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Short Note

“Passive surveillance” across species with cross-amplifying molecular markers: the potential of wolf (*Canis lupus*) genetic monitoring in tracking golden jackal (*C. aureus*) colonization and hybridization

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Abstract

From their historic ranges in southeastern Europe, the golden jackal (*Canis aureus*) distribution is expanding westward and northward, with range enlargement from Balkan and Caucasus source populations. Jackals can hybridize with dogs (*C. lupus familiaris*) and potentially also wolves (*C. lupus*), which is a conservation concern. Despite the emerging need for genetic monitoring of jackal expansion, such programs are nevertheless lacking. As microsatellite markers used for wolf monitoring cross-amplify across canids, we suggest exploiting profiles from wolf population monitoring schemes, where jackals can be detected as non-target species. Such “passive surveillance” can support rapid species identification and advance our understanding of jackal range expansion. We present an example from Slovenia and recommend inclusion of this cost-effective screening option as a standard procedure wherever jackal expansion is occurring or anticipated in the near future. This expansion can increase conflicts with humans and cause negative attitudes among certain interest groups, although preliminary results from Slovenia suggest hunters become accustomed to the presence of jackals and agree the species should be managed sustainably. As monitoring data are now routinely used to investigate the possible presence of wolf-dog hybrids, jackals can be included in such assessments with minor additional efforts, allowing more timely management responses and targeted public outreach.

The golden jackal (*Canis aureus*) is considered native to the southern Balkans and is known from the Adriatic Coast since at least 1491 (Galov et al., 2015). During the past century its distribution has expanded substantially (Arnold et al., 2012), and jackals from the Balkan and Caucasus regions have colonized northern Europe (Rutkowski et al., 2015; Pyšková et al., 2016; Kowalczyk et al., 2020). The first confirmed jackal observation in Finland was done in July 2019 (Ministry of Agriculture and Forestry of Finland, 2019). Hence, there is increasing interest in jackal ecology and their influence on prey species and competing carnivores (Arnold et al., 2012; Ćirović et al., 2016), and factors associated with their range expansion, ecological plasticity and environmental tolerance (Arnold et al., 2012; Deinet et al., 2013; Krofel et al., 2017).

Jackals typically appear to avoid wolves (*C. lupus*) and their range increase in many areas seems to mirror declines in the distribution of wolves (e.g., Kryštufek and Tvrtković, 1990; Krofel et al., 2017). However, jackals in some areas benefit from scavenging wolf kills (Jhala, 1993) and may thus, at times, be sympatric with wolves while maintaining spatiotemporal avoidance, as demonstrated for red foxes (*Vulpes vulpes*) and North American coyotes (*C. latrans*) (Paquet, 1992; Levi and Wilmers, 2012; Wikenros et al., 2017). Wolf and jackal distributions show increasing overlap, circa 7.1% during 1950–1970 and 22.7% after 2000; a situation where wolves are reclaiming historical ranges while jackals are colonizing new areas (Krofel et al., 2017).

Recent genetic results highlight conservation challenges by means of hybridization involving jackals and domestic dogs (*C. l. familiaris*) (Galov et al., 2015) and possibly also wolves (Freedman et al., 2014; Moura et al., 2014). Improved understanding of wolf and jackal range changes, and their interactions with each other and with free-ranging dogs, is vital for conservation of wild canids, with evolutionary (Moura et al., 2014; Galov et al., 2015) and legal (Trouwborst et al., 2015) implications. Genetic monitoring via noninvasive sampling provides essential information for management. Many European countries, including Slovenia, routinely perform wolf genetic monitoring, but we are unaware of similar programs for jackals. The European Union’s Habitat Directive protects both canids as species of community interest, with the wolf included under Annex II as a priority species (https://www.eea.europa.eu/help/glossary/european-species-listed-under-article/canis-lupus) and the jackal listed under Annex V (https://www.eea.europa.eu/help/glossary/european-species-listed-under-article/canis-aureus). Though fewer resources seem available for jackal monitoring, such efforts are urgently needed (see e.g. Kusza et al., 2018), also in Slovenia where the first resident jackals where confirmed in 2009 (Krofel, 2009).

Whereas it may be hard to obtain funds for targeted jackal genetic monitoring, much can be achieved with other sources of data. Of 26 microsatellite loci we have tested (details in Jelenčič, 2016; Potočnik et al., 2018 part 2.3), all cross-amplify among *Canis* species including wolves, dogs and jackals, and 20 loci amplify in red foxes (Jelenčič, 2016 Appendix C, Potočnik et al., 2018 part 2.7). Loci were organized in two multiplexes, each including a locus for mo-

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lecular sexing (1: C09.250, C20.253, CPH12, CPH5, CPH7, CPH8, CPH9, Cxx.121, FH2010, FH2145, and the sex-marker SRY, and 2: AHT137, AHTh260, AHTk211, AHTk253, CXX279, FH2054, FH2848, INRA21, INU030, INU055, REN162C04, REN169D01, REN169O18, REN247M23, REN54P11, AHTh171, and the sex-marker amelogenin (see also Pedersen et al., 2012 for multiplex 2). We used a multiple-tube approach with 2–8 amplifications following Karamanlidis et al. (2009). The fragment analysis was performed on an ABI 3130xl Genetic Analyzer (Applied Biosystems, USA), and we examined the output with GeneMapper software (version 4.0, Applied Biosystems, USA). The four species can reliably be distinguished, as shown with a principal component analysis (PCA) that separates dogs, wolves, red foxes, and jackals sampled in Slovenia (Fig. 1), where all jackal profiles included are noninvasive samples obtained from monitoring of other species. The PCA was done in adegenet v.2.1.1(Jombart et al., 2008) implemented in R v.3.5.3 (R Core Team, 2019) and with 11 microsatellites that amplified and showed high variability across the four species: AHT137, AHTh171, AHTk253, CXX279, FH2054, INRA21, INU055, REN162C04, REN169D01, REN247M23, and REN54P11 (details in Jelenčič, 2016, Potočnik et al., 2018 part 2.3). We nevertheless caution that allelic ranges may change as samples and data accumulate, particularly for areas receiving immigrants from several source populations (see e.g. Rutkowski et al., 2015), hence the possibility of overlapping allele ranges needs continuous attention. Moreover, we were in some cases able to identify jackal-specific alleles (at least for our region) but unable to obtain complete and reliable genotypes. Such samples were not included in further analyses but are nonetheless informative for monitoring of jackal presence and for identifying target areas for future sampling.

In noninvasive wolf monitoring programs, many samples from non-target species get collected and analyzed, especially when involving non-expert personnel, but red foxes, jackals and dog samples can provide useful data. In Slovenia, we established systematic wolf genetic monitoring in 2010–2013 within the LIFE SloWolf (LIFE08 NAT/SLO/000244), and yearly monitoring has continued since 2015. Species are first classified based on two or more loci with discriminant alleles (Skrbinšek et al., unpublished data). A panel of 25–35 loci is later produced for all putative wolves or wolf hybrids to examine population structure and possible hybridization. As our data accumulate and the public shows increasing interest in jackals, the value of "bycatch" samples is becoming increasingly evident. Since 2013, we have classified 32 noninvasive samples (scats and saliva collected at damage sites) from wolf monitoring as jackals, providing 13 profiles for further study. This included the first confirmed jackal record from the Alpine region of Slovenia. To date, no hybrids between jackals and other canid species have been detected in Slovenia.

Genetic monitoring via sources such as non-invasive sampling of damage cases combined with better understanding of public attitudes, beliefs and expectations can provide vital information for conservation management. Accurate species identifications may influence human attitudes to predators (Mihelič and Krofel, 2012), and reliable scientific results available to all stakeholders represent an important baseline for addressing human-carnivore conflicts (Wilson, 2016). Colonizing jackals can come into conflict with human interests, which may be compounded by conflicting management approaches and messages to the public in countries without historical records of these canids (Stratford, 2015; Trouwborst et al., 2015). We used a structured questionnaire to survey Slovenian hunters' attitudes, beliefs and expectations concerning jackals and their management. Hunters are a stakeholder group that usually is the first one to notice the presence of a new species, they are also most directly involved in species management, both through participating in field surveillance of the population and in implementing removals of the individuals from the population. Sampling was random and stratified with regards to whether jackals had been recorded by the national game informational system LISJAK (https://lisjak.lovska-zveza.si/login.aspx?ReturnUrl=%2f) in their hunting grounds.

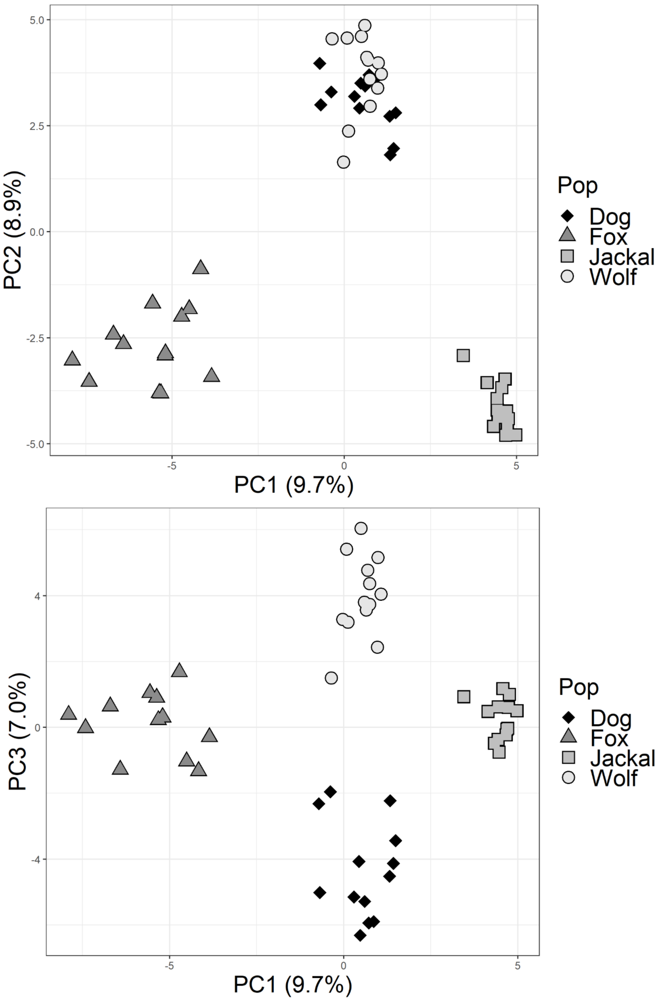

Figure 1 – Principal component analyses of dogs (n=13), wolves (n=13), red foxes (n=13), and jackals (n=13) with 11 autosomal microsatellite loci. The first panel shows that PC1, which explained 9.7% of the variation, separates foxes and jackals from the cluster including wolves and dogs, and PC2, which explained 8.9% of the variation, further differentiates the wolf-dog cluster from the other two species. The second panel shows that PC3, which explained 7.0% of the variation, differentiates between dogs and wolves.

We obtained 247 responses, 51.8% from areas without jackals (Potočnik et al., 2018 part 2.9). Circa 70% in both subsamples believed jackals strongly reduce game populations and cause problems for game management. A belief that jackals cause serious livestock damage was found among hunters from areas without jackals (54% agreed; 27% disagreed), whereas those from areas with jackals mainly disagreed with the statement (32.5% agreed; 46.2% disagreed). Slovenian hunters seem to view jackals as intruders but get accustomed to their presence (Potočnik et al., 2018 part 2.9). Nonetheless, many respondents agreed that the jackal does not belong to Slovenia and its colonisation needs to be stopped as soon as possible (65.1% and 59.1% agreement in areas with and without jackals, respectively). Notably, however, hunters agreed that jackals in Slovenia should be managed via sustainable hunting (92.3% and 87.1% agreement in areas with and without jackals). Slovenian hunters thus seem to prefer sustainable hunting of jackals over attempting to stop the colonisation process.

Monitoring data are now routinely used to assess wolf recolonization of historic ranges and the possible presence of wolf-dog hybrids, and relatively minor additional efforts, i.e. obtaining profiles from relevant reference populations, are needed to include jackals. This approach might be considered a parallel to the World Health Organisation's "passive surveillance" scheme for monitoring infectious diseases via "regular collection and reporting of surveillance data"(http://www.who.int/immunization/monitoring_surveillance/burden/vpd/surveillance_type/passive/en/). We recommend including "passive surveillance" for jackals and potential jackal hybridization as a standard procedure in wolf genetic monitoring, and further attention to wolf-jackal hybridization and possible simulation analyses to evaluate the extent to which signals of introgression will be visible after back-crossing to a parent species. At little extra expense, such efforts can offer vital data toward understanding jackal range expansion, ecology and evolution, allowing cost-effective contributions to multiple management objectives for large carnivore conservation, including targeted and effective public outreach.

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