Conservation Genet Resour
DOI 10.1007/s12686-016-0642-8

TECHNICAL NOTE

Evaluation of noninvasive genetic methods for Nubian ibex

Idan Goodman[1] · **Naama Shahar**[1] · **Gila Kahila Bar-Gal**[2] · **David Saltz**[1] · **Shirli Bar-David**[1]

Received: 29 August 2016 / Accepted: 23 October 2016

Abstract Noninvasive genetic methods enable the sampling of natural populations while minimizing detrimental effects on them. However, noninvasive methods are marred by DNA extraction and amplification difficulties that can be mitigated by improved methodology. Past studies have shown that noninvasive genetic protocols are system specific and highlighted the importance of pilot studies in the establishment of genetic monitoring programs. We tested, using a factorial design experiment, the effect of different methods for the collection-preservation and extraction on the amplification of DNA from Nubian ibex (*Capra nubiana*) fecal samples. We found collection-preservation using paper bags and extraction with QIAamp® fast DNA stool mini kit to significantly enhance success rate compared to collection-preservation in ethanol and extraction with QIAamp® DNA mini kit. Our results will contribute to the studies of natural populations of the vulnerable *C. nubiana*.

Keywords Fecal DNA · Collection-preservation · DNA extraction · *Capra nubiana* · Nubian ibex

Introduction

Non-invasive genetic sampling (NIGS) allows scientists to obtain genetic material without disturbing or even seeing their target species. Hence, studies of endangered and elusive species—that would otherwise require complicated capturing techniques—have become practical as molecular tools developed. The intestinal cells shed in animal feces have been found to be an effective source of DNA for many mammals (Beja-Pereira et al. 2009). Nevertheless, the low quantity and quality of DNA together with PCR inhibitors may result in high genotyping error rates and limit the accuracy and effectiveness of non-invasive studies (Waits and Leberg 2000). Comparative studies demonstrated the potential effects of seasonality (Maudet et al. 2004), time between feces excretion to DNA extraction (Woodruff et al. 2015) and different preservation (Tende et al. 2014) and extraction methods (Ramón-Laca et al. 2015) on amplification success and genotyping error rates. However, lack of consistency among studies emphasizes that NIGS protocols are system specific (Schwartz and Monfort 2008). Consequently, pilot studies are key for developing an efficient NIGS protocol on the target species in the research area before full-scale monitoring programs are deployed (Valière et al. 2007; Renan et al. 2012).

The Nubian ibex (*Capra nubiana*) is classified as vulnerable by the IUCN red list of threatened species (Alkon et al. 2008). The population in Israel is under threat and hence is a subject for behavioral, spatial and genetic studies aimed at its protection. Here, we assessed different methodologies to be used for genetic monitoring of wild ibex population using non-invasive samples. More specifically, we compared the amplification success of ibex fecal DNA under two collection-preservation methods and two commercial extraction kits, as a basis for a genetic monitoring project.

We used a factorial-design experiment in order to evaluate the effect the treatments have on amplification success of DNA extracted from ibex feces. Unlike the majority of studies that focus on a single experimental

✉ Idan Goodman
idangoodman@gmail.com

[1] Mitrani Department of Desert Ecology, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, 8499000 Midreshet Ben-Gurion, Israel

[2] The Robert H. Smith Faculty of Agriculture, Koret School of Veterinary Medicine, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel

Table 1 Source, polymorphism data and PCR conditions of the used primers

Locus	Source	Size range	No. of alleles	Annealing temp. (°C)
RM006	Kossarek et al. (1993)	115–145	10	56
CSRD247	Baumung et al. (2006)	227–281	9	56
ILSTS019	Kemp et al. (1995)	173–181	2	56
HSC	Blattman and Beh (1992)	274–308	10	60
OarFCB48	Buchanan et al. (1994)	153–161	5	55
SR-CRSP-26	Maudet et al. (2001)	134–140	3	55
BM1818	Crawford et al. (1995)	262–278	7	50
INRABERN185	Maudet et al. (2001)	264–278	6	50
TGLA122	Cockett et al. (1994)	152–202	14	55

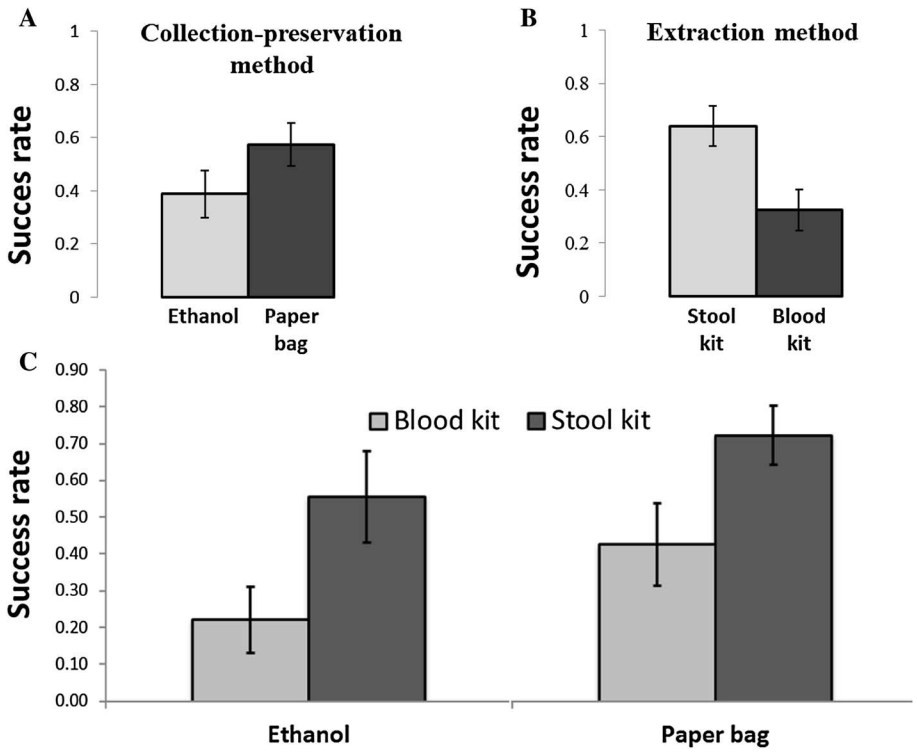

Fig. 1 Amplification success rates under the different collection-preservation methods (**a**), extraction methods (**b**) and combined stages (**c**) for Nubian ibex fecal samples. *Error bars* represent ± standard errors

stage (collection, preservation or extraction) this design enabled us to test also the interaction between the different treatments (Renan et al. 2012). The two collection-preservation treatments chosen were (a) paper bag and (b) 96% ethanol. The first provides a dry environment while the second actively dries the pellet. Both function to reduce activity of DNA-degrading enzymes (Beja-Pereira et al. 2009; Piggott 2004). Collected samples were kept in a cooling box and later stored in a freezer (−20 °C). The two extraction methods we tested were (1) QIAamp® DNA mini kit—designed for blood and tissue samples, and (2) QIAamp® fast DNA stool mini kit, specifically designed to diminish the effect of PCR inhibitors. For both extraction treatments we used a modified protocol that included an initial wash of the pellets for 45 min in AL and InhibitEX buffers (supplied with the kits) for kits (1) and (2), respectively.

Ibex were sampled in the Negev and Judean deserts in Israel, in dry environmental conditions during winter 2016. We collected 4 fecal pellets from each of the 6 sampled ibex immediately after defecation. This way, all 4 experimental combinations were performed on each of the ibex, and between individual variance could be measured. Success rate was estimated by averaging the amplification success of 9 microsatellites (Table 1) using 2.5% agarose gel visualized under UV transilluminator. DNA was amplified in a reaction volume of 20 µl, containing 1 µl of extracted DNA, 1 unit of *Taq* polymerase, 1× Taq Rxn buffer, 2mM $MgCl_2$, 200 µl of each dNTP and 0.5 µM of each primer. All 9 markers were amplified

using a touch-down PCR protocol (see annealing temperature, Table 1). Distilled water was used as negative control for each PCR reaction. DNA extracted from ibex blood was used as positive control, marking the band size of interest. We used a two-way ANOVA design that considered individuals as a random effect to test the significance of our results.

Results and conclusions

The different treatments had a significant effect on PCR amplification success in both the collection-preservation phase and the extraction phase. Between the two collection-preservation methods, the paper bag had a higher success rate (56%) compared to ethanol (38%) (two-way ANOVA; $F_{1, 15} = 11.91$, $P < 0.01$; Fig. 1a). Of the two extraction kits, the stool kit yielded a 62% success rate, whereas the success rate of the blood kit was only 32% (two-way ANOVA; $F_{1, 15} = 24.04$, $P < 0.001$; Fig. 1b). No significant interaction was found between the two treatments ($F_{1, 15} = 0.06$, $P = 0.80$). Hence, collection-preservation with paper bag and extraction using QIAamp® fast DNA stool mini kit proved to be the best combination as it yielded the highest success rate of 71%.

This factorial designed experiment enabled us to identify an efficient combination of methods, out of commonly used methods, to obtain DNA from fecal samples of Nubian ibex in Israel. This is an initial step in a genetic monitoring project aimed at the protection of the species.

Acknowledgements We would like to thank the regional biologists and rangers of the Israel Nature and Parks Authority for their support and assistance. We thank Ofer Ovadia and Sharon Renan for fruitful communication. This research was supported by the Israeli Nature and National Parks Protection Authority and the Israeli Ministry of Environmental Protection as part of the program for development and rehabilitation of the Dead Sea Basin. This is publication XXX of the Mitrani Department of Desert Ecology, Ben-Gurion University of the Negev.

References

Alkon P, Harding L, Jdeidi T et al (2008) *Capra nubiana*. The IUCN red list of threatened species. doi:10.2305/IUCN.UK.2008.RLTS.T3796A10084254.en

Baumung R, Cubric-Curik V, Schwend K et al (2006) Genetic characterisation and breed assignment in Austrian sheep breeds using microsatellite marker information. J Anim Breed Genet 123:265–271. doi:10.1111/j.1439-0388.2006.00583.x

Beja-Pereira A, Oliveira R, Alves PC et al (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. Mol Ecol Resour 9:1279–1301. doi:10.1111/j.1755-0998.2009.02699.x

Blattman AN, Beh KJ (1992) Dinucleotide repeat polymorphism within the ovine major histocompatibility complex. Anim Genet 23:392

Buchanan FS, Galloway SM, Crawford AM (1994) Ovine microsatellites at the OarFCB5. Anim Genet 25:60

Cockett NE, Jackson SP, Shay TL et al (1994) Chromosomal localization of the callipyge gene in sheep (*Ovis aries*) using bovine DNA markers. Proc Natl Acad Sci USA 91:3019–3023. doi:10.1073/pnas.91.8.3019

Crawford AM, Dodds KG, Ede AJ et al (1995) An autosomal genetic linkage map of the sheep genome. Genetics 140:703–724

Kemp SJ, Hishida O, Wambugu J et al (1995) A panel of bovine, ovine and caprine polymorphic microsatellites. Anim Genet 26:299–306

Kossarek LM, Grosse WM, Mcgraw RA (1993) Rapid communication: bovine dinucleotide repeat polymorphism RM006l. J Anim Sci 71:3176–3176

Maudet C, Luikart G, Taberlet P (2001) Development of microsatellite multiplexes for wild goats using primers designed from domestic Bovidae. Genet Sel Evol 33:S193–S203

Maudet C, Luikart G, Dubray D et al (2004) Low genotyping error rates in wild ungulate faeces sampled in winter. Mol Ecol Notes 4:772–775. doi:10.1111/j.1471-8286.2004.00787.x

Piggott MP (2004) Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. Wildl Res 31:485–493.

Ramón-Laca A, Soriano L, Gleeson D, Godoy JA (2015) A simple and effective method for obtaining mammal DNA from faeces. Wildlife Biol 21:195–203. doi:10.2981/wlb.00096

Renan S, Speyer E, Shahar N et al (2012) A factorial design experiment as a pilot study for noninvasive genetic sampling. Mol Ecol Resour 12:1040–1047. doi:10.1111/j.1755-0998.2012.03170.x

Schwartz MK, Monfort SL (2008) Genetic and endocrine tools for carnivore surveys. Noninvasive Surv Methods Carniv 228–262

Tende T, Hansson B, Ottosson U, Bensch S (2014) Evaluating preservation medium for the storage of DNA in African lion Panthera leo faecal samples. Curr Zool 60:351–358

Valière N, Bonenfant C, Toïgo C et al (2007) Importance of a pilot study for non-invasive genetic sampling: genotyping errors and population size estimation in red deer. Conserv Genet 8:69–78. doi:10.1007/s10592-006-9149-2

Waits JL, Leberg PL (2000) Biases associated with population estimation using molecular tagging. Anim Conserv 3:191–199. doi:10.1111/j.1469-1795.2000.tb00103.x

Woodruff SP, Johnson TR, Waits LP (2015) Evaluating the interaction of faecal pellet deposition rates and DNA degradation rates to optimize sampling design for DNA-based mark-recapture analysis of Sonoran pronghorn. Mol Ecol Resour 16:843–854. doi:10.1111/1755-0998.12362