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

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1 **REVIEW**

2 **SPECIAL ISSUE ON INVASIVE MAMMAL SPECIES**

3

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

6

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16

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18 generation sequencing

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26 **ABSTRACT**

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

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

56 INTRODUCTION

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

67

68 Efficient and cost-effective management of species invasions requires robust
69 knowledge of their demography, ecology and impacts, and genetic-based techniques
70 are becoming more widely adopted in acquiring such knowledge (Searle 2008,
71 Darling et al. 2017). The genetic tools that we have to study these processes have
72 developed dramatically over time, particularly over the last decade, and have
73 become more affordable, efficient and available for small to medium-scale
74 laboratories, providing new opportunities to study multiple aspects of invasions (Lee
75 2002). However, genetic tools are variable in methodology, design, price, complexity
76 and the resolution of results. The scope of this review is to provide an accessible
77 synopsis of the genetic techniques for the non-geneticist in order to enable
78 stakeholders, such as state and conservation managers, policy-makers, field
79 biologists and early-career researchers, to work collaboratively with geneticists to
80 address questions related to the prevention and management of mammalian

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

86

87 **DETECTION AND MONITORING**

88 The early and rapid detection of newly introduced mammals is vital to prevent further
89 spread that could subsequently result in a more costly eradication programme. Given
90 the elusive nature of many mammalian species, detection and monitoring often
91 requires indirect observations such as searching for latrines, faeces, hair, or tracks,
92 or direct observations such as live-trapping or camera-trapping surveys (Sales et al.
93 2020a). These can require differing levels of expertise and resources, but despite
94 high levels of expertise it is not always possible to assign indirect field signs correctly
95 to a species without further confirmation via DNA analysis (Harrington et al. 2010).

96

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

106

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

122

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

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

133

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

146

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

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

157

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

171

172 **ORIGINS AND INVASIVE PATHWAYS**

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

178

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

202

203 Given that we are now firmly entrenched within the genomics era of molecular

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

214

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

228 (2018) to determine that the most likely origins of the New York, USA, population of
229 brown rats were France and the British Isles.

230

231 **NEGATIVE IMPACTS**

232 **Diet and competition**

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

245

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

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

257

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

274

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

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

295

296 **Disease**

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

303 tools are becoming pivotal in disease management in wildlife (DeCandia et al. 2018):
304 PCR is currently used to verify morphological identification of pathogens and
305 parasites (Bagrade et al. 2016), and genetic tools can be used as detection methods
306 when there are difficulties in recreating optimal cell growing conditions to test for
307 prevalence levels (Heuser et al. 2017).

308

309 Different pathogen genotypes or strains can have different infection capabilities
310 (Nally et al. 2016). Sequencing actin genes of pathogenic *Cryptosporidium* revealed
311 that invasive raccoons harboured genotypes capable of infecting humans
312 (Leśniańska et al. 2016). To gain a higher resolution of bacterial population structure
313 and evolution, and to help understand the distribution of pathogenic species and
314 genotypes in novel areas invaded by mammalian hosts, multiple loci or genes can be
315 sequenced in multi-locus sequence typing (Margos et al. 2008). This method was
316 applied to *Borrelia* spp., an important pathogen in zoonotic ecology due to its
317 responsibility for Lyme disease. Sanger sequencing of the housekeeping gene (*clpA*)
318 and the infection-related gene (*ospC*) of *Borrelia burgdorferi* showed that invasive
319 grey squirrels in the UK are reservoirs for multiple *Borrelia burgdorferi* strains that
320 can affect multiple vertebrate clades (Millins et al. 2015). For larger-scale projects
321 and maximum efficiency, next-generation sequencing can be adapted for multi-locus
322 sequence typing from 100–200 samples in a cost-effective manner (Jacquot et al.
323 2014); this method was used to identify different *Borrelia* spp. lineages associated
324 with different small mammal host species (Jacquot et al. 2014).

325

326 Standardisation of sequence data is encouraged, and uploading data to online
327 databases allows combinations of multiple datasets to be incorporated into new and

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

332

333 **Hybridisation**

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

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

355

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

371

372 **MANAGEMENT AND ERADICATION**

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

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

379

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

393

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

402 success of management and eradication programmes, an important consideration is
403 the likely response of the invader to control or eradication measures, whether these
404 be chemical or biological. For example, using a genome-wide SNPs, Morgan et al.
405 (2018) demonstrated that invasive house mice on islands off North and South
406 America did not possess rodenticide resistance alleles that are present in parts of
407 Europe (even though the study also found that these house mice were of European
408 ancestry). This has important implications for subsequent eradication and control
409 measures.

410

411 Even when genetic tools are used to identify key populations to target for culling
412 programs, culls require a lot of effort and usually only have the power to manage a
413 population rather than eradicate it completely. Island populations of invasive mice
414 have been proposed as targets for trialling more elaborate eradication programmes
415 involving genome-editing techniques using **CRISPR-Cas9** (Breed et al. 2019). The
416 concept of gene drives, whereby the use of genetic engineering alters the probability
417 of how specific alleles are inherited in future generations of offspring, is being tested
418 for eradication programmes in multiple invasive species, particularly invertebrates
419 (see Breed et al. 2019 for examples). Mammals are now being considered, and well-
420 studied model organisms such as mice are an obvious starting point. Transgenic
421 delivery of the male sex-determining factor (Sry) has been proposed to skew the sex
422 ratio heavily towards male mice and thereby control population size (Backus & Gross
423 2016). This would require repeated releases of engineered males, which could be
424 feasible on small islands (Campbell et al. 2015). Prowse et al. (2019) demonstrated
425 that the Y chromosome can be 'shredded' using CRISPR technology in mouse
426 embryonic stem cells, and individual-based simulations show that this targeted

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

431

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

442

443 **CONCLUSION**

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

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

455

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

465

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

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

478

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741 conclusions. *Molecular Ecology* 28: 1857–1862.

742

743

745 **Glossary**746 **CRISPR-Cas9**

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

751

752 **DNA barcode**

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

757

758 **DNA metabarcoding**

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

761

762 **Environmental DNA (eDNA)**

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

767

768 **Microsatellite**

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

774

775 **Mitochondrial DNA**

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

782

783 **Next-generation sequencing**

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

788

789 **PCR**

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

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

797 **qPCR**

798 qPCR is a process by which the DNA fragment is amplified like in normal PCR, but
799 the amplification rate of the DNA fragment is continuously monitored using
800 fluorescent light. The starting amount of DNA can then be quantified against a set of
801 known standards. Droplet digital PCR (**ddPCR**) does not monitor the amplification
802 process, but it can accurately quantify the starting amount of DNA without the
803 necessity for standards.
804

805 **Reduced representation sequencing**

806 In reduced representation sequencing, restriction enzymes are used to cut (digest)
807 the genome at specific cut sites, defined by a specific sequence of nucleotides.
808 Sequencing and clustering of these DNA fragments allows the *de novo* discovery of
809 SNPs. Variations of this method of sequencing include Restriction-site Associated
810 DNA sequencing (RAD-seq), double digest RAD sequencing (ddRAD) and
811 genotyping-by-sequencing (GBS). See Andrews et al. (2016) for a detailed review.
812

813 **Reference databases**

814 Generated DNA sequences and barcodes need to be compared to existing
815 sequences that have been identified as belonging to a species (or at least as a
816 genus, depending on the taxonomic group) by an expert. Reference databases
817 provide public access to such sequences. Examples include Genbank, the Barcode
818 of Life Database and the CDC Bartonella Laboratory database. Sequences in
819 reference databases should have been subjected to quality control for taxonomic
820 accuracy, but this is not always the case (particularly for older records).
821

822 **Sanger sequencing**

823 A region of DNA is copied using a fluorescent dye unique to each nucleotide. The
824 colours read by the machine can determine the sequence of nucleotides in the
825 region. Sanger sequencing is a low throughput method suited to sequencing long
826 strands (~1000 base pairs) of a single region of DNA.
827

828 **Single Nucleotide Polymorphism (SNP)**

829 These are single base pair changes/variations (polymorphisms) spanning across
830 hundreds to thousands of locations (loci) along the genome. Deciphering patterns of
831 these changes between multiple individuals can be used to determine population
832 demographics such as gene flow and levels of inbreeding. They can provide higher
833 resolution information compared to other genetic markers such as microsatellites. In
834 addition, they can be used to identify signatures of selection/adaptation in
835 populations.
836

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