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The dispersion and detection patterns of mtDNA-assigned red fox *Vulpes vulpes* scats in Tasmania are anomalous

Clive A Marks[1]*, David Obendorf[2], Filipe Pereira[3], Ivo Edwards[4] and Graham P Hall[5]

[1]*Nocturnal Wildlife Research Pty Ltd, Wattletree Rd, Malvern, VIC 3144, Australia;* [2]*Veterinary Pathologist, 7 Bonnington Road, Hobart, TAS 7000, Australia;* [3]*Interdisciplinary Centre of Marine and Environmental Research (CIIMAR/CIMAR), University of Porto, Rua dos Bragas 289, Porto 4050-123, Portugal;* [4]*Padded Traps Ltd, 3777 Gordon River Rd, Maydena, TAS 7140, Australia; and* [5]*School of Environmental and Rural Science, University of New England, Armidale, NSW 2351, Australia*

Summary

1. Models used for resource allocation in eradication programmes must be based on replicated data of known quality and have proven predictive accuracy, or they may provide a false indication of species presence and/or distribution. In the absence of data corroborating the presence of extant foxes *Vulpes vulpes* in Tasmania, a habitat-specific model based upon mtDNA data (Sarre *et al*. 2012. *Journal Applied Ecology*, 50, 459–468) implied that foxes were widespread. Overall, 61 of 9940 (0·6%) surveyed scats were assigned as mtDNA fox positive by the fox eradication programme (FEP).

2. We investigated the spatiotemporal distribution of the 61 mtDNA-assigned fox scats and modelled the probability of replicating scat detection in independent surveys using detection dogs based upon empirically derived probabilities of scat detection success obtained by the FEP using imported fox scats.

3. In a prior mainland study, fox genotypes were recurrently detected in a consecutive four-day pool of scats. In Tasmania, only three contemporaneously collected scat pairs of unknown genotype were detected by the FEP within an area corresponding to a conservatively large mainland fox home range (639 ha) in a decade. Nearest neighbour pairs were widely spaced (mean = 7·0 km; circular area = 153 km^2) and generated after a mean of 281 days.

4. The majority of assigned mtDNA positive scats were found in urban and peri-urban environments corresponding to small mainland fox home ranges (30–45 ha) that imply higher scat density and more certain replication. Using the lowest empirically determined scat detection success for dogs, the failure to replicate fox scat detection on 34 of 36 occasions in a large (639 ha) home range is highly improbable (P = 0·00001) and suggestive of Type I error.

5. *Synthesis and applications*. Type I error, which may have various sources, should be considered when scat mtDNA data are few, accumulated over many years, uncorroborated by observations of extant specimens, inadequately replicated in independent surveys within an expected spatiotemporal scale and reported in geographically isolated environments unlikely to have been colonized.

Key-words: data quality, eradication, habitat-specific distribution model, red fox, scat DNA, Tasmania, Type I error

Introduction

Species distribution models have been used to predict the proliferation of biological invasions (Guisan & Thuiller 2005); however, their generalizability and usefulness is entirely dependent upon the quality of their training data (Vaughan & Ormerod 2005; Barry & Elith 2006). Obtaining presence data of sufficient quality to permit the modelling of new incursions is challenging, particularly if the target species is cryptic (Pearson *et al*. 2007) and exists at low population density (Darling & Mahon 2011). Genetic sampling has been favoured for the detection of rare species (Waits & Paetkau 2005) and is highly regarded for

*Correspondence author. E-mail: camarks@attglobal.net

confirming recent invasive species incursion given their greater specificity and sensitivity compared with many conventional approaches (Darling & Mahon 2011). Problematically, the precision of molecular data is rarely reported, and subjective assessments sometimes imply that they are sufficiently immune to error to preclude the need for qualitative assessments (Parkes & Anderson 2009; Blackman, Corcoran & Sarre 2013). This overlooks a range of methodological and technical considerations that can influence the accuracy and reliability of molecular data (Vaughan & Ormerod 2005; Waits & Paetkau 2005) and possible sources of Type I and Type II error that can provide misleading indications of the presence or absence of invasive species (Darling & Mahon 2011). Because DNA-based assays are incapable of discriminating between specimens arising from *post-mortem* or living specimens (Darling & Mahon 2011), a cautious interpretation is required if model data are assumed only to describe an extant population. When data precision is critical for defining unique species incursions, especially those uncorroborated by the detection of living specimens, qualitative assessments of molecular data are required prior to the generation of habitat-specific models as data error, bias and small sample size are well-known sources of modelling error (Barry & Elith 2006).

The European red fox *Vulpes vulpes* established in mainland Australia after at least nine separate introductions after 1845 (Abbott 2011) and presently inhabits much of continental Australia where it threatens the conservation status of a range of fauna (Bennett, Lumsden & Menkhorst 1989; Dickman 1996; Priddel & Wheeler 1997). Since 1843, a number of historical and anecdotal reports implied that red foxes were also released in Tasmania, yet no reports of a potentially establishing population were made until 2001 (Marks *et al.* 2014) when it was reported that 11–19 foxes had been deliberately released (Dennis 2002; Saunders *et al.* 2006; Sarre *et al.* 2007; Marshall 2011). Soon after, opportunistically acquired fox carcasses presented by members of the public, some of which were quickly attributed to hoaxing using foxes sourced from mainland Australia (Saunders *et al.* 2006; Marks *et al.* 2014), prompted the Tasmanian government to conclude that a fox eradication programme (FEP) was warranted (Wilkinson 2009). Molecular survey techniques targeting red fox mitochondrial DNA (mtDNA) were used after 2005 in an attempt to detect red fox scats in the Tasmanian environment (Berry *et al.* 2007) in contrast to the use of microsatellites that had previously been used to identify genotypes on the mainland (Piggott *et al.* 2008; Marks *et al.* 2009). Between 2002 and 2012, coordinated searches using volunteers and trained fox scat detection dogs (Smith *et al.* 2003; Vynne *et al.* 2011) collected 9940 putative fox scats of which 61 (0·6%) were initially assigned as mtDNA fox positive (Anon 2012) (Fig. 1). Thereafter, Sarre *et al.* (2012) retrospectively reported that from 7658 predator scats from which DNA was amplified, 56 had produced fox indicative sequences. Together with 9 unspecified cases of opportunistically acquired *post-mortem* evidence that are of equivocal evidentiary quality (Marks *et al.* 2014), the authors produced a habitat-specific model and concluded that foxes were now widespread in Tasmania. In the absence of data confirming the presence of extant foxes or independent data permitting the predictive capacity of the model to be tested, we undertook a qualitative analysis (Vaughan & Ormerod 2005) of the molecular data collected by the FEP.

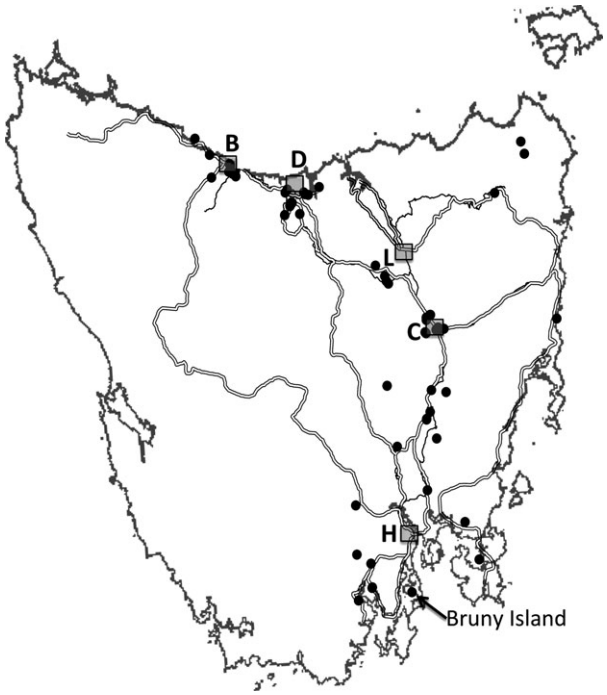

Fig. 1. Location of 61 mtDNA-assigned fox-positive scats in Tasmania relative to major highways (double lines), railway tracks (black line) and the urban centres of Burnie (B), Devonport (D), Launceston (L), Conara (C) and Hobart (H).

Materials and methods

We contrasted fox scat detection success on mainland Australia (Marks *et al.* 2009) with that found by the Tasmanian FEP. Thereafter, we examined the spatiotemporal distribution of molecular data collected in Tasmania and indicators of sample bias in the model provided by Sarre *et al.* (2012). Tasmanian data were then contrasted with a binomial model of expected fox scat detection probability based upon the results of experiments conducted by the FEP that had revealed the success of scat detection dogs in locating scats imported from the mainland.

REPLICATED DETECTION OF FOX GENOTYPES IN MAINLAND FOX SCAT SURVEYS

We reanalysed data published by Marks *et al.* (2009) that used DNA microsatellites and an analysis described by Piggott *et al.* (2008) in order to clarify the capacity to replicate the detection of known fox genotypes using only a four-day pool of scats collected from road transects by volunteers. The mainland site

(Werribee, Victoria) is approximately 33 km WSW of Melbourne and is a highly homogeneous rural study site of grazing pasture interspersed with remnant eucalypt woodland that supported a fox population naïve to recurrent lethal control. Mean home range size was previously estimated to be approximately 117 ha with a mean density of 2·3–5·8 foxes km^{-2} (Marks *et al.* 2009). In this prior experiment, scats were first removed from four 5-km-long and 2-m-wide road transects (total transect area = 1 ha), and thereafter, searches were conducted each morning over a 14-day period. Fox scats from the previous evening were collected after a slow walk that took approximately one hour to complete and stored for 1–3 months prior to DNA extraction and microsatellite genotyping (Piggott *et al.* 2008). The mean number of scats collected km^{-1} $week^{-1}$ was regressed against the total number of fox genotypes identified per km^{-1} from a consecutive four-day sample taken at the end of the two-week period. The frequency of genotypes detected more than once was reported for each transect and as an overall mean.

POOL OF ASSIGNED FOX-POSITIVE SCATS IN TASMANIA

Molecular data used to survey for the presence of foxes in Tasmania were presented by the Tasmanian Department of Primary Industries, Water, Parks and Environment (DPIEWE) on a dedicated web site (Anon 2012). The DPIEWE provided the mapped locations for the 61 scats assigned to be fox-positive reported as of 13 March 2012. Assigned fox-positive scats had been collected using detector dog searches (n = 36) or opportunistic and/or coordinated searches by FEP field staff and member of the public (n = 25) between 2002 and 2012. The revised analysis and tally of 56 fox-positive scats reported in late 2012 was retrospective (Sarre *et al.* 2012), yet taken from the same pool of scats. Because fox control and eradication programmes require the rapid detection of survivors and efficient monitoring of reinvasion (Gentle, Saunders & Dickman 2007; McLeod *et al.* 2011), we regarded the initial data to be of greater relevance.

SPATIOTEMPORAL DISTRIBUTION OF FOX ASSIGNED SCATS IN TASMANIA

Nearest neighbour analysis of scat pairs

The ability to replicate the detection of assigned fox-positive scats at each site was tested by assessing the number of nearest neighbour scat pairs that occurred within a 1·42-km radius (circular area = 639 ha). This limit corresponded to the mean 95% Kernel home range found for foxes in northern Victoria in mainland Australia (Carter, Luck & McDonald 2012) and was a conservatively large fox home range greater in area than reported in various habitats in south-eastern mainland Australia corresponding to forest, agricultural, peri-urban and urban habitats also present in Tasmania. The selected area exceeded the largest 100% minimum convex polygon (MCP: Kenward 1987) of 520 ha obtained in the Nadgee Nature Reserve (Phillips & Catling 1991); the largest 90% MCP utilization home range of 400 ha in central Victoria (Coman, Robinson & Beaumont 1991); a mean of 44·6 ha for 100% MCP estimates in semi-urban habitats of Melbourne (White *et al.* 2006); and a mean of 29·6 ha for 100% MCP in urban Melbourne (Marks & Bloomfield 2006). Given that the majority of Tasmanian scats assigned to be fox positive had been detected within urban environments (Parkes & Anderson 2009), the anticipated fox home range sizes in corresponding habitats would be expected to be much smaller than our chosen home range area appropriate for rural habitats. Mean nearest neighbour distance (di) with a 95% confidence estimate was calculated (Krebs 1999) for all scat pairs according to:

$$\bar{d} = \left(\sum_{i=1}^{N} d_i / N \right)$$

The duration over which each nearest neighbour pair was formed was determined by the difference between the collection dates in days for scat pairs.

Association of assigned fox-positive scats with roads

The position of each of 61 putative fox scats was plotted on GOOGLE EARTH PRO (version 7.1.1.1843, Google inc. http://www.google.com/earth/), and straight-line distances for each scat were measured to the nearest vehicle access road and highway. Traffic density based upon the average annual number of vehicles per day (AAVD) was obtained for the closest highway to each putative fox-positive scat (Anon 2005) as well as the annual tonnage of freight carried by Tasmanian roads to and from the Port of Burnie (Anon 2007) as an additional metric of traffic flow. Nearest neighbour scat distances at each point were correlated with AAVD and freight tonnage using a generalized linear model (Zar 1999). The model of fox habitat in Tasmania proposed by Sarre *et al.* (2012) was overlaid and registered with Google Earth imagery of Tasmania and imported into CARTOGRAPHICA (version 1.2.10, Clue Trust: Reston VA). One hundred random points were overlaid in 61 iterations and a single point that fell within the fox distribution model area was randomly selected in each iteration, and the distances from vehicle access roads, highways and railway tracks were determined. A t-test for equality of means with assumed unequal variance determined with Levine's test (Zar 1999) was selected to compare the distances of assigned fox-positive scats and random points placed in the model of fox distribution proposed by Sarre *et al.* (2012).

MODEL OF FOX SCAT DETECTION PROBABILITY

We based our model of fox scat detection probability on the following data and assumptions: (i). in FEP trials using fox scats imported from mainland Australia and placed in the field, scat detection dogs were reported to have 0·1–0·4 probability of finding a single fox scat known to be present in a standard 100-ha search area within a 30-min search period (Parkes & Anderson 2009); (ii). in captive studies, red foxes were reported to produce 8 scats day^{-1} irrespective of diet (Sadlier *et al.* 2004; Webbon, Baker & Harris 2004); (iii). viable nuclear DNA suited to microsatellite genotyping had been collected from one week and up to 12 weeks (Piggott & Taylor 2003; Piggott 2005). Given that mtDNA had far greater persistence (Berry *et al.* 2007), the presence of viable DNA in a pool of scats produced in the week prior to searching was assumed to be a highly conservative assumption; (iv). the total pool of scats available in a fox's home range was also assumed to correspond to only those produced in the week prior to searching, although a far greater number of scats could be realistically assumed to accumulate over a longer period (Sadlier *et al.* 2004; Webbon, Baker & Harris 2004) especially in

the absence of heavy rainfall (Belt, Delibes & Raw 1991). The binomial probability (P) of replicating scat detection in a 100-ha search area in a 30-min search using scat detection dogs (after the detection of a single scat) was found for home ranges 100–1000 ha in size based upon the number of scats estimated to be produced by one, two and a social group of six foxes by:

$$P(k,n) = \frac{n!}{k!(n-k)!} \times p_2^k q^{(n-k)}$$

A weekly pool of scats (n_s) was assumed to be apportioned evenly over a home range despite a greater likelihood of a clustering of scats due to recurrent marking (Belt, Delibes & Raw 1991; Gallant, Vasseur & Berube 2007) that had yielded replicated genotypes in molecular surveys (Marks *et al.* 2009). Nonetheless, even distribution was considered another highly conservative assumption for the purpose of the model, and the detection of each additional scat was assumed to be independent from the first and influenced only by the number of scats present in the search area. Binomial probability using an exact test was also calculated when n was the number of searches where a fox-positive scat had been detected by the FEP using scat detection dogs ($n = 36$), k was the number of times a pair of scats had been detected in one search within a nearest neighbour distance of 1·42 km ($n = 2$), and p_2 was our modelled estimate of the probability of replicating two or more scats detections in a 30-min search in a 100-ha search area using scat detection dogs against various home range sizes.

Results

REPLICATED DETECTION OF FOX GENOTYPES IN MAINLAND FOX SCAT SURVEYS

Overall, 49 of 64 scats (77%) collected by visual searches in the mainland study site yielded viable genomic DNA amplified by microsatellites after up to 12-week storage. Of 30 genotypes detected, 12 of 30 (40%) were detected at least twice (2–6 detections) from all transects combined. The mean number of fox scats collected km^{-1} week^{-1} correlated strongly ($r^2 = 0{\cdot}94$) with the mean lineal density of fox genotypes km^{-1} on each transect from the four-day pool of scats (Fig. 2).

SPATIOTEMPORAL DISTRIBUTION OF FOX ASSIGNED SCATS IN TASMANIA

Nearest neighbour distances

Mean nearest neighbour distances for all fox-positive scats collected between 2002 and 2012 ($n = 61$) was 7·0 km (±2·9 km, $P < 0{\cdot}05$) with a mean of 280·6 days (±124·4 days, $P < 0{\cdot}05$) to form each nearest neighbour pair. Only three scat pairs were found within a 1·26-km radius that were collected in a period <38 days apart, and these corresponded to two contemporaneously collected scat pairs (2 of 36) located using scat detection dogs and one pair (1 of 25) collected by visual searches without dogs.

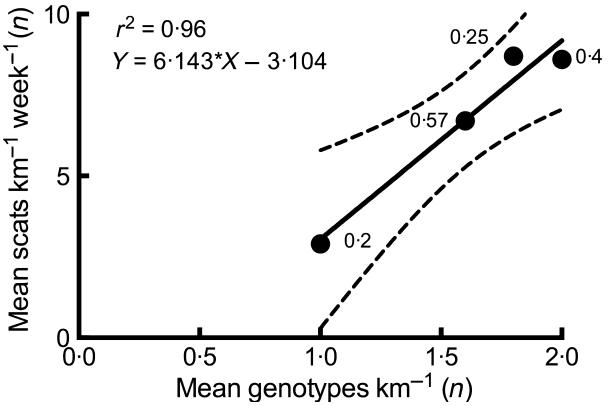

Fig. 2. Regression of mean scats collected km^{-1} week^{-1} with mean fox genotypes detected km^{-1} ($P = 0{\cdot}05$) determined from a four-day pool of genotyped scats collected at Werribee (mainland Australia) using visual detection of scats from four independent 5-km road transects (•). The frequency of genotypes detected twice or more is indicated for each transect.

Association of mtDNA fox scats with roads

Putative fox scats were found to be a mean distance of 82·3 m (±27·5 m, $P < 0{\cdot}05$) from vehicle access roads and 1·5 km (±0·57 km, $P < 0{\cdot}05$) from major highways. These distances were significantly larger for randomly placed points within the model of Tasmanian fox habitat proposed by Sarre *et al.* (2012) for vehicle access roads ($t = -4{\cdot}3$, d.f. = 70, $P < 0{\cdot}0001$) and highways ($t = -6{\cdot}95$, d.f. = 76, $P < 0{\cdot}0001$). Nearest neighbour scat distances correlated significantly with the index of average annual vehicles per day (AAVD) on adjacent highways ($r^2 = 0{\cdot}35$, $F = 30{\cdot}5$, d.f. = 1, $P < 0{\cdot}001$) and the average annual tonnage of freight shipped by road transport to and from the Port of Burnie ($r^2 = 0{\cdot}29$, $F = 24{\cdot}6$, d.f. = 1, $P < 0{\cdot}001$; Fig. 1).

MODEL OF FOX SCAT DETECTION PROBABILITY

Using the lowest empirically derived probability of detecting a single scats using dogs in a 30-min search ($p_1 = 0{\cdot}1$) for a single fox with an accumulation of one week of scats (56 scats week^{-1}) within a home range area of 400 ha (Coman, Robinson & Beaumont 1991), 520 ha (Phillips & Catling 1991) and 639 ha (Carter, Luck & McDonald 2012), the probability of detecting two or more scats each search was $P = 0{\cdot}53$, $P = 0{\cdot}41$ and $P = 0{\cdot}33$, respectively. Therefore, the binomial probability of recording only two incidences of replicated scat detection from 36 searches ranged between $P = 1{\cdot}26 \times 10^{-9}$ and $P = 0{\cdot}00001$. The probability of detecting two or more scats within an unexpectedly large 1000 ha home range was $P = 0{\cdot}2$, $P = 0{\cdot}44$ and $P = 0{\cdot}88$ for one, two and six foxes, respectively. Consequently, the binomial probability of detecting only two cases of replicated detection in 36 searches was $P = 0{\cdot}01$ (one fox), $P = 3{\cdot}35 \times 10^{-7}$ (two foxes) and $P = 2{\cdot}4 \times 10^{-29}$ (six foxes) (Fig. 3a). Using the highest

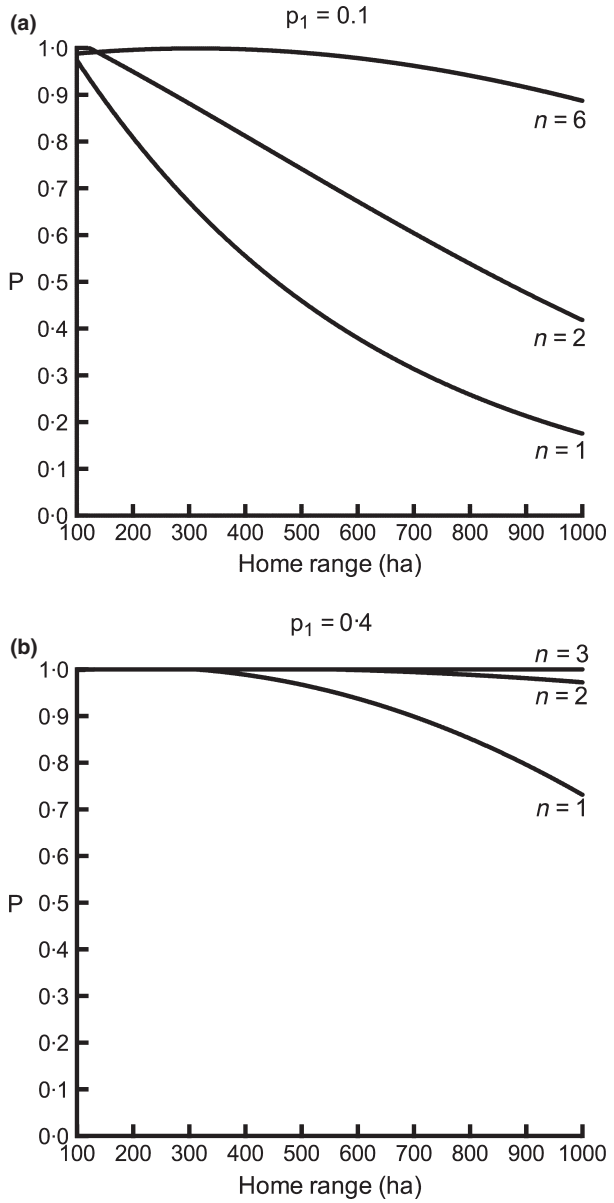

Fig. 3. Expected binomial probability (P) of replicating the detection of fox-positive scats after a single scat has been recovered using scat detection dogs in a standard search (100 ha area searched for 30 min) relative to home range size in hectares (ha) and a 7-day pool of scats produced by $n = 1$, 2 or 6 resident foxes. The binomial probability of replicated detection is based on the (a) lowest ($p_1 = 0·1$) and (b) highest ($p_1 = 0·4$) empirically derived probability of detecting a single scat.

empirically derived probability of detecting a single scat ($p_1 = 0·4$) replicated that scat detection was 100% probable for a single fox in a home range <400 ha (Fig. 3b).

Discussion

DISPERSION AND REPLICATION OF SCAT DETECTION

The placement of scats plays a key role in intraspecific communication and territorial demarcation (Macdonald 1980; White *et al.* 1989) and the defence of food resources (Macdonald 1980) for the red fox. The distribution of fox scats follows a bias in movement in proximity to reliable food, and scats are often used to mark free-feeding sites and bait stations (Marks *et al.* 2009). Foxes frequently return to the same locations in their home range (Carter, Luck & McDonald 2012), and the distribution of fox scats and those of other territorial mammals is clumped (Belt, Delibes & Raw 1991; Gallant, Vasseur & Berube 2007). This would increase the probability of encountering additional scats once single ones have been located and suggests that our model that assumes even scat distribution is likely to provide a conservative estimate of fox scat detection probability in this regard. In previous comparative mainland scat surveys conducted by Marks *et al.* (2009), replicated detection of fox genotypes was achieved with little collection effort using only a four-day pool of fox scats. A strong relationship also existed between the number of scats collected after visual detection and the known number of genotypes, where up to 57% of genotypes in one transect were repeat detections (Fig. 2). Visual scat detection is regarded as a far less efficient collection technique than the use of scat detection dogs where in one study dogs were capable of detecting up to 5·37 scats km^{-1} from transects (Smith *et al.* 2003). The probability of detecting maned wolf *Chrysocyon brachyurus* scats by dogs searching quadrants was calculated as 0·7 (Vynne *et al.* 2011) despite a much larger mean home range size (80·18 km^{-2}) for this species (de Almeida *et al.* 2009). Dogs have been able to detect scats in environments where experienced researchers report zero detection success (Oliveira *et al.* 2012).

In contrast to the previous mainland scat DNA surveys where multiple genotypes were detected routinely, only three contemporaneously collected mtDNA scat pairs (genotypes unknown) were reported within a 639-ha area in more than a decade in Tasmania. Overall, nearest neighbour pairs of fox-positive scats were widely spaced (mean = 7·0 km; circular area = 153 km^2) and generated after a protracted period (mean = 280·6 days). The failure to routinely replicate scat detections on 58 of 61 (95%) occasions in any one survey follows the collection of a large number of putative fox scats in Tasmania ($n = 9940$). Failure to replicate the detection of putative fox scats with dogs during 34 of 36 occasions appears to be a highly improbable outcome given that a high scat detection probability can be assumed for fox home range sizes typical of urban, peri-urban and rural environments and even atypically large 1000-ha ranges.

Overall, fox assigned scats in Tasmania were closely associated with urban and peri-urban environments, where home range sizes in similar mainland habitats for urban (Marks & Bloomfield 2006) and semi-urban habitats (White *et al.* 2006) have been reported to be <50 ha. In our model using the lowest empirically derived probability of scat detection ($p_1 = 0·1$) for 100-ha ranges containing a one-week pool of scats from a single fox, this

corresponds to an estimated probability of $P = 0{\cdot}98$ that two or more fox scats should be detected using trained dogs in each standard search (100-ha area searched for 30 min). Importantly, no fox baiting or other fox control had occurred in Tasmanian urban habitats by 2009 (Parkes & Anderson 2009, 2011), despite some authors indicating that Tasmanian foxes were widespread in these environments (Saunders *et al*. 2006; Parkes & Anderson 2011; Sarre *et al*. 2012). Accordingly, it is difficult to reconcile the failure to replicate scat detection in surveys and the absence of observations of extant foxes in environments not subjected to lethal control other than as an indicator of Type I error.

The 61 Tasmanian fox scats assigned by mtDNA were associated with vehicle roads and traffic flow that were not accounted for in the model proposed by Sarre *et al*. (2012) that associated 56 retrospectively assigned fox scats and nine cases of unspecified opportunistically acquired *post-mortem* evidence with habitats such as those defined by *Eucalyptus amygdalina* on sandstone geomorphology. However, such flora and geomorphology are widely distributed in the eastern half of Tasmania (Williams & Potts 1996), and the potential ecological significance accounting for red fox distribution remains unclear. Correlating fox assigned mtDNA scats with extremely general habitat classifications is difficult to justify without independent data and corroborative observations to demonstrate the predictive accuracy of this model.

WHAT OTHER HYPOTHESES COULD ACCOUNT FOR THESE DATA?

Are Tasmanian foxes itinerant?

Speculation that constant searches for breeding mates by establishing foxes may account for the failure to locate multiple fox-positive scats at any one location in Tasmania (Parkes & Anderson 2009, 2011) is not easily reconciled with fox breeding biology and the recruitment required to sustain a population for over a decade. Significant population turnover and an annual breeding strategy in foxes (Lindstrom 1989; Hone 1999; Harris 2008) give rise to intrinsic growth rates have been estimated to be between 0·84 (Pech & Hone 1988) and 0·65 (Hone 1999). Owing to neuro-endocrine regulation, breeding is strongly determined by photoperiod and the fox is an obligatory and predictably seasonal breeder where the vixen will produce only a single litter each year after a 52- to 53-day period of gestation (Lloyd & Englund 1973; Ryan 1976; Coman 1988; McIlroy, Saunders & Hinds 2001). This obligate breeding strategy requires the establishment of a breeding den (Lloyd & Englund 1973; Meia & Weber 1995), den attendance and a prolonged period of maternal care (Wright 2006) prior to sub-adult independence and possible, but not inevitable, dispersal of yearling foxes (Trewhella, Harris & McAllister 1988). Fox activity focuses upon the natal den within the maternal range during the breeding season (Meia & Weber 1995; Marks & Bloomfield 2006; Carter, Luck & McDonald 2012) so that large or itinerant movements of foxes are unlikely and the accumulation of multiple scats produced by approximately six foxes focused on the natal den is highly probable for many months after parturition.

Has the assumption of molecular data 'infallibility' overlooked causes of Type I error?

When survey data are uncorroborated, yet critical for affirming unique fox incursions or their distribution in eradication programmes, greater consideration of possible Type I error is warranted. The replication of independent wildlife survey data is a well-accepted requirement for assuring the precision of population estimates (Hurlbert 1984). Nonetheless, Parkes and Anderson (2009, 2011) assumed that mtDNA assays that assigned fox-positive status to scats (Berry *et al*. 2007) indicated the presence of an extant fox population with sufficient precision to counter 'rational doubt'. Other authors considered that it was inappropriate to doubt published molecular survey data (Blackman, Corcoran & Sarre 2013). However, although DNA evidence is highly regarded as an important technique for the molecular identification of invasive species (Paxinos *et al*. 1997; Piggott & Taylor 2003), it is not appropriate to consider the technique to be infallible. Increasingly, the evidentiary quality of molecular data used to define species incursions (Darling & Mahon 2011) and even criminal forensic evidence (Vecchiotti & Zoppis 2013) is considered in a wider context where various causes of error are considered. Particularly if the specificity and selectivity of the molecular test is not defined with precision and the provenance of the DNA detected is not known with certainty, in the absence of independent data replication, conservative conclusions are warranted. Inadequate site-specific spatial and temporal replication of mtDNA positive fox scats taken from a very large sample of mtDNA negative scats accumulated over a protracted period cannot be ignored by assuming an absence of Type I error.

A range of errors may influence the quality of molecular survey data such as sample (Darling & Blum 2007) and environmental contamination (Darling & Mahon 2011); hoaxing and use of samples of unknown provenance that have been submitted to the laboratory (Mills 2002; Stokstad 2002); or the use of assays with poor specificity or selectivity (Pompanon *et al*. 2005). Less stringent PCR conditions in particular may risk incorrect classification (Darling & Blum 2007; Gonçalves *et al*. 2014). Notably, the putatively fox-specific and rapid PCR assay designed to explicitly discriminate fox DNA from other species in Tasmania without sequencing (Berry *et al*. 2007) was found to amplify DNA from common species such as European rabbits *Oryctolagus cuniculus*, European hares *Lepus europaeus*, Tasmanian devils *Sarcophilus harrisii*, cows *Bos taurus* and pigs *Sus scrofa*, implying a

possibility of erroneous species classification and the generation of mixed species templates (Gonçalves *et al.* 2014). Moreover, the potential for predator scats to be contaminated by DNA arising from concurrently handled fox scats and other fox biological materials routinely sourced from the Australian mainland and used by the Tasmanian FEP to train scat detection dogs in the field appears to be a possible source of false positives that could account for poor independent data replication.

The detection of a fox-positive scat on Bruny Island (Anon 2012) is especially notable given that the island is separated from Tasmania by the D'Entrecasteaux Channel that is an approximately 2-km-wide water barrier at its closest point (Fig. 1). It is extremely unlikely that foxes could have colonized this offshore island and searches have so far failed to reveal any corroborating evidence of their presence. Such equivocal molecular data warrant confirmation using independent survey techniques that are capable of unequivocally affirming the presence of extant foxes (Marks *et al.* 2014).

CONCLUSIONS

Uncorroborated habitat-specific models may provide a misleading indication of the presence and distribution of an invasive species. If used to justify or inform eradication programmes, models that have used training data affected by Type I error may overstate risk and misdirect resources, particularly if *ad hoc* assessment of molecular data quality has overlooked sources of Type I error. Rigorous qualitative data analysis is appropriate when data are few yet relied upon to make critical decisions. Molecular data of unknown precision should be regarded as equivocal if collected with inadequate replication and particularly if sampling deviates from expected detection success and results in spatiotemporal patterns that are not easily reconciled with the presence of an extant population. Until the model proposed by Sarre *et al.* (2012) is corroborated with adequately replicated and independent observations, it has not convincingly demonstrated that living foxes were widespread in Tasmania as these current data appear to best fit a pattern accounted for by Type I error.

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