Diagnostic single nucleotide polymorphism markers to identify hybridization between dromedary and Bactrian camels

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Abstract The technique to produce hybrid Tulu or Nar camels from crosses between dromedary and Bactrian camels is common throughout Middle Eastern and Central Asian countries. Formerly, these hybrids were highly valued as strong and persistent pack animals but today are bred to improve milk or wool quality in the respective species and for camel wrestling. We developed a diagnostic single nucleotide polymorphism (SNP) panel to identify cryptic ancestry in F1 hybrids and their backcrosses by selecting loci from whole genome data, which were fixed for different alleles in either dromedary or domestic and wild Bactrian camel. With this SNP panel we are able to identify the hybridization patterns in camels with uncertain origins, support hybrid breeding management and to detect potential rare dromedary introgression in the last wild Bactrian camels in Mongolia and China.

Keywords Single nucleotide polymorphisms (SNPs) · Hybrid camel · Next generation sequencing (NGS) · Camelus bactrianus · Camelus dromedarius · Introgression

Technical note

Anthropogenic hybridization between livestock populations or between domestic species and their wild relatives has been practiced since the early phases of domestication.

In Old World camelids, hybridization between Bactrian camels (Camelus bactrianus) and dromedaries (Camelus dromedarius) was associated with the transportation of goods along the multiple routes of the Silk Road. This practice intended to produce animals with the robustness of the Bactrian camel, the endurance of dromedary, and the ability to tolerate sharply contrasting climatic conditions (Wilson 1984). Today, hybridization facilitates improved milk and wool yield in hybrid Tulu or Nar camels from Middle Eastern and Central Asian countries. Commonly, two hybridizing methods are recognized, Kurt-nar (dromedary female × Bactrian male) and Kez-nar (Bactrian female × dromedary male) followed by F1 backcrossing with either dromedary for increased milk productivity or Bactrian camel for wool and cold resistance (Faye and Konuspayeva 2012). F2 hybrids (F1 × F1) in Old World camels are usually not favoured because of a difficult character and weak progeny performance (Faye and Konuspayeva 2012). In western regions of Turkey, a relished sport is camel wrestling, where prized male Tulus compete against each other in heavily regulated fights (Vedat 2010). In this study, we developed diagnostic single nucleotide polymorphisms (SNPs) to identify F1 hybrids between dromedary and Bactrian camels and their backcrosses. These markers will facilitate the validation of non-invasive or historic samples with unclear or otherwise cryptic ancestry.

As part of an on-going research project, we sequenced the genomes of 25 Old World camelids [C. dromedarius (n = 9), C. ferus (n = 9), C. bactrianus (n = 7)] and aligned them to the C. ferus CB1 genome assembly (Genbank ID: GCA_000311805.2). To verify their species identity, these source individuals had previously been genotyped at 18 nuclear microsatellite loci and Sanger sequenced for 800 bp of mitochondrial DNA (unpublished...
We designed PCR primers around each SNP using PRIMER3 (Untergasser et al. 2012) and selected 26 primer pairs (Online Resource 2) for validation. We tested the 26 candidate loci using PCR and Sanger sequencing (see ‘Supplementary Methods’ in Online Resource 1) on a panel of control individuals including three Bactrian camels, three dromedaries, two hybrids (F1 and F1 backcross) and a two-humped individual with uncertain genetic background. All 26 candidate loci produced PCR products of the expected sizes, but we selected for sequencing only those 12 loci which produced single strong bands over consistent conditions (Table 1). Upon sequencing, all 12 loci were diagnostic between dromedaries and Bactrian camels (Table 2). However, in the F1 hybrid (Hyb56) only 10 (83.3 %) of the diagnostic loci were heterozygous and the remaining two loci were homozygous for the Bactrian allele (Table 2). This suggests that at these two loci (HP264 and HP288) the Bactrian allele may be present at low frequency in dromedaries as a result of being a shared ancestral polymorphism (not diagnostic) or from historical hybridization between the two species. The F1 backcross (Hyb55) was heterozygous for two (16.7 %) of the diagnostic loci and homozygous for the Bactrian allele in the remaining loci. In the two-humped individual with uncertain origin (DC575), four (33.3 %) of the diagnostic loci were heterozygous, demonstrating hybrid ancestry in this animal (Table 2).

With this panel of diagnostic SNPs it is possible to identify F1 hybrids between dromedary and Bactrian camels and to differentiate F1 backcrosses to either parental species. Thus, we can validate the status of individuals with unclear parental origins in regions where the two species are extensively crossbred (e.g. Kazakhstan, Turkey). Because we selected short PCR fragments (<150 bp) the panel can be applied in non-invasive, historical and archaeological (ancient) samples, where the morphology of the preserved materials cannot retrieve unambiguous classification (Galik et al. 2015). Moreover, with this marker set we will be able to detect potential, rare dromedary introgression in Bactrian camel populations in Mongolia and China. As hybridization between domestic Bactrian camels and their wild relatives has been demonstrated (Silbermayr and Burger 2012; Silbermayr 2009), the threat for dromedary introgression into the last wild two-humped camels (C. ferus) in the Mongolian Gobi (Yadamsuren et al. 2012) and Chinese Taklimakan and Lop Nor deserts (Lei et al. 2012) is small but existing. Therefore, this primer set is an important contribution to the conservation of the genetic integrity of this critically endangered species.

### Table 1 Primer sequences and information for the 12 diagnostic loci

| Locus  | Primer sequence (5’–3’)a | Scaffoldb | Positionb | Alleles | Tmb | Length |
|--------|--------------------------|-----------|-----------|---------|------|--------|
| HP206  | TGTCAGACGTGTTAGGCATTGC CATCCAAGTCTCTCATCTAACC CC | NW_006210212.1 | 501206 | C/G | 57 | 125 |
| HP379  | AGGATGCCATCATGTCAAG GAGGGACCTCTCATGAGTGG | NW_006210464.1 | 1151379 | G/A | 58 | 150 |
| HP405  | CCAGGACCTTTCTAGCTAGC CAGCACAGAACTCCTAGCG | NW_006211106.1 | 5030405 | G/C | 59 | 125 |
| HP429  | GCAGGCTACAAAATACCC GCTTTCTTTCTGGTCAGG | NW_006210666.1 | 2288429 | A/C | 57 | 125 |
| HP458  | TGTCAGACGACAGAAGCTTGGCTTTTATTGG | NW_006210457.1 | 11458 | T/C | 58 | 140 |
| HP501  | GAATAGATTGGGAGCAAGCTTCTCCTCATCCTATAGGC | NW_006211169.1 | 218501 | A/T | 59 | 125 |
| HP597  | ATGAACAGTGGTGTTGG GCGGATGCACCTTTATAGG | NW_006211126.1 | 6065597 | A/C | 59 | 125 |
| HP633  | CAGTGTAGAAGGTTGTAGCAGC CAGCCTTTCTGCATCTG | NW_006210489.1 | 4279633 | G/C | 57 | 125 |
| HP900  | CCACATGTCAGTTGTAGG GGGATCTTTCTCTATAGC | NW_006211075.1 | 294900 | G/C | 59 | 125 |
| HP930  | CTCCACGAAAAAACCTGCC TTTGGGACTTGCTTCTGCG | NW_006210745.1 | 3459920 | C/A | 59 | 125 |
| HP264  | TGGACAGAATTGTTTGCTTCTC TTTGTGAAGGCAATATCGC | NW_006211022.1 | 519264 | T/C | 59 | 125 |
| HP288  | GTCATGAGGCGTTCTCTG CAGCCTTCTGTGGTTCTCG | NW_006211252.1 | 214288 | T/A | 59 | 125 |

See Online Resource 2 for additional details on these and the remaining 14 primers examined

Tm: primer annealing temperature
Length: PCR product length

a Left (forward) primer given above the right (reverse) primer
b Accession number from GenBank and position of the SNP in the scaffold

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| Sample ID | Species      | Location     | HP206 (G/C) | HP379 (A/G) | HP405 (C/G) | HP429 (C/A) | HP458 (C/T) | HP501 (T/A) | HP597 (C/A) | HP633 (C/G) | HP900 (C/G) | HP930 (A/C) | HP264* (C/T) | HP288* (A/T) |
|-----------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|
| Drom155   | *C. dromedarius* | Australia   | GG          | AA          | CC          | CC          | CC          | TT          | CC          | CC          | CC          | AA          | CC           | AA           |
| Drom214   | *C. dromedarius* | Syria       | GG          | AA          | CC          | CC          | CC          | TT          | CC          | CC          | CC          | AA          | CC           | AA           |
| Drom814   | *C. dromedarius* | Sudan       | GG          | AA          | CC          | CC          | CC          | TT          | CC          | CC          | CC          | AA          | CC           | AA           |
| DC69      | *C. bactrianus*   | Mongolia    | CC          | GG          | GG          | AA          | TT          | AA          | AA          | GG          | GG          | CC          | TT           | TT           |
| DC158     | *C. bactrianus*   | Austria     | CC          | GG          | GG          | AA          | TT          | AA          | AA          | GG          | GG          | CC          | TT           | TT           |
| DC352     | *C. bactrianus*   | Iran        | CC          | GG          | GG          | AA          | TT          | AA          | AA          | GG          | GG          | CC          | TT           | TT           |
| Hyb56     | F1 hybrid       | Kazakhstan  | GC          | GA          | GC          | AC          | CT          | TA          | AC          | GC          | GC          | AC          | TT           | TT           |
| Hyb55     | F1 backcross    | Kazakhstan  | CC          | GA          | GG          | AA          | CT          | AA          | AA          | GG          | GG          | CC          | TT           | TT           |
| DC575     | uncertain origin | Kazakhstan  | CC          | GA          | GG          | AC          | TT          | AA          | AA          | GC          | GG          | CC          | CT           | TT           |

The parentheses indicate the reference alleles (dromedary allele | Bactrian allele) identified from the whole-genome sequencing.
Location: sample’s country of origin
HP206 ff.: SNP locus names

*a* These loci show patterns inconsistent with a diagnostic SNP in the F1 hybrid

*b* F1 (dromedary female × Bactrian male); F1 backcross (F1 female × Bactrian male)
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