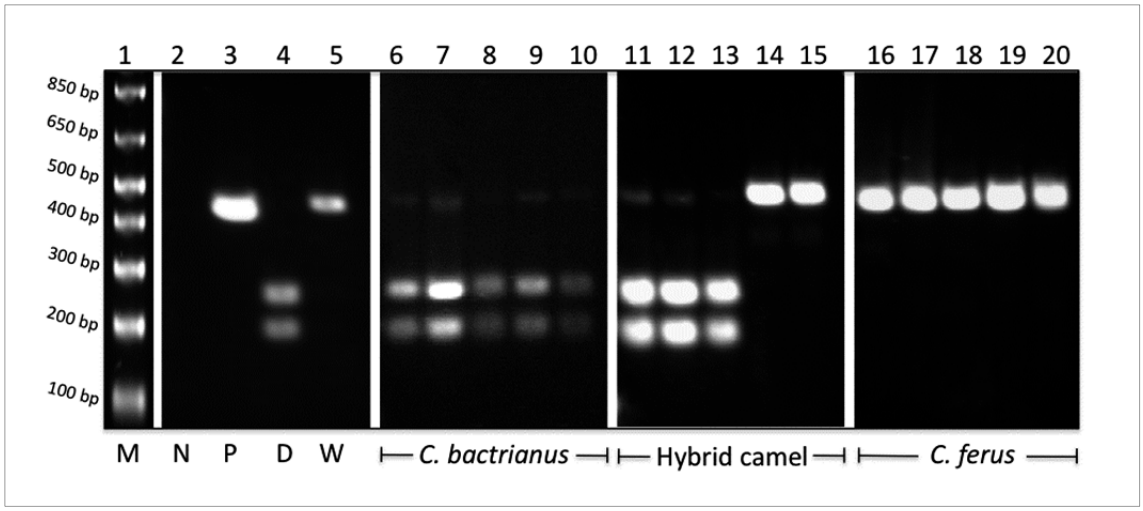
[5] Microsatellite markers developed for dromedary or llama are used – across species – in Bactrian camels.

[6] GenBank is an online database for genetic sequence data; each entry receives an individual accession number, like the Bactrian camel mitochondrial genomes used here (GI: 156615987, GI: 156615992, GI: 156615976, GI: 157011972, GI: 156615981, GI: 223972289, GI: 156615972, GI: 157011955, GI: 156615983).

analysis (Silbermayr et al. 2010a). For the analysis of 20 microsatellite loci, we tested a set of 42 domestic, 20 wild and four hybrid camels, in addition to three dromedaries and one alpaca for cross-species amplification and as an outgroup. The specimens originated from blood and were stored at minus 20°C. DNA isolation, PCR amplification, sequencing and sequence alignment was performed as described previously (Silbermayr et al. 2010a). Individuals were assigned to distinct clusters using a Bayesian approach implemented in the BAPS 5.2 computer program (Bayesian Analysis of Population Structure; Corander/Tang 2007). Microsatellite analysis was performed as described previously (Silbermayr 2009, Silbermayr et al. 2010b) and a neighbor-joining tree was generated based on the proportion of shared alleles with the Phylip 3.69 software (Felsenstein 1989). What we can do with the above-described methods is an initial rapid screening of wild camel samples using mtDNA followed by a more detailed analysis of their hybridization status by defining the individual genotypes with microsatellites.

RAPID SCREENING OF HYBRIDIZATION WITH MITOCHONDRIAL DNA (MTDNA)

In the field of wildlife conservation as well as in livestock management genetic monitoring has become an important tool, because it reveals information about genetic diversity, effective population size (i.e. how many breeding individuals exist) or inbreeding. For the effective genetic monitoring of a wild population it is essential to have an easy, fast and reliable screening method. Mitochondrial PCR-RFLP systems, in particular, meet these requirements and are therefore frequently used for species identification (Wolf et al. 1999, Kuehn et al. 2006). Based on the high levels of differentiation between wild and domestic Bactrian camels (see graph 13 in the article of P. Burger in this volume; Silbermayr et al. 2010a) we developed a PCR-RFLP system, which uses a particular enzyme to cut the targeted mtDNA fragment of domestic Bactrian camels into two pieces but leaves the mtDNA of wild camels uncut. With this easy and rapid method to discriminate between *Camelus bactrianus* and *Camelus ferus* we were responding to the demand for an inexpensive and reliable method to monitor the Mongolian wild camel population. To test the diagnostic value of the assay, we analyzed all collected samples including traditional (blood, skin), non-invasive (feces, hair) and museum specimen. The digestion treatment with the enzyme resulted in consistent banding patterns for domestic camel specimens yielding two bands (190/256 base-pair (bp)), while wild camels remained uncut (446 bp) (see graph 12). Captive-born hybrids with domestic mothers and the museum sample showed the domestic banding profile as expected (see graph 12; lanes 10–13). However, hybrids with a confirmed



Graph 12: Gel picture showing the banding patterns of a 446 (bp) long mitochondrial fragment digested with a restriction enzyme (XmiI; Fermentas). Domestic Bactrian camel samples are cut in two pieces visible as two bands of 190 and 256 bp, respectively (lanes 6–10), while wild camel specimen remain uncut (one band of 446 bp; lanes 16–20). The hybrid camels (lanes 11–15) show either the domestic or wild camel binding pattern depending on their

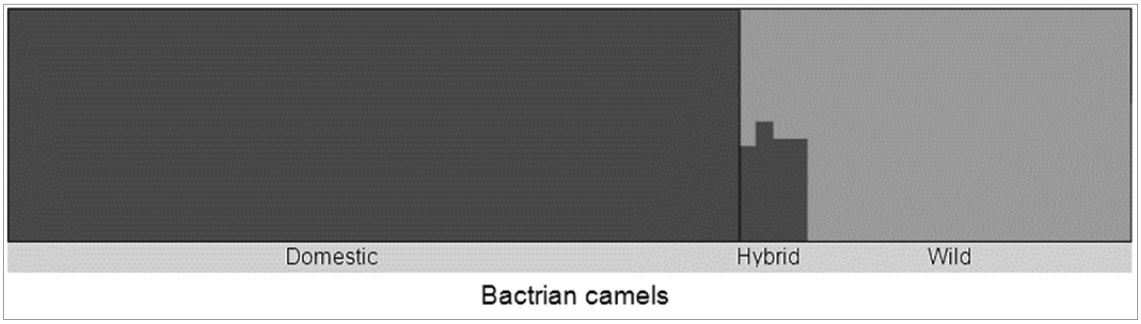
maternal origin. M: 1kb+ DNA ladder; N: negative control (without DNA); P: positive control (undigested PCR product); D: domestic Bactrian camel, cut (190/256 bp); W: wild camel, uncut (446 bp).

wild maternal ancestry as well as samples collected non-invasively during a monitoring trip in the national reserve GGSPAA showed the *Camelus ferus* pattern (see graph 12; lanes 14–15 and 17–20; Silbermayr et al. 2010a).

We applied this method successfully to non-invasively collected hair and fecal samples. Thus the system can be used for specimens collected during routine monitoring trips into the natural wild-camel habitat. Moreover, this technique can be established in a small genetic laboratory, as it requires relatively basic laboratory equipment and minimal training. In addition, we have used this marker to solve questions of taxonomy and nomenclature in a rare museum specimen, as we could assign one skin sample from the Natural History Museum Vienna as domestic Bactrian camel (Silbermayr et al. 2009).

IN-DEPTH INVESTIGATION OF HYBRIDIZATION USING NUCLEAR DNA

As mitochondrial markers give information only about the maternal genetic background, it is additionally important to use nuclear microsatellites for the investigation of paternal hybridization. The analysis of microsatellite data from 20 loci revealed the existence of hybrids between wild and domestic Bactrian camels. As displayed in the admixture analysis (see graph 13) the wild and domestic camels cluster together in separate groups while the hybrid camels harbor parts (domestic and wild) of both parental genomes.



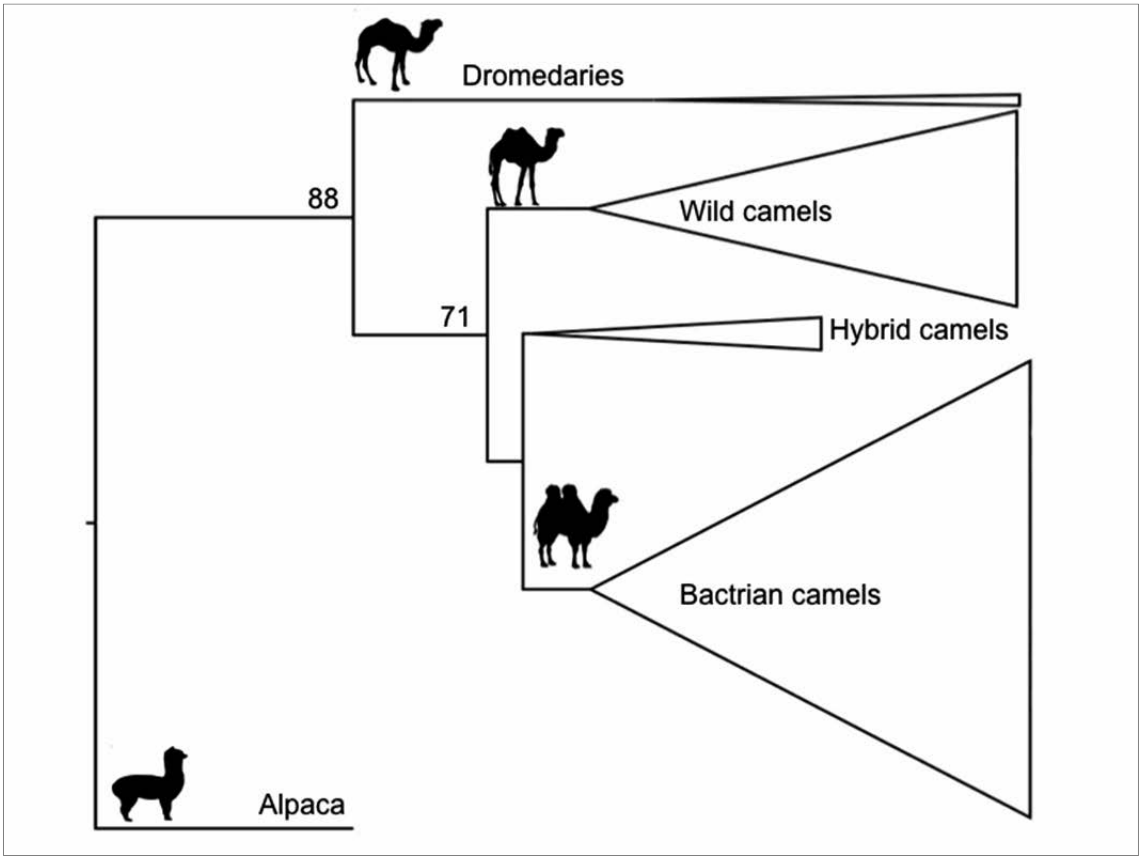
Graph 13: Admixture analysis using the BAPS 5.2 computer program. The pure domestic (left) and wild Bactrian camels (right) are assembled in distinct groups, while the hybrids show the genomic profiles of both (domestic and wild) parents.

Similar results were observed using a different (neighbor-joining tree) analysis, where the domestic and wild Bactrian camels grouped in separate branches (bootstrap support[7] 71%) and the hybrid camels formed a specific cluster (see graph 14). The analyses were carried out in three Bactrian camels with a known history of hybridization; in addition, however, we could detect another hybrid camel, which was originally presumed to be of wild origin.

In summary, the applied molecular markers comprise a reliable method for the genetic differentiation of wild and domestic Bactrian camel samples and for the detection of hybrids in the last wild camel populations of Mongolia and China. From the results obtained in this study we recommend using mitochondrial markers complementary to nuclear markers, as evidence for introgression can vary substantially in mitochondrial and nuclear genes (Halbert/Derr 2007). Hybridization has often been studied using mitochondrial sequence data coupled with information from other loci or geographic data. This has provided convincing evidence of introgression between domesticated species and their wild relatives such as alpaca (*Vicugna pacos*)/vicuna, llama/guanaco (Kadwell et al. 2001) or domestic goat/Markhor goat (*Capra falconeri*; Hammer et al. 2008) and domestic cattle/American bison (Halbert/Derr 2007). The process of

[7] In statistics, bootstrapping is a computer-based method for assigning measures of accuracy to sample estimates.

either anthropogenic or natural inter-specific hybridization is ongoing, e.g., as shown in hybridization frequencies of 75% in British ducks and 10% in European mammals (Mallet 2005). Similarly, a domestic horse (*Equus caballus*) mare is one of 13 founding individuals of the Przewalski's horse (*Equus przewalskii*) captive breeding program (Ryder 1994) in Mongolia. In spite of this hybridization, Przewalski's horses represent a distinct gene pool that is the object of continuing conservation efforts (Slotta-Bachmayr et al. 2004, Allendorf et al. 2001). Comparatively, the wild camels represent a genetically and morphologically (Sokolov/Orlov 1980, Tulgat/Schaller 1992) distinct population, which deserves international attention and conservation.



Graph 14: Collapsed (branches of individual camels were taken together) neighbor-joining tree based on the proportion of shared alleles between individuals. The domestic Bactrian camels are assembled distinctly and separate from the wild camels. The hybrids form a separate cluster. The tree is rooted with the alpaca as an outgroup. Numbers refer to bootstrap support (100 iterations).

CONCLUSIONS

The results of our genetic analysis highlight the importance of in-situ conservation actions for the last wild camel populations in Mongolia and China. Future hybridization assessment programs are needed to (i) record and protect the distribution range of non-hybridized wild camel populations and (ii) support the monitoring at the human-domestic-wildlife-interface in and adjacent to the national parks. This should help preserving the genetic integrity of wild camel populations and prevent a loss of biodiversity through hybridization between wild and domestic animals.

REFERENCES

Allendorf, FW/Leary, RF/Spruell, P/Wenburg, JK (2001): "The Problems with Hybrids: Setting Conservation Guidelines", in: *Trends in Ecology & Evolution* 16, pp. 613–622

Anderson, TM/vonHoldt, BM/Candille, SI/Musiani, M/Greco, C/Stahler, DR/Smith, DW/Padhukasahasram, B/Randi, E/Leonard, JA/Bustamante, CD/Ostrander, EA/Tang, H/Wayne, RK/Barsh, GS

(2009): "Molecular and Evolutionary History of Melanism in North American Gray Wolves", in: *Science* 323, pp. 1339–1343

Baack, EJ/Rieseberg, LH (2007): "A Genomic View of Introgression and Hybrid Speciation", in: *Current Opinion in Genetics & Development* 17, pp. 513–518

Barilani, M/Bernard-Laurent, A/Mucci, N/Tabarroni, C/Kark, S/Garridoe, JAP/Randi, E (2007): "Hybridisation with Introduced Chukars (*Alectoris chukar*) Threatens the Gene Pool Integrity of Native Rock (*A. graeca*) and Red-legged (*A. rufa*) Partridge Populations", in: *Biological Conservation* 137, pp. 57–69

Beaumont, MA/Barratt, EM/Gottelli, D/Kitchener, AC/Daniels, MJ/Pritchard, JK/Bruford, MW (2001): "Genetic Diversity and Introgression in the Scottish Wildcat", in: *Molecular Ecology* 10, pp. 319–336

Corander, J/Tang, J (2007): "Bayesian Analysis of Population Structure Based on Linked Molecular Information", in: *Mathematical Biosciences* 205, pp. 19–31

Cui, P/Ji, R/Ding, F/Qi, D/Gao, H/Meng, H/Yu, J/Hu, S/Zhang, H (2007): "A Complete Mitochondrial Genome Sequence of the Wild Two-Humped Camel (*Camelus bactrianus ferus*): An Evolutionary History of *Camelidae*", in: *BMC Genomics* 8, p. 241

Dovchindorj, G/Mijiddorj, B/Adiya, Y (2006): "Population Ecology of the Wild Camel in Mongolia", entry in: Adiya, Y/Lhagvasuren, B/Bayasgalan, A (eds): *Proceedings of the International Workshop on Conservation and Management of the Wild Bactrian Camel*. Ulaanbaatar, Mongolia, pp. 24–28

Edmands, S (2007): "Between a Rock and a Hard Place: Evaluating the Relative Risks of Inbreeding and Outbreeding for Conservation and Management", in: *Molecular Ecology* 16, pp. 463–475

Enkhbileg, D/Dovchindorj, G/Dorjgotov, A/Adiya, Y (2006): "Current Situation and Future Management of Hybrid Camel (*besreg*) in Buffer Zone Area of Great Gobi Protected Area A", entry in: Adiya, Y/Lhagvasuren, B/Bayasgalan, A (eds): *Proceedings of the International Workshop on Conservation and Management of the Wild Bactrian Camel*. Ulaanbaatar, Mongolia, pp. 36–46

Evdotchenko, D/Han, Y/Bartenschlager, H/Preuss, S/Geldermann, H (2003): "New Polymorphic Microsatellite loci for Different Camel Species", in: *Molecular Ecology Notes* 3, pp. 431–434

Goodman, SJ/Barton, NH/Swanson, G/Abernethy, K/Pemberton, JM (1999): "Introgression Through Rare Hybridization a Genetic Study of a Hybrid Zone Between Red and Sika Deer (Genus *Cervus*) in Argyll, Scotland", in: *Genetics* 152, pp. 355–371

Halbert, ND/Derr, JN (2007): "A Comprehensive Evaluation of Cattle Introgression into US Federal Bison Herds", in: *Journal of Heredity* 98, pp. 1–12

Hammer, SE/Schwammer, HM/Suchentrunk, F (2008): "Evidence for Introgressive Hybridization of Captive Markhor (*Capra falconeri*) with Domestic Goat: Cautions for Reintroduction", in: *Biochemical Genetics* 46, pp. 216–226

Hare, J (1997): "The Wild Bactrian Camel *Camelus bactrianus ferus* in China: The Need for Urgent Action", in: *Oryx* 31, pp. 45–48

IUCN (2011): *IUCN Red List of Threatened Species*. www.iucnredlist.org

Ji, R/Cui, P/Ding, F/Geng, J/Gao, H/Zhang, H/Yu, J/Hu, S/Meng, H (2009): "Monophyletic Origin of Domestic Bactrian Camel (*Camelus bactrianus*) and its Evolutionary Relationship with the Extant Wild Camel (*Camelus bactrianus ferus*)", in: *Animal Genetics* 40, pp. 377–382

Jianlin, H/Jiexia, Q/Zhenming, M/Yaping, Z/Wen, W (1999): "Rapid Communication: Three Unique Restriction Fragment Length Polymorphisms of EcoRI, PvuII, and ScaI Digested Mitochondrial DNA of Bactrian Camels (*Camelus bactrianus ferus*) in China", in: *Journal of Animal Science* 77, pp. 2315–2316

Johnson, WE/Onorato, DP/Roelke, ME/Land, ED/Cunningham, M/Belden, RC/McBride, R/Jansen, D/Lotz, M/Shindle, D/Howard, J/Wildt, DE/Penfold, LM/Hostetler, JA/Oli, MK/O'Brien, SJ (2010): "Genetic Restoration of the Florida Panther", in: *Science* 329, pp. 1641–1645

Kadwell, M/Fernandez, M/Stanley, HF/Baldi, R/Wheeler, JC/Rosadio, R/Bruford, MW (2001): "Genetic Analysis Reveals the Wild Ancestors of the Llama and the Alpaca", in: *Proceedings of the Royal Society B* 268, pp. 2575–2583

Kuehn, R/Kaczensky, P/Lkhagvasuren, D/Pietsch, S/Walzer, C (2006): "Differentiation of Meat Samples from Domestic Horses (*Equus caballus*) and Asiatic Wild Asses (*Equus hemionus*) Using a Species-Specific Restriction Site in the Mitochondrial Cytochrome B Region", in: *Mongolian Journal of Biological Sciences* 4, pp. 57–62

Lang, KDM/Wang, Y/Plante, Y (1996): "Fifteen Polymorphic Dinucleotide Microsatellites in Llamas and Alpacas", in: *Animal Genetics* 27, p. 293

Luzhang, R/Lixun, Z/Naifa, L/Zuhao, H/Muli, M/Kerong, X (2005): "Distribution and Population Status of the Wild Bactrian Camel (*Camelus bactrianus ferus*) in Gansu Province, China", in: *Journal of Camel Practise and Research* 12, pp. 59–63

Mallet J (2005): "Hybridization as an Invasion of the Genome", in: *Trends in Ecology & Evolution* 20, pp. 229–237

--- (2007): "Hybrid Speciation", in: *Nature* 446, pp. 279–283

Mariasegaram, M/Pullenayegum, S/Jahabar, M/Shah, SR/Penedo, MCT/Wernery, U/Sasse, J (2002): "Isolation and Characterization of Eight Microsatellite Markers in *Camelus dromedaries* and Cross-species Amplification in *C. bactrianus* and *Lama pacos*", in: *Animal Genetics* 33 pp. 377–405

Obreque, V/Coogle, L/Henney, PJ/Bailey, E/Mancilla, R/García-Huidobro, J/Hinrichsen, P/Cothran, EG (1998): "Characterization of 10 Polymporphic Microsatellites", in *Animal Genetics* 29, pp. 461–462

Penedo, MCT/Caetano, AR/Cordova, K (1999): "Eight Microsatellite Markers for South American Camelids", in: *Animal Genetics* 30, pp. 166–167

Oliveira, R/Godinho, R/Randi, E/Alves, PC (2009): "Hybridization Versus Conservation: Are Domestic Cats Threatening the Genetic Integrity of Wildcats (*Felis silvestris silvestris*) in Iberian Peninsula?", in: *Philosophical Transactions of the Royal Society B: Biological Sciences* 363, pp. 2953–2961

Randi, E (2008): "Detecting Hybridization Between Wild Species and their Domesticated Relatives", in: *Molecular Ecolology* 17, pp. 285–293

---/Pierpaoli, M/Beaumont, M/Ragni, B/Sforzi, A (2001): "Genetic Identification of Wild and Domestic Cats (*Felis silvestris*) and their Hybrids Using Bayesian Clustering Methods", in: *Molecular Biolology & Evolulation* 18, pp. 1679–1693

Ryder, OA (1994): "Genetic Studies of Przewalsky's Horses and their Impact on Conservation", entry in: Boyd, L/ Houpt, KA (eds): *Przewalski's Horse: The History and Biology of an Endangered Species*. New York: State University of New York Press, pp. 75–92

Schlötterer, C (1998): "Microsatellites", entry in: Hoelzl, AR (ed): *Molecular Genetic Analysis of Populations: A Practical Approach.* Oxford: IRL Press, pp. 237–261

Senn, HV/Pemberton, JM (2009): "Variable Extent of Hybridization Between Invasive Sika (*Cervus nippon*) and Native Red Deer (*C. elaphus*) in a Small Geographical Area", in: *Molecular Ecology* 18, pp. 862–876

Silbermayr, K (2009): *"The Detection of Hybridization on the Mongolian Wild Bactrian (Camelus ferus) Using Molecular Markers",* doctoral thesis, University of Veterinary Medicine, Vienna

---/Orozco-terWengel, P/Charruau, P/Enkhbileg, D/Walzer, C/Vogl, C/Schwarzenberger, F/Kaczensky, P, Burger, PA (2010a): "Mitochondrial Marker to Detect Maternal Hybridisation in the Mongolian Wild Bactrian Camel (*Camelus ferus*)", in: *Animal Genetics* 41, pp. 315–318

---/Tero, N/Charruau, P/Enkhbileg, D/Walzer, C/Burger, PA (2010b): "Isolation and Characterization of Nine New Microsatellite Loci in the Domestic Bactrian Camel (*Camelus bactrianus*) and Amplification in the Wild Bactrian Camel (*C. ferus*)", in: *Molecular Ecology Resources* 10, pp. 1106–1108

Slotta-Bachmayr, L/Boegel, R/Kaczensky, P/Stauffer, C/Walzer, C (2004): "Use of Population Viability Analysis to Identify Management Priorities and Success in Reintroducing Przewalski's Horses to Southwestern Mongolia", in: *Journal of Wildlife Management* 68, pp. 790–798

Sokolov, VE/Orlov, VN (1980): *Identification Guide to the Mammals of Mongolia.* Moscow: Nauka Press, p. 351

Tulgat, R/Schaller GB (1992): "Status and Distribution of the Wild Bactrian Camels *Camelus bactrianus ferus*", in: *Biological Conservation* 62, pp. 11–19

Walzer, C/Kaczensky, P/Enkhbileg, D/Adiya, Y/Khatanbaatar, I/Dorvchindorj, G (2006): "Capture and Anaesthesia of Wild Bactrian Camels – Working in a Freezer", in: *Proceedings of the European Association of Zoo and Wildlife Veterinarians*, *Budapest, Hungary* 6, pp. 61–65

Wolf, C/Rentsch, J/Huebner, P (1999): "PCR-RFLP Analysis of Mitochondrial DNA: A Reliable Method for Species Identification", in: *Journal of Agricultural and Food Chemistry* 47, pp. 1350–1355