REVIEW

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Genetic tools in the management of invasive mammals: recent trends and future perspectives

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ABSTRACT

1. Invasive non-native species are now considered to be one of the greatest threats to biodiversity worldwide. Therefore, efficient and cost-effective management of species invasions requires robust knowledge of their demography, ecology and impacts, and genetic-based techniques are becoming more widely adopted in acquiring such knowledge.
2. We focus on the use of genetic tools in the applied management of mammalian invasions globally, as well as on their inherent advantages and disadvantages. We cover tools that are used in: (1) detecting and monitoring mammalian invaders; (2) identifying origins and invasive pathways; (3) assessing and quantifying the negative impacts of invaders; and 4) population management and potential eradication of invasive mammals.
3. We highlight changes in sequencing technologies, including how the use of techniques such as Sanger sequencing and microsatellite genotyping, for monitoring and tracing invasive pathways respectively, are now giving way to the use of high-throughput sequencing methods. These include the emergence of environmental DNA (eDNA) metabarcoding for the early detection of invasive mammals, and single nucleotide polymorphisms or whole genomes to trace the sources of invasive populations. We are now moving towards trials of genome-editing techniques and gene drives to control or eradicate invasive rodents.
4. Genetic tools can provide vital information that may not be accessible with non-genetic methods, for the implementation of conservation policies (e.g. early detection using systematic eDNA surveillance, the identification of novel pathogens). However, the lack of clear communication of novel genetic

methods and results (including transparency and reproducibility) to relevant stakeholders can be prohibitive in translating these findings to appropriate management actions. Geneticists should engage early with stakeholders to co-design experiments in relation to management goals for invasive mammals.

INTRODUCTION

The introduction of species outside of their native range has escalated due to increased movement of people (Hulme 2009), and invasive non-native species are now considered to be one of the greatest threats to biodiversity worldwide (Bellard et al. 2016). Invasive species disrupt ecosystem services and lead to the introduction of novel diseases, ultimately impacting native wildlife, domesticated species and humans. In response to invasive non-native species, plans and policies are put into place to prevent their entry and reduce or eliminate their impact. Such measures are extremely costly in economic terms. For example, the European Union alone spends approximately €12 billion annually on the control and management of invasive non-native species and on mitigating their adverse impacts.

Efficient and cost-effective management of species invasions requires robust knowledge of their demography, ecology and impacts, and genetic-based techniques are becoming more widely adopted in acquiring such knowledge (Searle 2008, Darling et al. 2017). The genetic tools that we have to study these processes have developed dramatically over time, particularly over the last decade, and have become more affordable, efficient and available for small to medium-scale laboratories, providing new opportunities to study multiple aspects of invasions (Lee 2002). However, genetic tools are variable in methodology, design, price, complexity and the resolution of results. The scope of this review is to provide an accessible synopsis of the genetic techniques for the non-geneticist in order to enable stakeholders, such as state and conservation managers, policy-makers, field biologists and early-career researchers, to work collaboratively with geneticists to address questions related to the prevention and management of mammalian

invasions. We provide a brief overview of effective genetic techniques that are available for four management stages of a mammalian invasion: (1) detection and monitoring of non-native invasive mammals; (2) identifying invaders' origins and invasive pathways; (3) assessing and quantifying the negative impacts of invaders; and (4) population management and the potential eradication of invasive mammals.

DETECTION AND MONITORING

The early and rapid detection of newly introduced mammals is vital to prevent further spread that could subsequently result in a more costly eradication programme. Given the elusive nature of many mammalian species, detection and monitoring often requires indirect observations such as searching for latrines, faeces, hair, or tracks, or direct observations such as live-trapping or camera-trapping surveys (Sales et al. 2020a). These can require differing levels of expertise and resources, but despite high levels of expertise it is not always possible to assign indirect field signs correctly to a species without further confirmation via DNA analysis (Harrington et al. 2010).

Indirect field signs such as hair and faeces can be subjected to genetic non-invasive sampling (gNIS; Ferreira et al. 2018) to confirm species identification. gNIS has the benefit of collecting genetic information without handling animals, which may cause stress. Routine **PCR** (terms in **bold** are defined in the Glossary) methodologies can be applied as diagnostic tools for identifying species from ambiguous field signs such as hair or faeces. The required species-specific primers are already available to identify, for example, Iberian carnivores from faecal DNA, including invasive mammals such as the genet *Genetta genetta*, Egyptian Mongoose *Herpestes ichneumon* and the North American mink *Neovison vison* (Fernandes et al. 2008).

DNA obtained from gNIS may have degraded into smaller fragments due to prolonged exposure to environmental factors such as temperature fluctuations and ultraviolet light. Therefore, PCR detection or identification methods can be used to target short genetic regions (<1000 base pairs). **qPCR** is marginally more complex but has some benefits over traditional PCR for the identification of species from gNIS. qPCR can amplify shorter DNA regions (<100 base pairs) and is more sensitive to smaller starting amounts of DNA. It has been used to detect invasive mammals such as the greater white-toothed shrew *Crocidura russula* and grey squirrel *Sciurus carolinensis* from native pine marten *Martes martes* faeces (O'Meara et al. 2014). qPCR has the additional benefit of providing quality control to select optimal DNA samples for further analysis, such as sequencing and genotyping, thus allowing researchers to avoid wasting resources on poor-quality samples that are unlikely to yield results. Kierepka et al. (2016) used qPCR to screen feral pig *Sus scrofa* faecal-derived DNA prior to genotyping, to generate a robust capture-mark-recapture protocol in order to facilitate accurate estimates of abundance.

Physical samples such as faeces or hair are not always required for species detection. Organisms leave genetic material behind in the surrounding environment (e.g. in water bodies and soil) via excretions and secretions (Harper et al. 2019); this is referred to as **environmental DNA** (**eDNA**). Single-species detection from eDNA is possible using PCR, qPCR or droplet digital PCR (**ddPCR**). Research on invasive wild boar *Sus scrofa* in North America (Williams et al. 2018) has demonstrated the efficiency of a species-specific qPCR approach on samples from various water bodies in detecting the species, but has also highlighted that a minimum number of

individuals is required for detection. This clearly has implications for providing early detection of invasive species, which may initially be present in low numbers.

Single-species detection methods are relatively cheap, fast and robust, but require prior knowledge of the target species to design appropriate detection methods (e.g. O'Meara et al. 2014). If prior knowledge of the target species is unavailable, species can be identified from gNIS using **Sanger sequencing** to generate a **DNA barcode** (Hebert et al. 2003). In the Scottish Highlands, UK, experienced field surveyors used field signs such as faeces to identify 57 sites out of 147 as positive for the presence of invasive North American mink. Subsequent DNA sequencing of a standardised portion of **mitochondrial DNA** (mtDNA) showed that mink faeces were misidentified at all sites, and that they were commonly confused with native carnivore faeces (Harrington et al. 2010). Had management or eradication programmes been designed based on indirect observation, the result would have been a costly, time-consuming, and unnecessary eradication programme.

Next-generation sequencing can facilitate the simultaneous identification of entire communities (i.e. multiple species). **DNA metabarcoding** from environmental samples has the potential to be used as an early warning system for the detection of invasive non-native species, can be used for continuous monitoring programmes, and has been extensively applied for tracking biological invasions in aquatic ecosystems (Deiner et al. 2017). eDNA metabarcoding studies targeting mammalian communities were relatively rare in comparison to other taxonomic groups (Sales et al. 2020a), but this may change now that there are established metabarcoding

protocols for detecting and monitoring whole communities using vertebrate (Harper et al. 2019) or mammal-specific primer sets (Ushio et al. 2017, Sales et al. 2020a,b).

eDNA metabarcoding is an emerging technique for invasive mammal detection and monitoring, and there are important considerations for its use. For example, mammals with larger home ranges (e.g. invasive carnivores) have lower probabilities of detection than more abundant group-living mammals (Harper et al. 2019, Sales et al. 2020a). Due to the high sensitivity of metabarcoding, contamination is a concern (Sales et al. 2020a). It is therefore essential that specialised eDNA lab facilities (akin to working with ancient DNA) are used (Zinger et al. 2019). Another consideration is the existence of gaps in customised or online **reference databases** for identifying sequences to the appropriate species level in under-studied geographic regions (Sales et al. 2020b). However, with a carefully planned experimental design and the appropriate field and lab controls (Zinger et al. 2019), eDNA metabarcoding has the potential to be applied for early detection and ongoing surveillance of invasive mammals (Harper et al. 2019, Sales et al. 2020a).

ORIGINS AND INVASIVE PATHWAYS

Identifying the origins of invasions is a critical management strategy in controlling the spread of invasive species (Hulme 2009). When there is an absence of direct evidence indicating the routes of invasion (such as records from interception at ports), indirect methods such as the analyses of genetic data from invasive populations and putative sources becomes vital (Searle 2008, Gargan et al. 2016).

Studies initially relied upon sequencing mtDNA to track the transport of invasive species, because of the availability of universal primers for mammals (for mtDNA genes such as cytochrome *b* and the control region) and available sequences (from the native ranges for comparisons) in reference databases such as Genbank. For invasive mammals with a global distribution, such as house mice *Mus* spp. and rats *Rattus* spp., phylogenetic analyses of mtDNA have proven extremely useful in tracing multiple introductions to islands and different continents over recent millennia and centuries (Jones et al. 2013). The use of this type of mtDNA marker can be limited over the spatial and temporal scales required for tracking more recent invasions. Although mtDNA accumulates substitutions more rapidly than nuclear DNA, mtDNA markers are generally useful for investigating intraspecific relationships over tens to hundreds of thousands of years. Unless mtDNA variation is sufficiently high in the native range, it is not ideal for tracing most mammalian invasions (Gray et al. 2014) and may reveal the continent of origin as opposed to the country (Gargan et al. 2016). Raccoons *Procyon lotor* show limited mtDNA diversity within their invasive range in Europe, which originally led researchers to believe that they were descended from a small number of founding individuals (Frantz et al. 2013). However, the analysis of more rapidly evolving **microsatellites** led to the conclusion that there were potentially up to four separate sources for the raccoon's current distribution within its invasive range (Fischer et al. 2015). In the same vein, studies of house mice have revealed the importance of using a multiple marker approach (such as microsatellites) when inferring the origins of island populations, as many display admixed origins (e.g. Gray et al. 2014).

Given that we are now firmly entrenched within the genomics era of molecular ecology research, it is unsurprising that studies inferring the origins of invasive mammals are now switching to **Single Nucleotide Polymorphism (SNP)** marker-based approaches. Compared to microsatellite markers, SNPs usually span across a greater proportion of the genome, can determine population demographics to a finer scale, and do not require calibration between laboratories (Iacolina et al. 2016). Incorporating SNPs in a study previously required a huge investment of time and resources, usually applied only to economically important species (e.g. cattle, dogs, rodents, pigs). The *de novo* discovery of SNPs in non-model organisms is now achievable and affordable through **reduced representation sequencing** techniques (such as Restriction Site Associated sequencing or RAD-seq; Baird et al. 2008).

Puckett et al. (2016) used ~32000 SNPs (derived from ddRAD genotyping) to examine the population genomic structure of brown rats *Rattus norvegicus* throughout their worldwide geographic range. Brown rats were generally grouped into Asian and non-Asian groups, but fine-scale structuring was identified within regions, reflecting more recent invasion pathways. For example, mtDNA data revealed a European origin for contemporary New Zealand and western USA populations, but SNP data revealed ancestry from admixed Asian and non-Asian genomic clusters. In tracking the invasion of raccoon dogs *Nyctereutes procyonoides* in Denmark, Nørgaard et al. (2017) utilised genotyping-by-sequencing to identify over 4000 SNPs to trace their origins to Danish fur farms and reveal subsequent admixture with neighbouring German populations. Unlike with microsatellites, newly generated data on finer spatial scales can be compared with a global dataset of SNPs if a reference genome is available. For example, this allowed Combs et al.

(2018) to determine that the most likely origins of the New York, USA, population of brown rats were France and the British Isles.

NEGATIVE IMPACTS

Diet and competition

Invasive mammals may affect local flora and fauna through predation or ingestion (e.g. feral cats take terrestrial vertebrates; Doherty et al. 2017), or via increased competition (e.g. invasive American mink compete with native European mammalian carnivores Sidorovich et al. 2010). Mammals are notoriously elusive, making their diet difficult to document through direct observations, so that morphological diagnostics of prey remains from stomach contents and faeces are a popular method (Brzeziński et al. 2018). This methodology produces biased results due to variable degradation rates between species and body parts (i.e. soft body parts degrade faster than hard body parts), and residual body fragments that are found are difficult to identify to species level (Deagle et al. 2009). Stable isotope analysis shows promise, but has difficulties identifying prey species when isotopic signatures naturally vary between geographic locations (Chibowski et al. 2019).

Genetic tools require DNA to be extracted from faeces or gut contents using appropriate extraction kits capable of removing inhibitors associated with the digestive tract. Species-specific primers and PCR are straight-forward and cost-effective methods to measure predation rates of a single species of interest (Waraniak et al. 2018). However, invasive mammals can have a variable diet between native and introduced ranges (Ballari & Barrios-García 2014), making it difficult to predict what they will consume in their introduced range. DNA

metabarcoding is a promising method: it allows the identification of multiple dietary components of hundreds of individuals, and increases prey detection from 2% using morphological diagnostics to 70% using metabarcoding (Pompanon et al. 2012, Egeter et al. 2015a).

Not only can DNA metabarcoding accurately document an animal's impact on local resources, but it can also reduce ambiguity. Previous assessments of the impact of invasive rats *Rattus rattus* on endemic amphibians in New Zealand relied on abundance estimates of native frog species in comparison to arrival patterns of the invasive rat (Egeter et al. 2015b). Inconsistencies between observers caused doubt, but DNA metabarcoding clarified the rat's consumption of New Zealand's native frog species and its contribution to the population declines (Egeter et al. 2019). The sensitivity achieved from next-generation sequencing methods allows multiple prey items to be identified to the species level and generates a comprehensive account of multiple animals' resource use and overlap. Telfair's skink *Leiolopisma telfairii* was introduced to Ile aux Aigrettes, Mauritius, Indian Ocean, for conservation purposes, but unexpectedly met potential threats from the invasive Asian musk shrew *Suncus murinus*. Species-specific primers showed the two species did not predate one another (once adulthood was attained), but DNA metabarcoding identified significant prey overlap and resulted in the suggestion that controlling shrew populations would benefit the skink population (Brown et al. 2014).

Metabarcoding projects for dietary studies require some important considerations before they are started (also relevant to eDNA metabarcoding studies, see above). The first is targeting the appropriate genetic region for the target taxa in the diet,

such as vertebrates, invertebrates or plants (Kress et al. 2015). To know the full diet of an omnivorous invader (e.g. wild boar), multiple regions are required for the full taxonomic range within their diet (De Barba et al. 2014). Alternatively, highly degenerative (non-specific) primers can be used to capture a wider range of prey taxa, but this can result in over-representation of higher-quality host DNA (Zeale et al. 2011). The broader the primers' taxonomic range, the more likely the chance of amplifying non-target taxa and reducing the amount of information on a species' diet. Blocking primers can mitigate host DNA amplification, but require more time to design and test, as they may also block the amplification of some target prey taxa (Su et al. 2018). The high sensitivity of PCR and high-throughput sequencing can also result in the detection of taxa through secondary predation (i.e. detecting the food of the food; Sheppard et al. 2005). Another difficulty is the inference of biomass or the number of prey individuals from molecular diet analysis (Deagle et al. 2019). Estimates of prey proportion are biased towards harder-bodied organisms due to differential degradation rates. There are multiple ways to determine the importance of certain taxa within a predator's diet, such as frequency of occurrence or relative abundance (reviewed by Deagle et al. 2019).

Disease

The introduction of mammals into novel environments comes with the risk of co-introducing pathogens or parasites that local fauna have not yet developed resistance to (Paziewska et al. 2011). Mammalian invasions in Europe are likely to have been responsible for the transport of pathogens responsible for salmonellosis, toxoplasmosis and leptospirosis (Hulme 2014), and for the dissemination of the plague across continents via rodent introductions (Gage & Kosoy 2005). Genetic

304 tools are becoming pivotal in disease management in wildlife (DeCandia et al. 2018):
305 PCR is currently used to verify morphological identification of pathogens and
306 parasites (Bagrade et al. 2016), and genetic tools can be used as detection methods
307 when there are difficulties in recreating optimal cell growing conditions to test for
308 prevalence levels (Heuser et al. 2017).
309
310 Different pathogen genotypes or strains can have different infection capabilities
311 (Nally et al. 2016). Sequencing actin genes of pathogenic *Cryptosporidium* revealed
312 that invasive raccoons harboured genotypes capable of infecting humans
313 (Leśniańska et al. 2016). To gain a higher resolution of bacterial population structure
314 and evolution, and to help understand the distribution of pathogenic species and
315 genotypes in novel areas invaded by mammalian hosts, multiple loci or genes can be
316 sequenced in multi-locus sequence typing (Margos et al. 2008). This method was
317 applied to *Borrelia* spp., an important pathogen in zoonotic ecology due to its
318 responsibility for Lyme disease. Sanger sequencing of the housekeeping gene (*clpA*)
319 and the infection-related gene (*ospC*) of *Borrelia burgdorferi* showed that invasive
320 grey squirrels in the UK are reservoirs for multiple *Borrelia burgdorferi* strains that
321 can affect multiple vertebrate clades (Millins et al. 2015). For larger-scale projects
322 and maximum efficiency, next-generation sequencing can be adapted for multi-locus
323 sequence typing from 100–200 samples in a cost-effective manner (Jacquot et al.
324 2014); this method was used to identify different *Borrelia* spp. lineages associated
325 with different small mammal host species (Jacquot et al. 2014).
326
327 Standardisation of sequence data is encouraged, and uploading data to online
328 databases allows combinations of multiple datasets to be incorporated into new and

broad meta-analyses (Maiden 2006). Phylogenetic analysis of openly available sequence data from online reference databases allowed Hayman et al. (2013) to decipher the origins, dissemination and diversification of the zoonotic pathogen *Bartonella* spp. in mammalian clades and introductions.

Hybridisation

Hybridisation among species which are naturally separated is undoubtedly increasing due to anthropogenic impacts, including species' invasions (McFarlane & Pemberton 2019). Extensive introgression from invading populations can put already endangered native populations at risk (Senn et al. 2019). Identifying hybrids based on phenotypic characteristics is problematic due to intermediate phenotypes and observer biases (McDevitt et al. 2009). To increase the efficiency of hybrid identification, molecular markers have long been deployed; microsatellites have been used since the 1990s. The increased use of assignment-based analysis in the early 2000s (e.g. Randi et al. 2001) allowed researchers to identify the proportion of the genome (usually inferring from ≥10 microsatellites) assigned to each species in each individual, which individuals exhibited an admixed genotype and could therefore be labelled as hybrids, and the percentage of the population consisting of hybrids. This type of analyses has been important in providing initial indications of the level of hybridisation between invasive sika deer *Cervus nippon* and red deer *Cervus elaphus* in Europe (e.g. McDevitt et al. 2009), domestic/feral cats and wildcats *Felis silvestris* in Europe (e.g. Randi et al. 2001) and domestic/feral pigs and wild boar in multiple geographic regions (e.g. Scandura et al. 2011). While microsatellite markers can be informative in detecting first or second-generation hybridisation events, their low coverage means that they cannot detect extensive

backcrossing over several generations between parental species (McFarlane & Pemberton 2019).

In order to improve resolution in detecting hybrids and their backcrosses, there is clearly a need to use higher-density and diagnostic SNPs (Mattucci et al. 2019). Several recent studies have highlighted improvements in hybrid detection by using thousands of SNPs rather than 10 - 25 microsatellites. For example, a study on wolf-dog hybridisation showed that only 1-5% of individuals were identified as hybrids when 16 or 18 microsatellites were used (Randi 2008). A later study used 61000 SNPs to infer that 62% of Eurasian wolves *Canis lupus* had some level of admixture with domestic dogs *Canis familiaris* (Pilot et al. 2018). In a well-studied hybrid zone between sika deer and red deer in Kintyre, Scotland, an increased panel of 45000 SNPs reclassified 26% of individuals as hybrids that had originally been assigned to one of the parental species from a previous study based on 22 microsatellites (McFarlane et al. 2019). In attempting to preserve Scottish wildcats from extensive introgression with feral/domestic cats, only wildcat individuals with both high genetic scores (using a SNP panel) and high phenotype scores of wildcat ‘purity’ are selected for the captive breeding and reintroduction programmes (Senn et al. 2019).

MANAGEMENT AND ERADICATION

Given the financial and social commitments required from stakeholders to undertake long-term eradication programmes of species such as grey squirrels and American mink, it is important to be able to gauge the success and impact of these efforts. Microsatellites are very effective in determining recent changes in invasive mammal

population demographics in order to assess the progress of management and control schemes (Velando et al. 2017).

Culling programs are well-established for the control of American mink in several European countries. Fraser et al. (2013) used microsatellites to divide the Scottish mink population into genetic clusters (sub-populations) which were classified as management units. These units were formed through a combination of historical fur-farm escapes and subsequent natural movement through a mosaic landscape throughout Scotland. The genetic analysis of Scottish mink populations corresponded to the habitat characteristics, and allowed Fraser et al. (2013) to create an informed proposal on how to reduce the spread of the species and decide where to direct eradication efforts. However, Oliver et al. (2016) used similar data to identify a possible mechanism for populations in mainland Scotland remaining relatively stable despite culling. They identified an increase in long-distance immigration and an almost three-fold increase in male immigration into culled areas, providing evidence of compensatory immigration during these culling efforts.

As with identifying invasive pathways with genetic markers, SNPs can provide higher resolution of population demographics, and have been implemented instead of traditional capture-mark-recapture methods to show connectivity and dispersal in brown rat populations in an urban area (Combs et al. 2018). Piertney et al. (2016) used 299 SNPs to identify genomic clusters of brown rats on the island of South Georgia in order to identify the appropriate number of target areas for baiting operations. Although these types of data (microsatellites and SNPs) and analyses (population structure and gene flow) are useful for planning and assessing the

403 success of management and eradication programmes, an important consideration is
404 the likely response of the invader to control or eradication measures, whether these
405 be chemical or biological. For example, using a genome-wide SNPs, Morgan et al.
406 (2018) demonstrated that invasive house mice on islands off North and South
407 America did not possess rodenticide resistance alleles that are present in parts of
408 Europe (even though the study also found that these house mice were of European
409 ancestry). This has important implications for subsequent eradication and control
410 measures.
411
412 Even when genetic tools are used to identify key populations to target for culling
413 programs, culls require a lot of effort and usually only have the power to manage a
414 population rather than eradicate it completely. Island populations of invasive mice
415 have been proposed as targets for trialling more elaborate eradication programmes
416 involving genome-editing techniques using **CRISPR-Cas9** (Breed et al. 2019). The
417 concept of gene drives, whereby the use of genetic engineering alters the probability
418 of how specific alleles are inherited in future generations of offspring, is being tested
419 for eradication programmes in multiple invasive species, particularly invertebrates
420 (see Breed et al. 2019 for examples). Mammals are now being considered, and well-
421 studied model organisms such as mice are an obvious starting point. Transgenic
422 delivery of the male sex-determining factor (Sry) has been proposed to skew the sex
423 ratio heavily towards male mice and thereby control population size (Backus & Gross
424 2016). This would require repeated releases of engineered males, which could be
425 feasible on small islands (Campbell et al. 2015). Prowse et al. (2019) demonstrated
426 that the Y chromosome can be ‘shredded’ using CRISPR technology in mouse
427 embryonic stem cells, and individual-based simulations show that this targeted

deletion of a sex chromosome has the potential for eradicating an island population of rodents. However, it would require >90% efficiency to produce high probabilities of eradication success, and would be highly susceptible to changes in mating systems and population size (Prowse et al. 2019).

There are additional concerns if genetically altered individuals are ever accidentally released or spread beyond their target areas of control (a hallmark of effective invaders), as gene drives are self-sustaining (Noble et al. 2018). Despite these justifiable concerns, plans are already underway to bring this technique to the field and to select an appropriate island for trials (Scudellari 2019). The use of the technique is clearly complex in terms of scientific, social, regulatory and ethical issues (Breed et al. 2019), and it remains to be determined how effective gene drives will be over large geographic areas. However, gene drives offer a potentially more targeted approach than the use of chemicals, which could impact non-target, native species.

CONCLUSION

The impact and challenges of surveillance programmes related to invasive species are recognised internationally and have led to the creation of policies aimed at preventing and managing invasions. For example, the European Union has created Regulation 1143/2014 on Invasive Alien Species (IAS Regulation) that contains three measures to combat invasive species which include: (1) prevention, (2) early detection and rapid eradication and (3) management. This review has highlighted that genetic tools have multiple applications for the active management of invasive mammalian species. Not only this, but they are reliable, robust, and provide vital

information, that may not be accessible with non-genetic methods, for the implementation of conservation policies (e.g. early detection using systematic eDNA surveillance and the identification of novel pathogens).

However, there are technical challenges associated with the standardisation of genetic methodologies and bioinformatic pipelines used between laboratories, even when researchers are attempting to address similar questions (Zinger et al. 2019). For example, how the samples have been collected (e.g. gNIS or tissue) and stored (e.g. in ethanol or frozen) has implications for what techniques can be performed downstream in the laboratory (e.g. single gene or whole-genome approaches). Another significant challenge is the availability of appropriate funding and expertise. These factors can all limit what questions can be addressed that will translate into management actions and decisions.

In addition, the lack of clear communication of novel genetic methods and results (including transparency and reproducibility) to relevant stakeholders can be prohibitive in translating these findings to appropriate management and eradication action on the ground (Mosher et al. 2019, Ward et al. 2020). These communication challenges have been well documented in relation to the marine sector (e.g. Darling et al. 2017), but little coordination has taken place in relation to invasive mammals, despite the environmental and economic consequences that invasive non-native species pose to native species, habitats and the agricultural industry. Geneticists should engage early with stakeholders in relation to project costs, duration and management goals for invasive mammals. This will allow for robust experimental

477 design using existing genetic tools, and the development of new technologies that
478 can be tailored towards specific management issues (Mosher et al. 2019).
479

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Glossary

CRISPR-Cas9
A targeted genome-editing tool comprised of the programmable Cas9 endonuclease, which introduces double-strand breaks into DNA; and a guide RNA, which targets the Cas9 nuclease to a specific DNA sequence. This allows for a portion of a target organism's genome to be modified by adding, removing or altering a DNA sequence.

DNA barcode
A DNA barcode is a standardised fragment of the genome that can be used to identify a species. Cytochrome c oxidase I was traditionally the mtDNA marker of choice in barcoding studies. The region is highly conserved throughout the animal kingdom but is variable enough to differentiate between species (Hebert et al. 2003).

DNA metabarcoding
The use of universal primers to amplify multiple DNA barcodes from bulk samples containing multiple species, such as stomach contents or environmental samples.

Environmental DNA (eDNA)
Extra-organismal DNA molecules that are shed in the environment. In animals, eDNA can originate from skin, mucous, saliva, sperm, secretions, eggs, faeces, urine and blood. eDNA can be used to detect the presence of species from samples of soil, water, or other substances from the environment.

Microsatellite
Microsatellites are regions of nuclear DNA which have tandemly repeated regions. These tandem repeats are generally 2–6 base pairs in length and have a very high mutation rate. The variation of microsatellites between individuals and populations can be used to determine population demographics such as gene flow, relatedness and genetic diversity.

Mitochondrial DNA
Mitochondrial DNA (mtDNA), found in the mitochondria as opposed to in the nucleus, has a number of favourable properties for phylogeographic and phylogenetic studies, such as the absence of recombination (which results in an effectively clonal inheritance from the maternal side) and a lack of both pseudogenes and repetitive DNA. mtDNA tends to accumulate base pair substitutions at a higher rate than nuclear DNA.

Next-generation sequencing
Next-generation sequencing, also known as high-throughput sequencing, is a broad term used to describe a number of different modern sequencing technologies. A large number of sequences (millions to billions of sequence reads) are generated on a single sequencing run.

PCR
The **Polymerase Chain Reaction (PCR)** is the exponential amplification (i.e. makes thousands of copies) of a specifically targeted region of DNA through repeated

heating and cooling cycles. It is an essential component in most genetic methodologies as more copies of the region provides a stronger signal for downstream analysis such as sequencing. Primers are required to target the region of interest and can be designed to be species-specific or to work on a broad range of species.

qPCR

qPCR is a process by which the DNA fragment is amplified like in normal PCR, but the amplification rate of the DNA fragment is continuously monitored using fluorescent light. The starting amount of DNA can then be quantified against a set of known standards. Droplet digital PCR (**ddPCR**) does not monitor the amplification process, but it can accurately quantify the starting amount of DNA without the necessity for standards.

Reduced representation sequencing

In reduced representation sequencing, restriction enzymes are used to cut (digest) the genome at specific cut sites, defined by a specific sequence of nucleotides. Sequencing and clustering of these DNA fragments allows the *de novo* discovery of SNPs. Variations of this method of sequencing include Restriction-site Associated DNA sequencing (RAD-seq), double digest RAD sequencing (ddRAD) and genotyping-by-sequencing (GBS). See Andrews et al. (2016) for a detailed review.

Reference databases

Generated DNA sequences and barcodes need to be compared to existing sequences that have been identified as belonging to a species (or at least as a genus, depending on the taxonomic group) by an expert. Reference databases provide public access to such sequences. Examples include Genbank, the Barcode of Life Database and the CDC Bartonella Laboratory database. Sequences in reference databases should have been subjected to quality control for taxonomic accuracy, but this is not always the case (particularly for older records).

Sanger sequencing

A region of DNA is copied using a fluorescent dye unique to each nucleotide. The colours read by the machine can determine the sequence of nucleotides in the region. Sanger sequencing is a low throughput method suited to sequencing long strands (~1000 base pairs) of a single region of DNA.

Single Nucleotide Polymorphism (SNP)

These are single base pair changes/variations (polymorphisms) spanning across hundreds to thousands of locations (loci) along the genome. Deciphering patterns of these changes between multiple individuals can be used to determine population demographics such as gene flow and levels of inbreeding. They can provide higher resolution information compared to other genetic markers such as microsatellites. In addition, they can be used to identify signatures of selection/adaptation in populations.