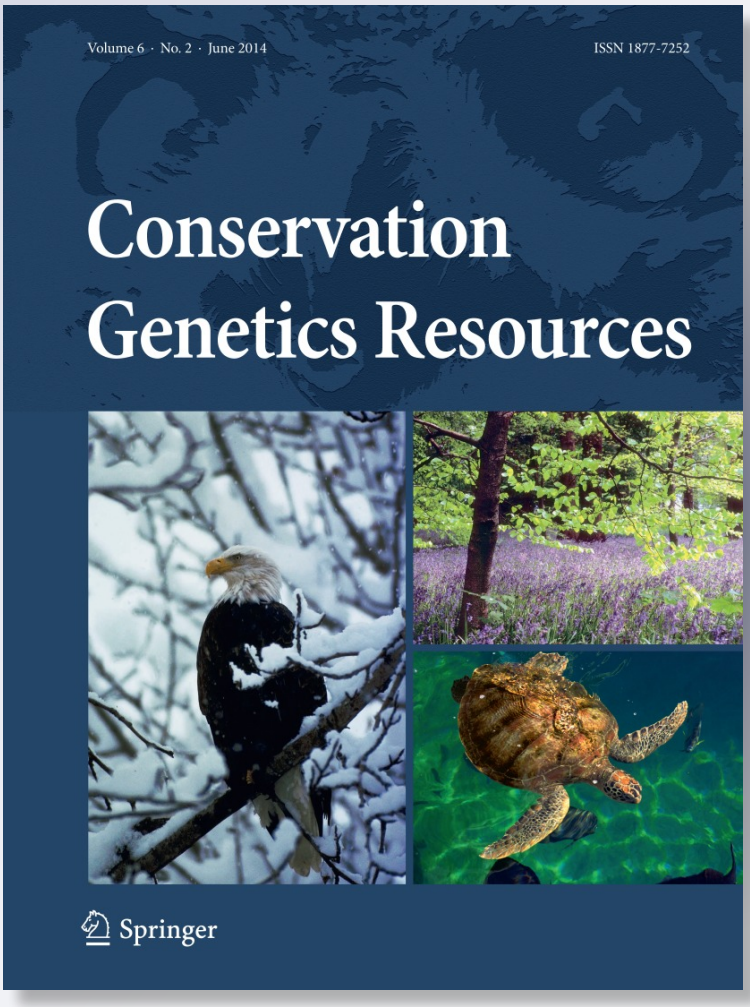
*PCR-based Y chromosome marker for discriminating between golden jackal (*Canis aureus*) and domestic dog (*Canis lupus familiaris*) paternal ancestry*

Ana Galov, Magda Sindičić, Tomislav Gomerčić, Haidi Arbanasić, Matea Baburić, Ivica Bošković & Tihomir Florijančić

Conservation Genetics Resources

ISSN 1877-7252
Volume 6
Number 2

Conservation Genet Resour (2014)
6:275-277
DOI 10.1007/s12686-013-0110-7



Springer

Conservation Genet Resour (2014) 6:275–277
DOI 10.1007/s12686-013-0110-7

TECHNICAL NOTE

PCR-based Y chromosome marker for discriminating between golden jackal (*Canis aureus*) and domestic dog (*Canis lupus familiaris*) paternal ancestry

Ana Galov · Magda Sindičić · Tomislav Gomerčić · Haidi Arbanasić · Matea Baburić · Ivica Bošković · Tihomir Florijančić

Received: 26 August 2013 / Accepted: 13 December 2013 / Published online: 18 December 2013

Abstract Numerous reports of hybridization between wild *Canis* species and domestic dog suggest that hybridization between golden jackal and dog is likely. Here we present an assay of a Y chromosome marker for discriminating between golden jackal (*Canis aureus*) and dog (*Canis lupus familiaris*) paternal ancestry. Taking advantage of an insertion found in a dog *Zfy* intron haplotype but not in a golden jackal haplotype, we developed a three-primer PCR system in which species are differentiated based on the number of amplicons. Two amplicons are produced from domestic dog DNA template, while one amplicon is produced from golden jackal DNA template. Both amplicons can be analyzed in a single agarose gel electrophoresis run, while the longer amplicon also serves as an internal control. The method was validated using 27 golden jackal and 25 dog samples; all samples showed the expected amplicons. This simple and cost-effective method should prove useful for determining the direction of golden jackal–dog hybridization.

Keywords *Canis aureus* · *Canis lupus familiaris* · Y chromosome · Hybridization

A. Galov · H. Arbanasić · M. Baburić
Department of Biology, Faculty of Science, University of Zagreb, Rooseveltov trg 6, Zagreb, Croatia

M. Sindičić (✉)
Department for Game Biology, Pathology and Breeding, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia
e-mail: magda.sindicic@vef.hr

T. Gomerčić
Biology Department, Faculty of Veterinary Medicine, University of Zagreb, Heinzelova 55, Zagreb, Croatia

I. Bošković · T. Florijančić
Department for Hunting, Fishery and Beekeeping, Faculty of Agriculture, Josip Juraj Strossmayer University of Osijek, Trg Svetog Trojstva 3, Osijek, Croatia

The golden jackal (*Canis aureus*) is one of the least studied canids in Europe despite its recent extensive expansion from the Balkan Peninsula (Arnold et al. 2012). It is likely that higher population density favors the encounters between golden jackals and feral dogs possibly resulting in hybridization. Furthermore, numerous reports of hybridization between wild *Canis* species and domestic dog (e.g. Randi and Lucchini 2002) suggest that hybridization between golden jackal and dog is highly likely.

Molecular techniques are commonly used to identify closely related species and detect hybridization. Using a combination of maternal and paternal genetic markers can even reveal the direction of hybridization (Vilá et al. 2003). One mtDNA control region haplotype has been identified so far in European golden jackals (Zachos et al. 2009; Fabbri et al. 2013) and is effective at identifying golden jackal–dog hybrids through the maternal line.

The aim of the present study was to develop a reliable, simple, fast and inexpensive Y chromosome marker for discriminating between male golden jackals and male domestic dogs. Such a marker should prove useful for investigating hybridization between golden jackals and dogs through the paternal line.

Genomic DNA was extracted from 27 male golden jackal tissue samples and 25 male dog blood samples using a Wizard Genomic DNA Purification Kit (Promega, USA). A 594-base pair (bp) fragment of the *Zfy* intron was

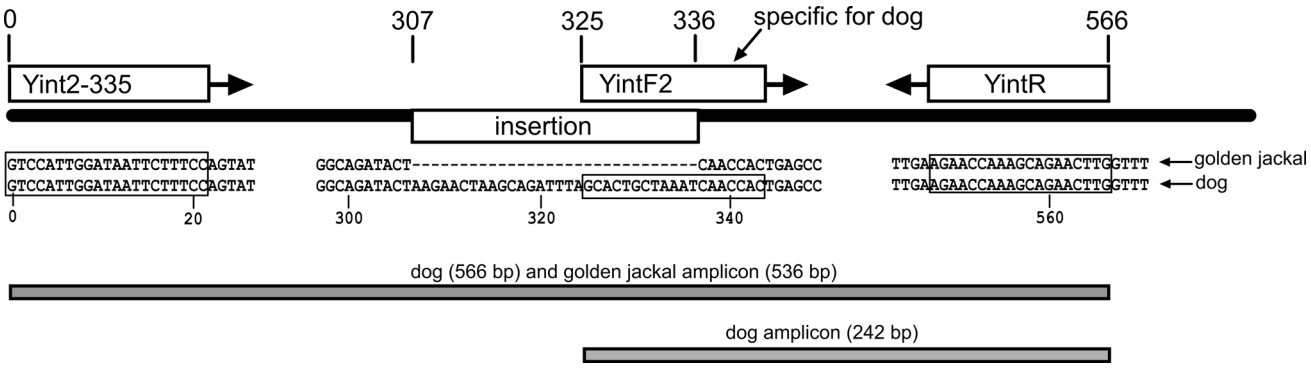


Fig. 1 Three-primer system based on an intron within the *Zfy* gene. Two amplicons (566 and 242 bp) are produced from domestic dog DNA template, while one amplicon (536 bp) is produced from golden jackal DNA template. The 30 bp insertion is exclusive to dog Zfy gene

amplified in five golden jackals and two dogs using the primers Yint2-335 (Shami 2002, as cited in Rutledge et al. 2010) and LGL-331 (Shaw et al. 2003). Amplification conditions were as described in Rutledge et al. (2010). Sequences were deposited in the GenBank under accession numbers KF021269 (golden jackal) and KF021271 (dog). Sequence alignment using BioEdit software (Hall 1999) revealed a 30-bp insertion in dog sequences. Based on this insertion we developed a three-primer PCR system for distinguishing golden jackals and dogs. This system uses the forward primer Yint2-335 (5′-GTCCATTGGATAATTCTTTCC-3′) designed by Shami (2002) in addition to two specially designed primers: a forward primer YintF2 (5′-GCACTGCTAAATCAACCAC-3′) and reverse primer YintR (5′-CAAGTTCTGCTTTGGTTCT-3′). Primers were designed using the online tool Primer 3 (Ye et al. 2012). Primer YintF2 is partially complementary (12 bases of 19) to the insertion found only in the dog haplotype, so using it together with YintR amplifies a 242-bp fragment only in dogs. In addition, using primers Yint2-335 and YintR together amplifies longer fragments with characteristic lengths for both species: 566 bp in dog and 536 bp in golden jackal (Fig. 1). Thus the species of a sample depends on the number of amplification bands (two in dogs, one in golden jackals).

PCR reactions (10 µl) contained 1 µl of extracted DNA, 1 × Qiagen Multiplex PCR Master Mix (Qiagen, Germany) and 0.5 µM of each of the three primers. Amplification was achieved with 35 cycles of initial denaturation at 95° C for 15 min, 94° C for 40 s, 55° C for 50 s, and 72° C for 60 s, followed by a final extension at 72° C for 10 min in a Perkin Elmer Gene Amp PCR System 9700. Amplified products and 100-bp DNA ladder (Promega, USA) were electrophoresed in a 1 % agarose gel at 90 V for approximately 45 min in TBE buffer (45 mM Tris borate, 1 mM EDTA, pH 8). PCR products were stained with SYBR Safe™ DNA gel stain (Invitrogen, USA) and visualized under ultraviolet light.

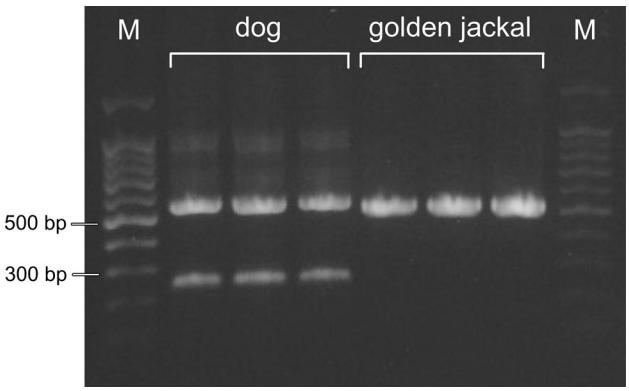


Fig. 2 Amplicons of *Zfy* intron obtained using the three-primer system in three male dog samples and three male golden jackal samples

As expected, golden jackal samples showed one band of 536 bp, whereas dog samples showed a diagnostic fragment of 242 bp in addition to an internal control fragment of 566 bp (Fig. 2). The expected species patterns shown in Fig. 2 were obtained for all 27 golden jackals and all 25 dog samples.

The ability to genetically distinguish admixed individuals of closely related species can provide invaluable resources for wildlife management (Oliveira et al. 2008). Furthermore, complete understanding of the dynamics of hybridization in natural populations requires not only detection of hybrids but also determination of the direction of hybridization (Godinho et al. 2011). Therefore we developed a reliable method for testing paternal ancestry of potential golden jackal–dog hybrids. Identifying species-specific Y chromosome markers is challenging because of the low levels of Y chromosome diversity, especially in related taxa like golden jackals and dogs (Gomerčić et al. 2013). Our three-primer test is simple and cost-effective, requiring only PCR amplification and agarose gel electrophoresis. A second, longer amplicon observed in DNA

samples from both species serves as an internal control that rules out PCR inhibitors or low quality or quantity of template DNA as a potential cause for the absence of a dog-specific band. This method proved useful for investigating paternal ancestry of potential golden jackal–dog hybrids, which should help fill in important gaps in golden jackal biology and help satisfy the growing interest in these animals following their expansion into new European territories.

References

Arnold J, Humer A, Heltai H, Murariu D, Spassov N, Hacklander K (2012) Current status and distribution of golden jackal *Canis aureus* in Europe. Mammal Rev 42:1–11

Fabbri E, Caniglia R, Galov A, Arbanasić H, Lapini L, Bošković I, Florijančić T, Vlasseva A, Ahmed A, Mirchev RL, Randi E (2013) Genetic structure and expansion of golden jackals (*Canis aureus*) in the north-western distribution range (Croatia and eastern Italian Alps). Conerv Genet. doi:10.1007/s10592-013-0530-7

Godinho R, Llaneza L, Blanco JC, Lopes S, Álvares F, García EJ, Palacios V, Cortés Y, Talegón J, Ferrand N (2011) Genetic evidence for multiple events of hybridization between wolves and domestic dogs in the Iberian Peninsula. Mol Ecol 20:5154–5166

Gomerčić T, Sindičić M, Florijančić T, Bošković I, Huber Đ, Galov A (2013) Differentiating between Y chromosome sequences in Croatian canids. Vet Arhiv 83:571–579

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/97/NT. Nucleic Acids Symp Ser 41:95–98

Oliveira R, Godinho R, Randi E, Alves PC (2008) Hybridization versus conservation: are domestic cats threatening the genetic integrity of wildcats (*Felis silvestris silvestris*) in Iberian Peninsula? Philos Trans R Soc Lond B Biol Sci 363(1505): 2953–2961

Randi E, Lucchini V (2002) Detecting rare introgression of domestic dog genes into wild wolf (*Canis lupus*) populations by Bayesian admixture analyses of microsatellite variation. Conserv Genet 3:31–45

Rutledge LY, Garroway CJ, Loveless KM, Patterson BR (2010) Genetic differentiation of eastern wolves in Algonquin Park despite bridging gene flow between coyotes and grey wolves. Heredity 105(6):520–531. doi:10.1038/hdy.2010.6

Shami K (2002) Evaluating the change in distribution of the eastern timber wolf (*Canis lycaon*) using the Y-chromosome. Masters thesis, McMaster University

Shaw CN, Wilson PJ, White BN (2003) A reliable molecular method of gender determination for mammals. J Mammal 84(1):123–128

Vilá C, Walker C, Sundqvist AK, Flagstad Ø, Andersone Z, Casulli A, Kojola I, Valdmann H, Halverson J, Ellegren H (2003) Combined use of maternal, paternal and bi-parental genetic markers for the identification of wolf-dog hybrids. Heredity 90:17–24

Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T (2012) Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics 13:134

Zachos FE, Cirovic D, Kirschning J, Otto M, Hartl GB, Petersen B, Honnen A-C (2009) Genetic variability, differentiation, and founder effect in golden jackals (*Canis aureus*) from Serbia as revealed by mitochondrial DNA and nuclear microsatellite loci. Biochem Genet 47:241–250