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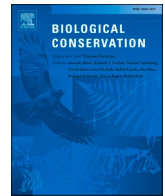


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Perspective

Wolf-dog admixture highlights the need for methodological standards and multidisciplinary cooperation for effective governance of wild x domestic hybrids

Astrid Vik Stronen^{a,*,1}, Jouni Aspi^b, Romolo Caniglia^c, Elena Fabbri^c, Marco Galaverni^d, Raquel Godinho^{e,f}, Laura Kvist^b, Federica Mattucci^c, Carsten Nowak^{g,h}, Alina von Thaden^{g,h}, Jenni Harmoinen^{b,i,**,1}

^a Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia

^b Ecology and Genetics Research Unit, P.O. Box 3000, University of Oulu, 90140 Oulu, Finland

^c Unit for Conservation Genetics (BIO-CGE), Department for the Monitoring and Protection of the Environment and for Biodiversity Conservation, Italian Institute for Environmental Protection and Research, Bologna, via Ca' Fornacetta 9, 40064 Ozzano Emilia, Italy

^d Science Area, WWF Italy, Via Po 25/c, 00198 Rome, Italy

^e CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, Universidade do Porto, Campus de Vairão, 4485-661 Vairão, Portugal

^f Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Porto, Portugal

^g Wildlife Genetics Center, Senckenberg Research Institute and Natural History Museum Frankfurt, Clamecystrasse 12, 63571 Gelnhausen, Germany

^h LOEWE Centre for Translational Biodiversity Genomics (LOEWE-TBG), Senckenberganlage 25, 60325 Frankfurt am Main, Germany

ⁱ Natural Resources Institute Finland, Latokartanonkaari 9, 00790 Helsinki, Finland

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ABSTRACT

Hybridisation between wild and domestic taxa raises complex questions for conservation. Genetic advances offer new methods for hybrid identification, yet social and cultural factors can influence study design, and the interpretation, application, and communication of results. A relevant illustration is hybridisation between domestic dogs (*Canis lupus familiaris*) and wild canids, such as grey wolves (*C. lupus*). For regional European monitoring programs in areas with expanding wolf populations, priorities include shared genetic markers and inclusion of all relevant reference populations to ensure dispersing wolves are identified as such and not classified as wolf-dog hybrids, which may cause harmful management decisions. Beyond technical developments, hybrid research and conservation management can benefit from improved integration of legal and policy perspectives, recognition of phenotypic traits as broadly unreliable for identification, and attention to the drivers of, and responses to, evolution in human-dominated landscapes. Additionally, the proliferation of unsubstantiated reports about hybrids in popular and social media shows that communication based on verified findings of hybridisation is essential. Hybridisation requires more constructive discussion on how to balance potentially competing conservation objectives, and the integration of multidisciplinary perspectives. These encompass the welfare of individual animals and preservation of historical predator-prey relationships. Conservation measures centred on preserving the ecological function of wild canids likely offer the most sustainable prospects but require improved understanding of the extent to which their behavioural ecology might differ from that of hybrids. Accurate genetic identification is required to fill this critical knowledge gap, advance public discourse, and initiate relevant conservation actions.

* Correspondence to: A. V. Stronen, Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1000 Ljubljana, Slovenia.

** Correspondence to: J. Harmoinen, Ecology and Genetics Research Unit, P.O. Box 3000, University of Oulu, 90140 Oulu, Finland.

E-mail addresses: astrid.stronen@gmail.com (A.V. Stronen), jenni.harmoinen@luke.fi (J. Harmoinen).

¹ Except for the first and final author, all others are listed in alphabetical order.

1. Introduction

Hybridisation is a complex evolutionary process which can increase the fitness of wild taxa or reduce their survival and long-term persistence (e.g., McFarlane and Pemberton, 2019; Quilodrán et al., 2020; Hirashiki et al., 2021; Klemme et al., 2021). Hybridisation between wild and domestic taxa is nonetheless an increasing concern worldwide, and recent genome-wide studies have shown complex spatio-temporal hybridisation patterns in several species including Atlantic salmon (*Salmo salar*, Wringe et al., 2018), wild boar (*Sus scrofa*, Iacolina et al., 2018), sheep (*Ovis* sp., Cao et al., 2021), wildcat (*Felis silvestris*, Mattucci et al., 2019), and the dingo (referred to as *Canis dingo*, *C. lupus dingo*, or *C. familiaris*, van Eeden et al., 2019; Crowther et al., 2020). Human-induced (anthropogenic) hybridisation generates difficult questions for conservation and management, including forensic, legal, and policy issues (Trouwborst, 2014; Amorim et al., 2020; Salvatori et al., 2020; Cairns et al., 2021a). Associated human-wildlife conflicts raise ethical concerns about wildlife control (Dubois et al., 2017; van Eeden et al., 2019) and protection of individual animals versus populations (Dubois et al., 2017; Wallach et al., 2018; Callen et al., 2020). The study of natural and anthropogenic hybridisation also involves complex and rapidly advancing topics in evolutionary research (vonHoldt et al., 2018; Senn et al., 2019; Stanton et al., 2019; Taylor and Larson, 2019) that are often demanding for public outreach and science communication.

A prerequisite for navigating the intricate and interdisciplinary questions around anthropogenic hybridisation is the ability to accurately identify wild-domestic hybrid individuals (McFarlane and Pemberton, 2019). Monitoring of genetic introgression typically becomes increasingly difficult with the time since hybridisation, but, at minimum, it is essential to identify the first generation of hybrids (henceforth F1) and the first generation of their backcrosses to wild parental taxa (BC1). There is an urgent need for standardised methods of hybrid assignments that are open for peer-review by independent scientists and

can be implemented across national borders to help design conservation management plans at relevant scales, and that can help advance further research such as study of hybrid behavioural ecology.

A good illustration of the problem with anthropogenic hybridisation is the situation for grey wolves (hereafter wolves, *C. lupus*, Pilot et al., 2018). In recent decades, wolves have recovered parts of their historical range across Europe (Chapron et al., 2014), recolonising even strongly human-altered environments (e.g., Schley et al., 2021). The recent availability of entire genomes of both wolves and domestic dogs (*C. l. familiaris* or *C. familiaris*), which descend from – and hybridise with – wolves, has produced new insights about the timing and diffusion of wolf-dog hybridisation (e.g., Galaverni et al., 2017; Pilot et al., 2018; Fig. 1). Genome-wide analyses have found wolf-dog hybridisation to be recurrent on multiple timescales, albeit with considerable regional variation (see e.g., Smeds et al., 2021; Stenøien et al., 2021), and wolf populations generally appear to have retained their genetic integrity (Pilot et al., 2018). Whereas genomic analyses indicate historical gene flow across the genus *Canis* (Gopalakrishnan et al., 2018), ongoing anthropogenic hybridisation is nonetheless deemed a serious threat to wolves (Hindrikson et al., 2017). There is therefore increasing attention toward the need for consistent management actions to reduce the prevalence of free-ranging dogs and wolf-dog hybrids (Salvatori et al., 2020), particularly for populations in southern and parts of eastern Europe, where feral and free-ranging dogs are widespread (Boitani et al., 2015).

The objective of this perspective article is to discuss technical, analytical, and societal factors of relevance for hybridisation. We reflect on areas where more interdisciplinary collaboration can help promote an evolutionary enlightened (sensu Ashley et al., 2003) and practical direction forward, with focus on conserving the ecosystem role of wild canids such as wolves and other wildlife species in human-dominated landscapes. We first consider possible ecological and evolutionary consequences, and next address different methodological, governance, and communication aspects likely to influence how society defines and



Fig. 1. The problem of wolf-dog hybridisation requires improved genetic monitoring tools, but also prompt conservation management actions. This image shows one of the hybrid individuals identified in the central Apennines, Italy, and captured during the LIFE M.I.R.CO-Lupo project. Photo credit: Marco Antonelli.

responds to anthropogenic hybridisation.

2. Potential ecological and evolutionary consequences of hybridisation

Below we discuss how co-dependent influences on behaviour and ecological function in human-dominated areas, and a possible cycle of reinforcement, or 'hybridisation vortex', might affect wolf evolution. Although speculative, this discussion addresses potential long-term effects on predator-prey relationships. Preserving these relationships will require thoughtful conservation management, centred on how wolves and other large carnivores can continue to maintain their ecological function in increasingly anthropogenic landscapes.

2.1. Ecological function

Increased hybridisation between wolves and domestic dogs could influence ecological function, if the process results in a canid less suited to the role wolves have historically played as social predators of large ungulates, hunting by pursuit. Predators that shift from hunting live prey toward persisting on anthropogenic food sources and scavenging could be affected in multiple ways, including changes in territoriality, life history, and individual traits such as boldness and innovation (reviewed in Parsons et al., 2021). Human provision of food sources can limit the motivation of wolves to hunt, which may reduce historical selection pressures and favour more dog-like canids (Ordiz et al., 2013; Ciucci et al., 2020). Conversely, maintaining selective forces that promote the ecological role of large predators hunting in social groups could be vital in preserving long-term ecological function (Pilot et al., 2018), although more empirical research is needed to address these different hypotheses. At present, little is known about the general ecology and behaviour of wolf-dog hybrids (Lescureux and Linnell, 2014; Pilot et al., 2018), but the ecological role of hybrids is receiving more attention. A first study indicated no significant differences in diet composition compared to that of wolves (Bassi et al., 2017), and similar results have been reported for dingoes affected by domestic dog introgression (Crowther et al., 2020). Yet domestic dogs may not have the same ability to persist in remote areas without access to anthropogenic resources (Cairns et al., 2021a). Dingoes have been found to play a major ecological role by suppressing mesopredators and promoting the conservation of native small mammals (Letnic et al., 2009a, 2009b), and may thus provide key ecosystem services (Colman et al., 2014; van Eeden et al., 2020). As hybridisation is a particular concern for small populations (Muñoz-Fuentes et al., 2010) there is an urgent need for more data on these topics, although such research might inherently present difficult legal and ethical questions.

2.2. Bold behaviour

If dog-like hybrids are allowed to persist and increase in abundance, a central question is whether they may become more dependent on human resources and show bolder behaviour near humans. Potential bold behaviour could also occur in the vicinity of domestic animals, refuse sites, and in localities where humans approach animals to provide them with food. Such situations could result in negative long-term consequences for wolf-human relations (Linnell et al., 2008). Recent findings have suggested that human-directed selection for sociability in dogs may have influenced several linked behavioural genes (vonHoldt et al., 2017), and behavioural or cognitive traits derived from dogs could benefit wolves (Pilot et al., 2021). Yet, whether selection may favour dog vs. wolf genes in shaping hybrid behaviour will need further study in environments with various types of prey, ecological conditions, and degrees of human influence. A recent European Court of Justice decision (C-88/19) confirmed that the strict protection of species listed in the European Union's Habitats Directive Annex IV (a), including wolves, also applies to individuals that stray into human settlements (Curia,

2020). Albeit important, this clarification of protection may amplify negative human attitudes toward free roaming canids (both wild and hybrids), potentially further elevating human-caused mortality, disruption of social structure, and the prevalence of hybridisation, and highlights the need to accurately identify hybrids and investigate their behaviour.

2.3. Hybridisation vortex

Earlier findings suggest that the loss of breeding members may contribute to the break-up of wolf packs (Brainerd et al., 2008), and human-caused mortality and high population turnover can disrupt the social structure of wild canids and augment hybridisation (Wallach et al., 2009; Rutledge et al., 2012; Leonard et al., 2014; Randi et al., 2014; Cairns et al., 2021a). If human-caused mortality increases, this could augment hybridisation and generate further lethal control (van Eeden et al., 2019), thereby hampering the ecological function of wild canids. Such a scenario might produce a negative spiral – a hybridisation vortex – parallel to the extinction vortex described for populations at risk and supported by analyses across taxa (Fagan and Holmes, 2006). Such scenarios have also been described as 'extinction via hybridisation' (Rhymer and Simberloff, 1996). Although the process could result in a canid better-adapted to current human-dominated habitats (Newsome et al., 2017), the disruption of the ecological and evolutionary relationships between wolves and their large ungulate prey would represent a major loss for the ecosystem (Ripple et al., 2014).

3. Defining and responding to hybridisation

Reliable detection of hybrids and admixture patterns are essential, as hybridisation and subsequent introgression of domestic genetic variation into wildlife could have serious implications for long-term ecosystem conservation and human-wildlife relationships. Yet other concerns around hybridisation are not simply technical challenges, but instead require attention to human dimensions and communication. Despite recent technical progress, careful study design and data analyses are needed to produce meaningful results, which must (still) be interpreted with caution, especially for species that are known as long-distance dispersers (e.g., Wabakken et al., 2007). We discuss methodological issues and solutions, and then societal, governance, and communication aspects likely to be important for a balanced and sustainable response to anthropogenic hybridisation.

3.1. Methodological considerations

3.1.1. Standardising and sharing genetic markers and data

National genetic monitoring programs for wildlife have traditionally employed microsatellite genetic markers, where the responsible organisations have accumulated multi-year databases that provide context for spatio-temporal analyses on the status of local populations. However, integration of standard microsatellite genotypes across different laboratories and countries requires calibration, which is costly and time consuming. This extra step can impede timely conservation management, especially for species that disperse over long distances and national boundaries, although this limitation can be alleviated by the exchange of reference samples among laboratories, to help identify dispersing individuals and their origin. Recent work on high-throughput sequencing of microsatellite markers (De Barba et al., 2017) and single nucleotide polymorphism (SNP) panels (e.g., Kraus et al., 2015) suitable for non-invasively collected samples have provided important advancements (von Thaden et al., 2017). These new markers do not require calibration among laboratories, although it is important to mention that not all markers developed for specific populations will be suitable (i.e., polymorphic) in other populations (Giangregorio et al., 2019).

The detection of wolf-dog hybridisation has nevertheless continued

to be a problem for monitoring efforts. However, in a recent study, [Harmoinen et al. \(2021\)](#) designed a panel of 96 SNP loci selected for their power to distinguish between dogs and wolves across Europe, suitable for DNA from non-invasively collected samples that are the typical source of data for monitoring programs. The panel of diagnostic SNPs provides standardisation in analyses and reporting, which will help resolve cases of putative hybridisation and determine the distribution and prevalence of hybridisation across the continent.

3.1.2. Inclusion of relevant reference populations and documentation of analytical parameters

Highly mobile species often cross jurisdictional and management borders, a situation which has important implications for regional and national monitoring programs. For genetic analyses of wolves and other species affected by anthropogenic hybridisation (e.g., wild boar; [Iacolina et al., 2018](#); wildcat; [Tiesmeyer et al., 2020](#)), it is thus vital to include reference samples from all putative source populations. This will help ensure that dispersers are correctly identified, and that they and their descendants are not erroneously classified as hybrids ([Harmoinen et al., 2021](#)). Such errors can occur because wild canids and dogs have very similar genomic backgrounds that mostly differ in the frequency of the genetic variants (alleles). In principle, the new European diagnostic SNP panel for wolf-dog hybridisation eliminates the need for regional reference samples in hybrid identification, because the panel was designed and successfully tested across European populations ([Harmoinen et al., 2021](#)). However, monitoring programs in many regions still rely on traditional microsatellite markers. In addition to being well-established in local labs, these traditional markers provide a valued temporal connection in areas where older DNA sources have been exhausted and legacy data exist only as microsatellite profiles. Here, reference samples from local wolves and dogs, and, crucially, neighbouring wolf populations with different allelic frequencies, should be included to ensure that dispersing wolves are not accidentally misclassified as wolf-dog hybrids.

Population structure and geographic variation have been suggested as possible confounding factors in analyses of dingo-dog hybridisation (reviewed in [van Eeden et al., 2019](#) and [Crowther et al., 2020](#)). Accurate detection of population structure and dispersers is also increasingly relevant for wolves, which are recolonising their historical ranges across Europe where their populations continue to reconnect ([Ražen et al., 2016](#); [Hulva et al., 2018](#); [Szewczyk et al., 2019](#)). For example, wolves from the long-isolated and genetically divergent Italian population have recently dispersed into nearby countries including Slovenia, Switzerland, and Germany ([Bartol et al., 2018](#); [Dufresnes et al., 2019](#); [Harmoinen et al., 2021](#)). Reference samples from Italy and other distinct wolf populations are thus needed where dispersers are found, or may appear, in the future, and will aid monitoring programs in categorising local and dispersing wolves. Any unresolved profiles can then be genotyped on the diagnostic SNP panel to investigate possible hybrid ancestry. Although genetic analysis costs for hybrid detection may be a concern (e.g., [Dziech, 2021](#)), genotyping of samples on the reduced panel is cost-effective (around 8 €/sample for high-quality tissue samples and 24 € considering three replicates per sample for non-invasively collected samples, not considering working costs, taxes, initial costs for machine acquisition and maintenance ([von Thaden et al., 2017, 2020](#)).

Moreover, various other steps can limit bias on inferred admixture proportions in population analyses, including equalised sample sizes ([Toyama et al., 2020](#)) and estimators accounting for uneven sample sizes ([Puechmaille, 2016](#)). Reference population allele frequencies should also be representative and updated where needed, especially for expanding populations ([Caniglia et al., 2020](#)). Natural long-distance dispersal is considered essential for the viability of small, isolated populations, but many long-distance dispersers die before reproducing in their new home range ([Kojola et al., 2006](#); [Bartón et al., 2019](#)). Correct identification of dispersers is thus of great practical importance for conservation. Conversely, studies that include neither relevant reference

populations nor transparent reporting on reference populations and methods used may create confusion about scientific results. Such cases could reduce public trust in science and management, and limit constructive scientific and public discourse on how to address long-term consequences of human-induced hybridisation.

3.2. Governance and societal issues

3.2.1. Hybrid definition and management: Integration of scientific, legal, and policy perspectives

The definition of hybrid is relevant for legal and conservation management ([van Eeden et al., 2019](#); [Amorim et al., 2020](#); [Dziech, 2021](#)) and requires integration of genetic and legal information. Importantly, this definition may differ from the long temporal perspective often considered in evolutionary research (e.g., [Galaverni et al., 2017](#); [Schweizer et al., 2018](#)) and must focus on achieving effective conservation outcomes ([Lorenzini et al., 2014](#); [Senn et al., 2019](#); [Salvatori et al., 2020](#); [Cairns et al., 2021a](#)). For example, backcrosses into wolf populations may in some instances be too difficult to detect and too abundant to allow their effective removal ([Wayne and Shaffer, 2016](#); [Salvatori et al., 2019](#)). The ability to detect hybridisation at increasing levels of resolution amplifies the normative challenges surrounding policies for conservation management, such as the level at which individuals – including those with traces of historical introgression – should be considered for protection under existing wildlife and habitat legislation ([Wayne and Shaffer, 2016](#); [Galaverni et al., 2017](#); [Senn et al., 2019](#)). Findings from Australia also suggested that use of the term ‘wild dog’ may have confounded members of the public about management actions that also affect dingoes and the ecosystem services they provide as apex predators ([van Eeden et al., 2020](#)), underscoring the importance of language and communication in wildlife management. Although hybrids are not typically addressed in international conservation legislation, wild-living wolf-dog hybrids appear to be protected by legal frameworks such as the European Union's Habitat Directive and the Bern Convention on European Wildlife and Natural Habitats ([Trouwborst, 2014](#)). [Trouwborst \(2014\)](#) stated that although preventive measures to address hybridisation seem to be permitted under these frameworks and may even be required, it is not fully clear whether hybrids that present a potential threat to wolf populations could be managed in an effective and consistent manner. It would seem a priority to ensure good integration of legal and genetic considerations, and to assure that hybrid regulations and guidelines reflect categories that genetic analyses can realistically achieve. [Trouwborst \(2014\)](#) noted that according to CITES guidelines, the protection of wolves also covers wolf-dog hybrids with wolf ancestry within the last four generations (backcrosses to dogs), although this level of ancestry can be difficult to discern even with high-density genome-wide profiles ([Galaverni et al., 2017](#); [Pilot et al., 2018](#)). Moreover, although second and third backcrosses to wolves (BC2, BC3) were in most instances detectable with the Europe-wide 96-SNP panel – and conservation management is generally concerned with backcrosses to wolves or other wild species, not to their domestic counterparts – reliable detection of all individuals with wolf ancestry within the last four generations is not currently feasible ([Harmoinen et al., 2021](#)). If we cannot clearly discern the number of hybrid generations identified in legal and management guidelines, there is a mismatch in law and practical feasibility that may confound and limit conservation decisions. Focusing hybrid management guidelines on the recent-generations such as F1 and BC1_{wolf} that have higher potential to spread dog genetic variants than back-crossed individuals with more limited dog ancestry (BC2_{wolf} and beyond), could be a practical objective at the current state of the art ([Caniglia et al., 2020](#)). If advances in routine genetic methods can improve resolution in the future, guidelines could later be updated to allow implementation of new knowledge.

3.2.2. Phenotypic traits in hybrids

Findings from several taxa suggest that possible individual

phenotypic and genetic indicators of hybridisation often differ (Iacolina et al., 2018; Kusak et al., 2018; Senn et al., 2019; Cairns et al., 2021b). Dingoes, for instance, showed a wide variation in coat colour and it was not possible to evaluate hybrid ancestry based on this trait, where certain unusual patterns also appear to be ancestral variants (Cairns et al., 2021b). In wolves, some individual phenotypic differences may simply represent natural variation in morphology, such as the presence of a black stripe on the foreleg (Pulliainen, 1965). In contrast, other traits have been associated with hybridisation or ancient introgression, exemplified by black coat colour (Anderson et al., 2009; Galaverni et al., 2017). Importantly, genome-wide analyses of Italian canids show that black coat colour can occur in individuals assigned as hybrids and in individuals fully assigned to wolves (Galaverni et al., 2017). Although often unreliable on their own as indicators of hybridisation (Lorenzini et al., 2014), phenotypic traits might help identify priority areas for more detailed investigation of hybridisation, e.g., by camera-trapping. Areas where individuals with atypical phenotypic traits are observed can then be prioritised for non-invasive genetic monitoring. Another important outcome of such research is to understand whether certain phenotypic traits are *not* useful hybrid indicators for conservation management, but unreliable measures that risk producing harmful decisions (Galaverni et al., 2017; Cairns et al., 2021b). Moreover, the persistence of traits such as black coat colour in canids with genome-wide profiles fully assigned to wolves can offer insights about environmental selection and ecological function following introgression. An allele at the CBD103 gene in wolves, derived from historical wolf-dog hybridisation, is associated with black coat colour and immune function (Schweizer et al., 2018), thus illustrating a situation where hybridisation and subsequent introgression may have provided fitness benefits.

3.2.3. Identify drivers of, and responses to, evolution in anthropogenic landscapes

Hybridisation can represent both fitness gains and costs for wild species (e.g., vonHoldt et al., 2018; McFarlane and Pemberton, 2019; Quilodrán et al., 2020; Dziech, 2021) and these effects may occur simultaneously, suggesting complex effects that are still poorly understood (Klemme et al., 2021). Although hybridisation is typically considered to have detrimental effects on the ecological function of wolves, introgression from dogs or other canids better adapted to human-dominated landscapes might, at times, increase survival and permit the use of additional habitats (Coulson et al., 2011; Godinho et al., 2011; Lescureux and Linnell, 2014). Notably, Schweizer et al. (2016) reported signs of selection in wolf ecotypes linked to diet and metabolism, and several dog breeds appear to have adaptations toward a diet rich in starch (Axelsson et al., 2013; Arendt et al., 2016). Such adaptations, if transferred to wolves, might promote wolf survival in habitats where animals depend increasingly on human-provided resources, but could thereby also augment human-wildlife conflicts. Another question concerns the extent to which ecological differences between wolves and wolf-dog hybrids might be observed in areas with medium-sized or large ungulates, which are typical prey species for wolves in many regions. Here, selection may favour larger individuals (MacNulty et al., 2009) and more wolf-like phenotypes. Similarly, few wild-living domestic dogs were reported in the arid interior of Australia, where environmental conditions may favour dingoes (Cairns et al., 2021a). Natural selection based on historical ecological function could help restore wild phenotypes and genotypes (Pilot et al., 2018) and such a process might co-occur with selection on the immune system. For example, in response to canine distemper virus (Schweizer et al., 2018) or resistance against infectious disease associated with human impacts, such as canine parvovirus, which was first detected in dogs some decades ago and has since spread to other carnivore species (Allison et al., 2013). Carefully designed studies and targeted genetic markers could help clarify how the genomes of wild canids and hybrids respond to such diverse evolutionary forces. The amount of habitat needed to ensure

long-term sustainable populations of large carnivores may require them to occupy areas used by humans to various extent, and thereby adapt to some level of human presence throughout portions of their range (Carter and Linnell, 2016; López-Bao et al., 2017). Further investigations are needed across various environments, including southern and south-eastern Europe where high numbers of free-ranging dogs share their environments with wolves (Galaverni et al., 2017; Salvatori et al., 2020) and where dog-jackal hybridisation has also been confirmed (Galov et al., 2015). Parallel concerns exist in other areas where wild canids hybridise with dogs, including Africa (Mallil et al., 2020), and Australia (Claridge et al., 2014).

3.2.4. Promote communication of peer-reviewed scientific findings

The publication of genetic findings is frequently done in specialised journals behind paywalls, and articles include technical language that is often poorly accessible to non-scientists (or non-geneticists). This can make peer-reviewed scientific results difficult to access on several levels. In contrast, claims and reports of hybrids are at times offered high-profile publication in the media, even if based on unverified sources rather than results that have been subject to (or have been submitted for) peer-review by independent scientists. Such cases are deeply problematic, given the potential for management actions based on erroneous results, and because dissemination of erroneous results can create persistent public perceptions that can be difficult to reverse (Pivetti et al., 2020). For all laboratories involved in hybrid analysis, we therefore advocate for the use of standardised scientific techniques for investigating and reporting new cases, and peer-review publication of results where methodological details – including choices of reference populations, and discussion of possible limiting factors (e.g., sample quality and sample size) – are included in the research description. Where financial resources are available, open-access publication is recommended to promote scientific outreach, although the long processing time for peer-reviewed scientific literature remains a challenge for scientists, conservation managers, and dissemination of scientific results to the public and various interest groups. Yet, where possible, we encourage journalists writing popular articles about research on anthropogenic hybridisation to provide links to the original peer-reviewed scientific studies, to pre-print results awaiting such appraisal (see e.g., <https://www.biorxiv.org/>), or a note stating that the reported findings have not yet been subject to peer-review. Similarly, we encourage readers to consider such contextual information – or the lack thereof – before citing or (re)publishing reports of hybridisation, for example on social media. Social media coverage about carnivores is often focused on sensationalistic reports that can increase sharing but might also increase fear and reduce support for conservation, underlining the need to disseminate more accurate and objective information (Nanni et al., 2020).

3.2.5. Encourage constructive discussion while targeting changes in human behaviour

The risks that hybrids and feral domestic animals may present to the genetic makeup, ecological function, and evolution of wild taxa, and the extent to which humans should mitigate human-induced hybridisation have been subject to extensive discussions (e.g., Wallach et al., 2018; Callen et al., 2020). These have highlighted ethical questions around possible sterilisation, capture, or killing of individuals (animal control) to advance conservation goals and offered insights on the – often conflicting – societal aims in setting conservation objectives, and how we can start changing *human* behaviour to promote sustainable solutions (Dubois et al., 2017; Donfrancesco et al., 2019; Cairns et al., 2021a). For hybridisation, tackling the underlying problem of free-ranging dogs is fundamental to achieving meaningful long-term change (Lorenzini et al., 2014; Donfrancesco et al., 2019). However, addressing this critical step has frequently been avoided by conservation managers because of the – frequently complex – legal framework regarding domestic animals, and the risk of invoking strong ethical and emotional questions

(Donfrancesco et al., 2019). Efforts to control animals should be done on a case-by-case basis and only after consideration of all available options (Dubois et al., 2017), also for the removal of hybrids or their reproductive potential (Donfrancesco et al., 2019; Caniglia et al., 2020). Crucially, it is recognised that *not* taking action on hybrids and abundant feral animals also entails important risks (Callen et al., 2020; Salvatori et al., 2020). Typically, there is no uniform answer but a need to tailor solutions to local circumstances, and acceptance for hybrid removal may depend on the specific conservation context, local laws, and human attitudes (Lescureux and Linnell, 2014; Fitzpatrick et al., 2015; Wayne and Shaffer, 2016; Donfrancesco et al., 2019; Caniglia et al., 2020). Public sentiment toward hybrids may influence how policies are implemented, and managers need to consider the practical realities of public response to, and enforcement of, species-based or other ecological protections (Fitzpatrick et al., 2015; van Eeden et al., 2019). A reliable and commonly agreed-upon protocol for genetic analyses is the basis for a scientific assessment (Donfrancesco et al., 2019), and we contend that this step is critical for the subsequent decision processes that may involve scientists, conservation managers, NGOs, and other public interest groups. However, there are currently no commonly accepted best practices for how to manage hybrids and the surrounding debate is often highly emotional (Lescureux and Linnell, 2014; Donfrancesco et al., 2019). This is also why the spread of any incorrect claims about hybridisation can be so detrimental for conservation efforts, and why accurate scientific data, including standardised genetic identification, is needed for meaningful discussion, prioritisation of resources, and broadly supported conservation actions.

3.2.6. Integration of multidisciplinary perspectives

Long-term efforts to mitigate hybridisation need to involve collaboration across disciplines, to address technical, ethical, legal, ecological, and evolutionary aspects and adapt these to local circumstances. Important priorities include (a) conserving the ecological function of wolves and other wild canids, and (b) limiting the proliferation of free-ranging dogs. Another key priority is to (c) better understand hybrid behaviour, especially whether hybrid ecological function and behaviour toward humans and livestock may differ from that of wolves. This research will need to be achieved while balancing many concerns, including how to best spend scarce conservation resources while obtaining essential data to inform hybrid management. In certain areas, returning hybrids that are collared and sterilised (i.e., retaining hormonal functions and social behaviour) to the wild might offer important insights into their interactions with humans, domestic animals, and wild prey species, while assisting conservation managers in protecting vulnerable populations of wolves and other wild canids. Results from a recent project in Italy suggested that sterilisation and release of hybrids did not appear to affect their ability to re-join their original pack (LIFE M.I.R.CO-Lupo, 2020).

In the red wolf (*C. rufus*) recovery program in the US, sterilised hybrids between coyotes (*C. latrans*) and red wolves have been released and managed as 'placeholder packs', thereby acting as a buffer around red wolf packs to discourage hybridisation (Gese and Terletzky, 2015). Although this intervention concerns hybridisation between wild species, the conservation management context would be similar for wolf-dog hybrids. Such invasive procedures toward animals may encounter considerable objections. On the other hand, the current management response in several European countries is lethal removal of wolf-dog hybrids to prevent further introgression, although certain jurisdictions have contemplated and/or used sterilisation and release (Salvatori et al., 2020 Table 2). Mitigation of hybridisation will need to consider national and regional legislation, wolf population size and conservation status, economic costs, and social attitudes (Donfrancesco et al., 2019; van Eeden et al., 2019; Caniglia et al., 2020). Local environmental factors are also essential for understanding *why* hybridisation is occurring in the first place. The focus must be on changing human activities that lead to animal control (Dubois et al., 2017) and avoiding makeshift solutions

whereby we kill or manipulate animals without addressing the underlying problems. As with the complex questions surrounding genetic management of threatened species (e.g., Liddell et al., 2021), there is probably no ideal and universal solution to anthropogenic hybridisation. The best (or least undesirable) outcome with broad support is therefore likely to be achieved by including local environmental knowledge and perspectives from diverse disciplines.

3.3. Conclusion

Whereas hybridisation in wild canids may represent an extreme showcase for conservation management in terms of their high public profile and broad geographic distribution, they offer a good example given that future environmental changes and species range shifts are expected to augment hybridisation (Scheffers et al., 2016). Mitigation of hybridisation caused or influenced by human activities may therefore increasingly require international collaboration across disciplines. Humans must impose a management boundary on what is an evolutionary gradient of wild-domestic hybridisation, and decisions may vary depending on perceived cost-benefits for conservation and the feasibility of removing hybrids or their reproductive potential without negative consequences for local ecosystems, while ensuring social acceptance (Allendorf et al., 2001; Lescureux and Linnell, 2014; Fitzpatrick et al., 2015; Wayne and Shaffer, 2016). Conservation strategies are more likely to succeed where they focus on ecological function over genetic purity (Claridge et al., 2014; Fitzpatrick et al., 2015; Quilodr n et al., 2020). Actions that help maintain natural social structure in wild canids, and their historical ecological role as top predators, could therefore help wild populations resist or limit introgression (Rutledge et al., 2012; Galaverni et al., 2017; Cairns et al., 2021a).

Although genetic methods and the resolution of individual genomic profiles have seen rapid advances, improved resolution of hybrid profiles in the future will only shift, not eliminate, the conservation decision processes. As a starting point, we propose the use of standardised, shared, and transparent genetic methods, and further international collaboration across disciplines. These important steps will help ensure that genetic analyses of wide-ranging species, intraspecific genetic lineages and populations can distinguish (I) recent wild-domestic hybrids from (II) introgressed individuals that carry limited amounts of historical domestic ancestry, and (III) immigrant individuals and their offspring with admixed ancestry from divergent wild populations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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