

Faecal DNA detection of invasive species: the case of feral foxes in Tasmania

Oliver Berry^{1,2}, Stephen D. Sarre¹, Lachlan Farrington^{1,3}, Nicola Aitken¹

Correspondence:

E-mail. ofb@cyllene.uwa.edu.au

Tel. +61 8 6488 4509

Fax. +61 8 6488 1029

¹Institute for Applied Ecology, University of Canberra, ACT, 2615, Australia

²Current address: Invasive Animals Co-operative Research Centre and School of Animal Biology (M092), The University of Western Australia, Crawley, WA, 6009.

³Current address: School of Earth and Environmental Sciences, The University of Adelaide, SA, 5005

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1 **Abstract**

2 Early detection of biological invasions is critical to reducing their impact, but because
3 invading organisms are initially at low densities, detection and eradication can be challenging.
4 Here, we demonstrate the utility of faecal DNA analysis for the detection of an elusive
5 invasive species - the red fox, *Vulpes vulpes*, which was illegally introduced to the island of
6 Tasmania in the late 1990s. Foxes are a devastating pest to both wildlife and agriculture on
7 the Australian mainland, and would have a similarly serious impact in Tasmania if they
8 became established. Attempts to eradicate foxes from Tasmania have been hampered by
9 unreliable distribution data derived mostly from public sightings. In response, we developed
10 a highly accurate and reliable DNA-based PCR-multiplex test that identifies foxes from field-
11 collected faeces. We also developed a sexing test, but it was reliable only for faeces less than
12 three weeks old. Faeces are a useful target for DNA-based diagnostics in foxes because they
13 are deposited in prominent locations and are long-lasting. The species identification test
14 formed a key component of a Tasmania-wide detection and eradication program. One
15 thousand one hundred and sixty geo-referenced carnivore scats were analysed. Seventy-eight
16 percent contained DNA of sufficient quality for species identification. A single scat from the
17 north-east of the island was identified as belonging to fox, as was a nine week old roadkill
18 carcass from the north coast, and a blood sample from near Hobart, triggering increased
19 control and surveillance in these regions. The accuracy, reliability, and cost-effectiveness of
20 non-invasive tests make them a critical adjunct to traditional tools for monitoring cryptic
21 invasive species that are at low density in the early stages of invasion and when eradication is
22 still an option.

23

1 **Introduction**

2 Biological invasions are a major cause of world-wide species endangerment and extinction
3 (Caughley 1994) and also contribute massive economic costs (Perrings *et al.* 2002). Early
4 detection of invading organisms is a key to their effective management, by enabling better
5 targeting of control measures (Myers *et al.* 2000; Simberloff 2003). However, invasive
6 species are typically scarce and difficult to locate during the early phases of an invasion,
7 making eradication problematic (Mack *et al.* 2000).

8

9 Exemplifying this situation, the red fox (*Vulpes vulpes*) was introduced to the island of
10 Tasmania in the late 1990s. Foxes are a devastating pest to both wildlife and agriculture on
11 the Australian mainland (Saunders *et al.* 1995), costing AUD\$227.5 million annually in
12 biodiversity and economic losses (McLeod 2004). In contrast, the native fauna of Tasmania,
13 a large (64,332 km²) island south of the Australian mainland, is relatively unchanged by
14 European settlement - in large part because of the absence of the fox (Short and Smith 1994).

15

16 Three fox carcasses have been discovered in Tasmania since 2001, and further evidence has
17 suggested that these individuals were part of a deliberate illegal introduction of between 12
18 and 19 individuals of unknown sex to the island around 1999/2000 (Saunders *et al.* 2006).
19 The threat that this invasion poses led to the establishment of a major control program centred
20 on widespread baiting with the poison 1080 (sodium fluoroacetate). 1080 is toxic to native
21 wildlife, and to minimise non-target deaths, baits must be set by hand, which is expensive and
22 time consuming. A baiting program has focused on regions where sightings of foxes have
23 been most concentrated. Over 1000 sightings have been reported by the public since 2001
24 (Figure 1) However, most have been difficult to verify, and many are believed to be
25 erroneous (Saunders *et al.* 2006).

26

27 To bolster evidence from sightings, earlier phases of this program employed analysis of faecal
28 (scat) morphology and hair to monitor fox distributions. This approach makes use of the
29 tendency of foxes to defecate in prominent locations such as tracks and track junctions
30 (Macdonald 1980), and the potentially long life of canid scats (weeks or months, Kohn *et al.*
31 1999). Scats are regularly sampled by ecologists, because they provide a non-invasive and
32 practical monitoring tool for cryptic animals (e.g. Triggs 1996; Sadlier *et al.* 2004). However,

1 in many cases definitive diagnosis can be difficult and lead to ambiguous results of limited
2 usefulness (Davison *et al.* 2002).

3
4 (*Figure 1 placed around here*)

5
6 DNA analysis of scats provides a solution to this problem, by combining the benefits of
7 sampling offered by scats with the robust detection provided by approaches based on the
8 polymerase-chain-reaction (PCR) (Kohn and Wayne 1997). It potentially also enables more
9 sophisticated data to be obtained, such as the determination of sex-ratios (Dallas *et al.* 2000),
10 identification of individuals (Piggott *et al.* 2006), and analysis of diet (Farrell *et al.* 2000).
11 These approaches have been primarily used to study the ecology of native wildlife. However,
12 the same principles could equally be applied to the detection and distribution mapping of
13 elusive invasive species, such as the feral fox. With appropriate verification of the
14 methodology (Taberlet *et al.* 1999), non-invasive DNA-based methods could provide the high
15 quality distribution data that is required for effective control.

16
17 Here, we describe our approach to determining the extent of the fox incursion in Tasmania by
18 developing, testing, then applying a PCR-based species identification test to 1160 putative fox
19 scats collected from throughout the island between 2003 and 2005, as part of the fox
20 eradication program. The identification of positive fox traces triggers management action
21 such as extra baiting, scat collections, and spot-lighting surveys. Such extra actions are
22 expensive, so it is critical that any markers developed are reliable and accurate. We show our
23 method to be highly accurate and more reliable than morphological analyses of scats. In
24 addition, as an aid to determining the likelihood of fox reproduction in Tasmania we
25 developed a sex-diagnostic DNA test. We identify a single fox scat from north-east
26 Tasmania, and positively identify highly degraded roadkill remains from the north coast of
27 Tasmania, as well as a blood sample from near Hobart, enabling control and surveillance to
28 be intensified in these regions.

1 **Methods**

3 *Principles of marker and assay development*

4 Given the potentially large number of scats that would have to be screened to detect foxes in
5 Tasmania, our focus was on developing rapid and cost-effective tests, rather than more
6 involved DNA sequence analysis (e.g. Adams *et al.* 2003). Our preferred method involved
7 species-specific PCR, which generally permits amplification of shorter PCR fragments than
8 restriction digestion methods (e.g. Paxinos *et al.* 1997), and requires minimal processing of
9 samples, thus increasing PCR robustness and reducing opportunities for sample mix-ups.

11 *Multiplex PCR fox-diagnostic test*

12 Primers were designed to amplify a 134 bp PCR product in the presence of fox DNA only.
13 To ensure species-specificity we aligned the entire mitochondrial cytochrome *b* sequences of
14 the seven mammalian carnivores that produce morphologically similar scats to those of foxes
15 (fox, *Vulpes vulpes* genbank ac. X94929; domestic dog, *Canis familiaris*, genbank ac.
16 X94920; domestic cat, *Felis catus*, genbank ac. AB004238; eastern quoll, *Dasyurus*
17 *viverrinus*, genbank ac. U07582; spotted-tailed quoll, *Dasyurus maculatus*, genbank ac.
18 M99461; Tasmanian devil, *Sarcophilus harrisii*, genbank ac. M99465, and; thylacine,
19 *Thylacinus cyanocephalus*, genbank ac. M99452), and designed primers that maximised
20 mismatches between the fox and the remaining species (9-11 mismatches/species). Primer
21 sequences are: *VV-cytb F* GACCTTCCCGCACCATCAAATAT, and *VV-cytb R*
22 GAAAGCAGTAGCTGTGTCAGA. These primers have no mismatches with western
23 European *V. vulpes* sequences on genbank (including samples from The United Kingdom,
24 Sweden, Spain).

26 We also designed ‘universal’ primers that would serve as a positive amplification control and
27 amplify a PCR product in the presence of any of the candidate mammalian carnivores and
28 also co-amplify with the fox-specific primers (Figure 2). To do so we aligned sequences from
29 the mitochondrial 12s ribosomal gene from the candidate carnivore species (genbank
30 accession numbers: Y08508, Y08507, AY012149, U87402, U87401, AF009893, U87405)
31 and designed primers GAGGGTGACGGGCGGTGTGT (coinciding with the position of
32 primer H1478, Kocher *et al.* 1989), and 12SL-1 CATAAAAAMGTTAGGTCAAGGTGT,
33 which produce a 183 bp PCR product.

1

2 (*Figure 2 placed around here*).

3 We combined these primer pairs in a 10 µL PCR multiplex reaction as follows: PCR Buffer
4 (160 mM (NH₄)₂SO₄, 670 mM Tris-HCl pH 8.0, 0.1% Tween-20; Bioline), 2.5 mM MgCl₂,
5 0.4 mg/mL bovine serum albumin, 0.2 mM each dNTP, 0.5 U Taq (Bioline RedTaq), primers
6 (all at 0.4 µM), and 20 ng DNA template. The reaction was cycled through 94 °C 2 minutes,
7 (94 °C 30 seconds, 58 °C 20 seconds, 72 °C 15 seconds) x 30, 72 °C 2 minutes. The
8 specificity of these primers was confirmed by running a series of PCRs with 20 ng of
9 genomic DNA from ten individuals of each other candidate species sourced from across their
10 geographic range or from multiple breeds (except thylacine). In addition, we trialed the test
11 on DNA obtained from multiple foxes from each Australian state in which they are present (n
12 = 26 overall, n = 3-7 per state). All amplifications were successfully diagnostic for fox.
13 Finally, as a test of the risk of scoring error, we conducted a blind trial involving 10 fox, 5
14 dog and 5 cat samples.

15

16 *Multiplex PCR sex-diagnostic test*

17 We also used a multiplex-PCR approach to determine the sex of foxes from their scats. To do
18 so we obtained sequences from the fox testis-determining *SRY* gene (deposited in genbank ac.
19 AY600298), and designed primers *VV-sry F* and *VV-sry R* within and adjacent to the HMG
20 box. These primers maximised mismatches with prey or other species that may potentially
21 contaminate scats (mice rats, humans, dasyurid marsupials, macropod marsupials), and
22 amplified a 78 bp PCR product. We confirmed the specificity of the *SRY* primers by
23 attempting PCR amplification from genomic DNA of multiple male rats and mice (n=2 each).
24 As an amplification control we designed primers to the sequence of the *HPRT* gene of the
25 domestic dog (Meyers-Wallen *et al.* 1995), which produces an X-linked product of 150 bp:
26 *CF-hprt F* AGTCAACGGGGGACATAAAAG, *CF-hprt R*
27 ACCATTTTTGGATTATACTGC. The PCR setup for the sexing multiplex was the same as
28 for the species identification test, except that the *HPRT* primers were at a final concentration
29 of 0.2 µM and the *SRY* primers were at 0.8 µM.

30

31 *Robustness of the test under field conditions*

32 To determine the robustness of our test under field conditions we conducted a scat ageing trial

1 during winter in Canberra, ACT. We collected 200 fresh scats from captive foxes over a 14-
2 day period, and stored them at -80°C for 1-3 weeks until the ageing trial began. The scats
3 were then randomly allocated to one of five ageing treatments (40 scats per treatment, 0 days,
4 1 week, 3 weeks, 6 weeks and 12 weeks) and were placed in a grassed outdoor enclosure. In
5 addition, we allocated an equal number of male and female fox scats to each ageing treatment.

7 *Effect of different DNA extraction methods*

8 Because of the potentially high cost and effort involved in extracting DNA from many scats
9 (Piggott and Taylor 2003), we conducted a field experiment to examine the performance of
10 these tests when using DNA extracted by two methods: one involving a commercial DNA
11 extraction kit, which is labour intensive and costly, and the other a relatively rapid and
12 inexpensive chelex based technique. At each time interval (ageing treatment), scats were
13 collected, split roughly in half (to enable direct comparison of PCR success between methods)
14 and each half placed into a different plastic bag ready for DNA extraction and stored at room
15 temperature until extraction.

17 *DNA extraction protocols*

18 DNA extractions took place within two days of collection. All scat extractions took place in a
19 fume cupboard within a laboratory that had not previously been used for processing fox tissue
20 or DNA, and all pipetting was done with aerosol-resistant tips. Extraction blanks were run
21 with each extraction at the ratio of 20 samples to each blank. DNA was stored at 4 degrees
22 until PCR analysis (usually < 2 weeks). The commercial kit protocol involved gently
23 washing scats in SLP buffer (500mM Tris-HCl pH 9.0, 50mM EDTA, 10mM NaCl; Deuter *et*
24 *al.* 1995) to release epithelial cells, followed by overnight digestion and purification with a
25 Qiagen DNeasy kit (see Banks *et al.* 2002 for details). The second chelex-based method
26 involved an initial wash with PBS buffer (pH 7.4) as above, then 500 μL of supernatant was
27 combined with an equal volume of SET buffer (10 mM Tris-HCl pH 8.0, 0.5 mM EDTA,
28 0.2% SDS) containing 5 % chelex 100 beads (Biorad), and digested overnight with 0.25 mg
29 proteinase K at 55°C . The tubes were then boiled for 5 minutes and centrifuged at 13,000
30 rpm for 5 minutes before 200 μL of supernatant was removed and stored at 4°C .

31

1 *PCR amplification, scoring of results and data analysis*

2 PCRs for scat DNA were as described above except that 2 μ L of scat extract was used as
3 template, and they were run for 35 cycles. PCR products were visualised on 3 % high-
4 resolution TBE agarose gels (Amresco 3:1 HRB™) after running for 35 minutes at 90 volts,
5 and then stained with ethidium bromide. For the fox-diagnostic PCR, we loaded 2.5 μ L of
6 PCR product and for the sexing protocol, 10 μ L of PCR product. Negative controls (no
7 template added) were run in each PCR and size standards of known DNA concentration were
8 run on each gel (Hyperladder II™; Biorad). Gel images were captured on a Geldoc 2000 with
9 QUANTITYONE software (Biorad). We used the volumes tool from the QUANTITYONE
10 software to determine the average intensity of PCR products in optical density units. Intensity
11 was corrected for differences in staining between gels by reference to the 200 bp standard
12 band (=20 ng DNA). Repeatability of intensity was high (data not shown).

13

14 *The effect of scat age and method of DNA extraction on species and sex diagnostic PCR*

15 All species-diagnostic PCRs were successful on known fox scats (n = 199 for each extraction
16 method). Therefore, we tested whether the age of scats or the method used to extract DNA
17 from scats had an effect on the amplification efficiency (intensity) of the universal PCR
18 product with generalised linear modelling implemented with SAS (SAS Institute, NC, USA).
19 We also tested whether method of DNA extraction or age of scat affected the amplification
20 success of the sexing multiplex (correct vs. null result or incorrect) using log-linear models
21 (Sokal and Rohlf 1995).

22

23 *Tasmanian samples*

24 Possible fox scats were collected opportunistically in Tasmania by members of the Fox-Free
25 Tasmania Taskforce during routine trapping and searches for foxes in areas where sightings
26 were most common. Because carnivore scats are commonly misidentified (Davison *et al.*
27 2002), and fox scats are morphologically similar to at least five other medium sized
28 carnivores present in Tasmania (Triggs 1996), the scats collected comprised all medium sized
29 carnivore-like scats.

30

31 The majority were collected from the north-east of Tasmania, where sightings have been most
32 common (Figure 1). Scats were given a unique identity number, a GPS co-ordinate was

1 obtained, and they were then sealed in a paper bag, and dried at room temperature before
2 being sent by mail for analysis. Following the success of the field trials (see results), DNA
3 from all scats collected from Tasmania were extracted using the chelex method in a laminar
4 flow hood that had not been used to process any fox DNA, scats or tissue. The work area was
5 subject to UV irradiation between extractions to destroy any potential residual DNA. PCRs
6 were run with a positive and negative control.

7

8 In addition to scats, roadkill remains that were suspected to be fox were collected from a
9 locality in northern Tasmania (Lillico Beach; Figure 1), and a blood sample was collected
10 from a wooden surface adjacent to a chicken shed near Old Beach, north of Hobart. Both
11 samples were subjected to the species identification and sexing tests following DNA
12 extraction via standard phenol-chloroform methods (Sambrook *et al.* 1989). The roadkill
13 remains were collected at least nine weeks after they were first sighted. They consisted of
14 skin and bone fragments, and their poor state of preservation meant that their species of origin
15 could not be identified from gross morphology.

1 **Results**

2
3 *Test verification: viability of scats in ageing trial*

4 Scats persisted for extended periods in the field, with one hundred percent of scats lasting for
5 12 weeks of the field trial (n = 40) and all but one lasting until collection (i.e. 1-6 weeks).
6 The average weight of scats declined from 10.4 g (\pm 0.86 SE) at time zero to 3.04 g (\pm 0.30
7 SE) at twelve weeks.

8
9 *Test verification: fox-diagnostic testing of scats of different ages.*

10 One hundred percent of the samples used in the blind trial were correctly identified as either
11 fox or non-fox (cat or dog). All known fox scats were correctly identified, regardless of the
12 method of DNA extraction or their age, and in all cases both the universal and fox-specific
13 PCR products were amplified (n = 199 for each extraction method). However, the intensity of
14 PCR products declined significantly with scat age (F = 46.58, df = 4, p < 0.01), and DNA
15 extracted with the Qiagen method on average produced significantly more intense PCR
16 products than chelex-extracted scats of the same age (F = 32.01, df = 1, p < 0.001).

17
18 *Test verification: sex diagnostic testing of scats of different ages*

19 All sexing tests on scats equal to or less than one week old were successful if DNA was
20 extracted using the Qiagen kit, while tests using chelex extracts were *ca.*10-25 % less robust
21 (Figure 3). There was a decline in the ability to sex fox scats as they became older - at six
22 weeks between 60 and 70 percent of scats could be sexed correctly and at 12 weeks only 25%
23 of scats could be accurately sexed. The main reason for failure of the sexing test was null
24 results, where no diagnostic PCR products were produced, but up to 5 percent of tests
25 identified sex incorrectly in each ageing treatment (Figure 3). Log-linear modelling showed a
26 significant three-factor interaction term, indicating that the difference in amplification success
27 between Qiagen and chelex extracts also depended on scat age (G = 17.38, df = 4, p < 0.05).
28 Separate G-tests for scats of each age showed that Qiagen extracts were significantly more
29 likely to produce a correct sex diagnosis than chelex extracts, if the scats were 0, 1, or 3
30 weeks old (p < 0.05), but neither method was significantly better at 6 or 12 weeks old. G
31 values for each time are 7.26, 13.62, 12.56, 0.96, 0.00 for scats of 0, 1, 3, 6, 12 weeks of age
32 respectively, and the critical $\chi^2_{.05,1}$ value is 3.841. G-tests also showed that the age of the scat
33 had a significant effect on the success of sex-diagnostic PCR for both chelex and Qiagen

1 extracts (Qiagen $G = 101.81$, chelex $G = 43.54$, $df = 4$, $p < 0.05$).

2

3 (*Figure 3 placed around here*).

4

5 *In practice: testing of possible fox scats from Tasmania*

6 A total of 1160 carnivore scats were collected from throughout Tasmania. The ‘universal’
7 PCR product was successfully amplified from 78 % of scats, indicating that sufficient DNA
8 was present for species identification. The vast majority of carnivore-like scats amplified the
9 ‘universal’ PCR product only, indicating that they were not from fox. However, a single scat
10 from Conara in the north-east of the island produced the diagnostic fox PCR product (Figure
11 1). This result was replicated by PCR three times on two independent DNA extractions, and
12 confirmed through direct sequencing of the fox-diagnostic PCR product, which showed a
13 perfect match to *Vulpes vulpes* cytochrome *b* sequence (genbank ac. X94929). The sexing test
14 did not amplify DNA from this scat. Two independent DNA extractions from the roadkill
15 remains and blood samples were identified as fox, and this diagnosis was confirmed by
16 sequencing of the cytochrome *b* PCR product. The sexing test did not amplify any PCR
17 products from either the roadkill or the blood sample.

1 **Discussion**

2 Early detection of an invasive species is a key to successful eradication (Myers *et al.* 2000).
3 Species with low dispersal capabilities or strong affinities to particular habitats are typically
4 good candidates for eradication because they are readily observed (e.g. Kuris and Culver
5 1999). Mobile, adaptable species such as the red fox, which typically occurs at low densities
6 (Saunders *et al.* 1995), require novel detection strategies. Illustrating this, in Tasmania the
7 analysis of DNA from carnivore scats has provided robust evidence for the presence of at
8 least one fox, whereas the evidence from public sightings and morphological analysis of scats
9 has often been ambiguous.

10
11 *Foxes are present in Tasmania*

12 Our aim was to identify fox scats in Tasmania with high accuracy and so provide a robust
13 trigger for expensive and time consuming control effort. Better targeting of baiting should
14 reduce both expense and the poisoning of non-target native species. The number of field-
15 collected scats that we processed (1160) is one of the largest such sample that we are aware
16 of, and produced a single positive result from north-east Tasmania, in an area where sightings
17 have been common. The significance of these results are twofold. First, in the context of
18 growing public scepticism and political pressure to downscale control operations (Saunders *et*
19 *al.* 2006), our results provide independent evidence for the presence of foxes in Tasmania,
20 and given the potentially massive biodiversity and agricultural costs, reaffirms the importance
21 of an eradication program. It also has enabled control operations to be better targeted in this
22 region (C. Parker, TDPIWE, pers. comm.).

23
24 Second, our results emphasise the large effort required to survey adequately for widely
25 dispersed and mobile invasive species (cf. Sadler *et al.* 2004). Undertaking such a large
26 screening project requires a cost-effective protocol that is both highly accurate and reliable,
27 and this is best achieved through extensive field testing prior to implementation. Our field
28 trials provided three significant results. First, fox scats are surprisingly persistent in the field.
29 This, coupled with a method for species identification, greatly increases the opportunity to
30 “sight” animals and to take management action, yet little was previously known of the
31 persistence of canid scats in the field (but see Kohn *et al.* 1999). Second, sufficient DNA is
32 contained within scats for 100 percent accuracy in species identification, even after three
33 months of weathering, and probably longer, even though their external morphology was often

1 greatly modified. Reliability, measured as the proportion of fox scats for which identity can
2 be diagnosed, was also 100 percent. Third, DNA-based species identification is robust, no
3 matter what method is used to extract DNA from scats. This is important, because DNA
4 extraction with the commercial kit was the most costly (about AUD\$6.0, Euro€3.6, US\$4.4
5 per sample in consumables) and time consuming aspect of scat processing (compare less than
6 AUD\$0.10 per sample for chelex). Use of a cheaper and more straightforward extraction
7 protocol places fewer constraints on the number of scats that could be processed. In
8 combination, these findings enable a high level of confidence to be put in the analysis of field
9 collected scats from Tasmania. This credibility has real political and management
10 consequences in situations such as the fox in Tasmania, where public scepticism and political
11 pressure to abandon control programs are high (see Lapidge and Berry 2004; Saunders *et al.*
12 2006).

13
14 The value of extensive validation of the DNA-based method of testing is highlighted by
15 earlier morphological analyses of carnivore scats from Tasmania, which was of limited
16 usefulness as a monitoring tool, because a large proportion of scats could not be definitively
17 identified (N. Mooney TDPIWE, pers. comm.), a finding that mirrors experience elsewhere
18 (Davison *et al.* 2002). Indeed, several of the scats that we processed and identified as non-fox
19 were independently identified as “possible fox” by morphological analysis (L. Overend,
20 TDPIWE, pers. comm.). Without recourse to a second method of diagnosis, this tentative
21 appraisal could result in significant effort being wasted. Moreover, the scat that yielded a
22 positive fox result via the DNA-based test was not initially identified as fox based on hair and
23 morphology.

24
25 In addition to species identification, DNA analysis of scats can reveal additional ecological
26 information about invasive species that cannot be obtained from morphological analysis. For
27 example, knowing the sex of feral predators could enable the estimation of sex ratios (Dallas
28 *et al.* 2003) and the likelihood of reproduction. In the case of the fox in Tasmania, until a
29 female fox was discovered dead in late 2003, there had been some evidence that only male
30 foxes were introduced (N. Mooney, TDPIWE, pers. comm.). As expected from the results of
31 Frantzen *et al.* (1998), our field trials demonstrated that such information is more difficult to
32 obtain than species identification from all but the freshest scats. Yet, success was still
33 reasonably high from fresh scats (≤ 3 weeks old, 60-90 %), and can be maximised by use of a

1 more expensive and labour intensive DNA extraction protocol. If acquiring such information
2 were particularly important for control of an invasive species, a two-tier process, where initial
3 rapid and inexpensive screening for species is followed by the more involved sexing protocol,
4 would provide the best scaling of costs and effort. However, as our results from the fox-
5 positive scat, the roadkill and blood sample show, where the age of the sample is unknown
6 and the degree of degradation is high, obtaining such results is not guaranteed.

7

8 In conclusion, we developed and tested a DNA-based method of species and sex identification
9 for the feral fox, and applied it to an extensive field collection of carnivore scats from
10 Tasmania, as well as some highly degraded roadkill remains and a blood sample. We
11 demonstrated that scats are useful for monitoring elusive invasive species because they persist
12 for long periods in the field and contain sufficient DNA for accurate and reliable species
13 identification. Significantly, we identified a single fox scat from north-east Tasmania – a
14 result that other detection approaches have been unable to achieve, and also positively
15 identified roadkill remains and a blood sample from widely separate localities. Because foxes
16 are likely to continue to be introduced to Tasmania both deliberately and accidentally
17 (Saunders *et al.* 1995; Saunders *et al.* 2006), this non-invasive approach should remain an
18 important part of the ongoing management program.

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9 collection.

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1 **Figure legends**

2

3 **Figure 1.**

4 Map showing location of putative sightings of feral foxes reported in Tasmania between 2002
5 and 2005 (black circles), and areas where intensive scat searches took place (stars). Arrows
6 mark localities referred to in the text. Data source: Fox-Free Tasmania Taskforce.

7

8 **Figure 2.** Agarose gel showing diagnostic banding pattern for the fox (Vv) and other
9 candidate carnivores present in Tasmania (Sh Tasmanian devil, Dv eastern quoll, Dm spotted-
10 tailed quoll, Cf domestic dog, Fc domestic cat). The left and right lanes are 100bp size
11 standards.

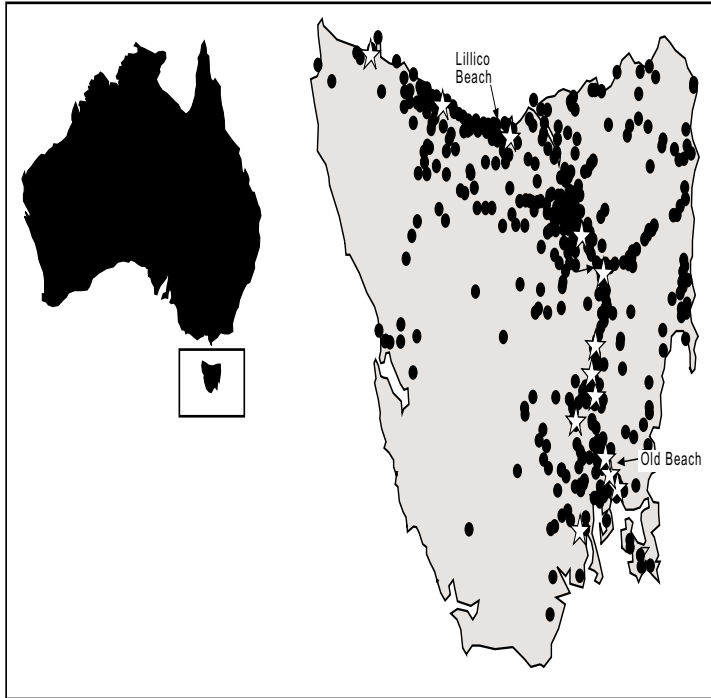
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13 **Figure 3.**

14 Percentage of sex-diagnostic PCR tests that produced correct sex, incorrect sex, or null results
15 (no amplification). * indicates DNA extraction methods were significantly different.

16

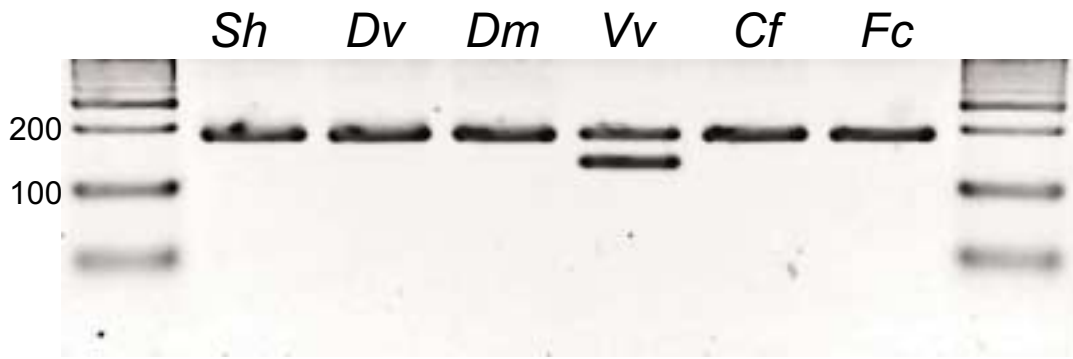
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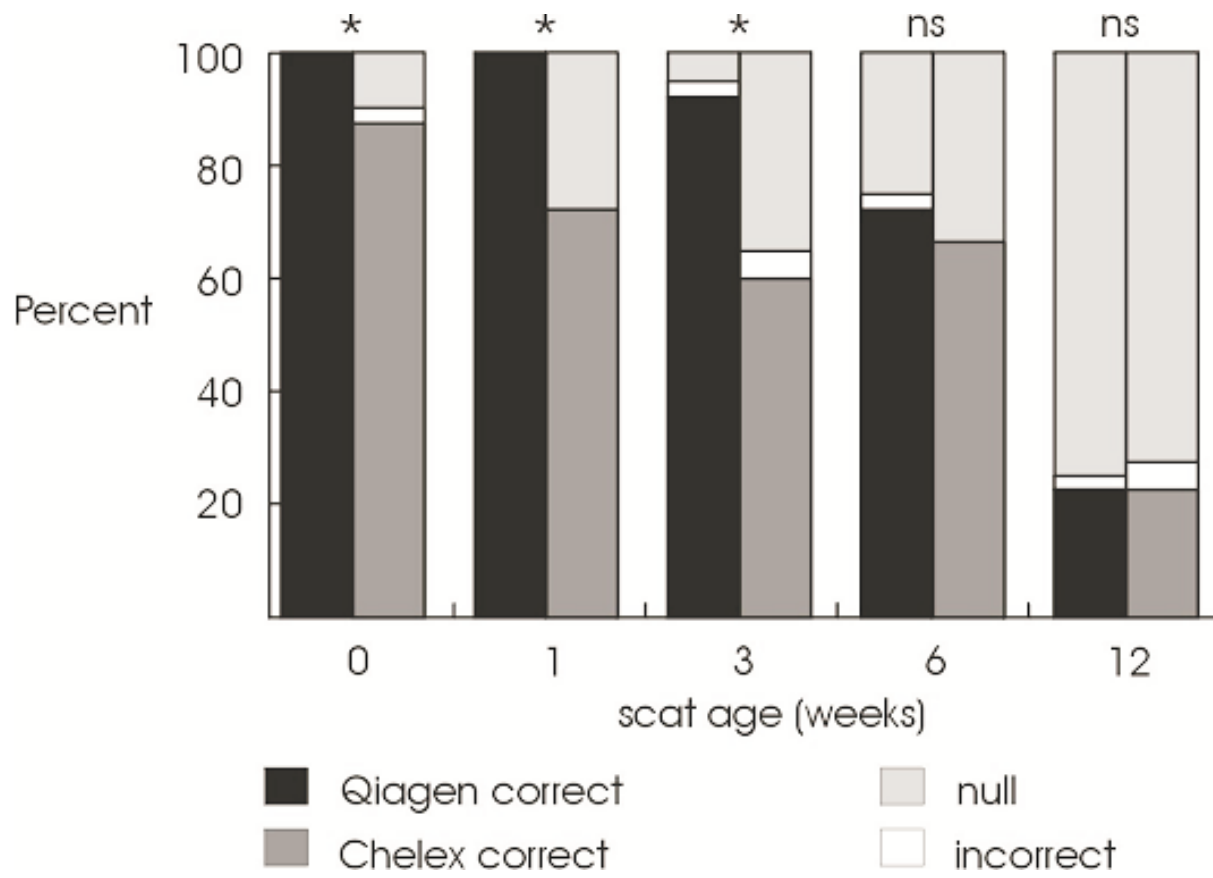
3 Figure 1



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3 Figure 2



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2 Figure 3
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