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The truth about scats and dogs: Next-generation sequencing and spatial capture–recapture models offer opportunities for conservation monitoring of an endangered social canid

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ABSTRACT

Obtaining accurate population counts of endangered species is central to conservation biology, with implications for gaining ecological insights, informing management strategies, and judicial use of conservation funds. Despite decades of progress in methodological developments in the realm of population ecology, reliable density estimates are unavailable for many species of conservation concern. The dhole (Asiatic wild dog *Cuon alpinus*) is one such endangered large carnivore found in the tropical forests of south and southeast Asia. Here, we (i) develop next-generation sequencing resources to identify individual dholes from genetic samples, (ii) apply these methods to identify individuals in the wild, from scat (fecal) samples collected through systematic field surveys and (iii) generate reliable estimates of dhole densities in Wayanad Wildlife Sanctuary (Western Ghats, India) using Spatial Capture–Recapture 'SCR' models. We estimate dhole densities to be 12–14.2 individuals/100 sq. km based on a set of SCR models, with ~50 individuals within Wayanad's administrative boundary. Our study presents a methodological improvement in generating population estimates of an important apex predator while also offering ecologically informative insights on a species in dire need of science-based management efforts. Replicating this study across connected reserves and over time can serve as a unified framework for understanding population dynamics, population structures, landscape connectivity and metapopulation-level conservation requirements. We propose that the approach presented here may be adopted as an economically and logistically feasible protocol for conservation monitoring of dholes and other ecologically important species plagued by similar issues of data-deficiency, and insufficient funding and resources.

1. Introduction

Conservation monitoring of endangered species often rests squarely on obtaining estimates of their population size, or density – perhaps the most crucial metric for ascertaining conservation status (Williams et al., 2002; Witmer, 2005). Estimates of abundance are equally important for gaining ecological insights, monitoring population trends and evaluating effectiveness of management interventions aimed at recovery of rare or threatened species (Nichols and Williams, 2006). In this context, terrestrial carnivores present a good case study. They are among the most threatened taxonomic groups (Schipper et al., 2008), with governments, public institutions and conservation organizations spending enormous resources towards their conservation (Treves and Karanth, 2003). Certain charismatic carnivore species are also flagships

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for a great number of ecosystem conservation programs across the globe (Macdonald and Loveridge, 2010; Smith et al., 2012). But estimating abundances of these carnivores remains a challenge because most of them are elusive, nocturnal and typically occur in low numbers. Furthermore, methodological challenges and wide application of flawed, index-based population counts continue to undermine management decisions drawn from conservation research (Karanth et al., 2003; Hayward et al., 2015). As a consequence, rigorous and statistically sound long-term population studies are also virtually non-existent for many of these species (Ripple et al., 2014; Wolf and Ripple, 2017).

Heralding a significant shift away from count-based methods that do not account for partial detectability of individuals, the development of mark–recapture models has been a prominent line of research in the pursuit of reliably estimating animal populations (Williams et al., 2002). Capture–recapture analysis of photographic data obtained from camera-trap surveys have been particularly effective in estimating abundance of species with natural body markings or pelage patterns (e.g., Royle et al., 2009a; Sollmann et al., 2011; Alexander et al., 2016). Spatial extensions of these models, or, Spatial Capture–Recapture (SCR) models have further enhanced the reliability of population size and density estimates by incorporating geographic locations of individual encounters in the modelling process (Royle et al., 2009b, 2013). For species lacking natural body markings or pelage patterns, genetic typing of DNA extracted from their hair, tissue or feces is an alternative option to identify individuals (Waits and Paetkau, 2005). Non-invasive genetic sampling, combined with SCR models can be applied to estimates of population sizes or densities, demographic parameters, and thereby offers a powerful tool for conservation monitoring of carnivores (e.g., Chandler and Clark, 2014).

Mark–recapture models and their extensions are among the most sophisticated methods available for estimating animal populations. But the approach relies on identifying 'marked' individuals without ambiguity (Otis et al., 1978). In practice, varying levels of uncertainty are associated with the process, depending on the type of survey and the nature of data collected (Augustine et al., 2020; Johansson et al., 2020). In genetic mark–recapture studies, individual identities have long been assigned using microsatellite data through DNA extracted from non-invasively collected samples (e.g., Fuller et al., 2016; Morin et al., 2018). Recent developments in high-throughput amplicon sequencing using Single Nucleotide Polymorphisms 'SNPs' (vonHoldt et al., 2011) offer greater promise in circumventing challenges inherent in sparse and low-quality samples, which are commonplace with microsatellite data (von Thaden et al., 2020). These sequencing-based methods using SNPs could therefore provide better opportunities for application to carnivore population studies that usually generate low sample sizes, which are degraded or of low quality (Natesh et al., 2019).

Dholes (or Asiatic wild dogs, *Cuon alpinus*) are social carnivores found in the forests of south and southeast Asia (Kamler et al., 2015). Despite their 'Endangered' status (IUCN Red List) and purportedly declining numbers, statistically robust estimates of their abundance are lacking from most parts of their distribution range (but see Ngoprasert et al., 2019). Since dholes do not have uniquely identifiable pelage markings, population assessments have hitherto relied on surrogate metrics like encounter rates and relative abundance indices (Venkataraman, 1998; Selvan et al., 2014), or distribution and habitat associations examined at various spatial scales (Srivathsa et al., 2014, 2020a; Punjabi et al., 2017; Singh et al., 2019). India harbors the largest population of the species, with key metapopulations clustered in three landscapes– Western Ghats, Central India and Northeast India. Dholes are habitat-sensitive and occur mostly within protected forest areas (Kamler et al., 2015; Srivathsa et al., 2020a, 2020b). Unprotected multi-use forest fragments and agroforests adjoining protected reserves likely support smaller populations, while also aiding movement and dispersal of individuals (Gangadharan et al., 2016; Srivathsa et al., 2019a, 2019b).

Estimating abundance of animals that do not have natural/artificial marks, particularly those that cannot be easily captured and tagged, has been a long-standing issue in population studies (Gilbert et al., 2020). This is particularly a challenge for social/group-living carnivores; relatively fewer studies have used a combination of genetic tools and capture–recapture models for such species (e.g., Cubaynes et al., 2010; Caniglia et al., 2014; Murphy et al., 2018). Given the purported benefits of using high-throughput sequencing in genotyping individuals, our study focuses on the dhole as a model species and addresses three specific objectives:

(a) develop high-throughput sequencing based methods (as demonstrated by Natesh et al., 2019) to identify individual dholes using genetic information (SNPs);
(b) assess the field applicability by implementing these methods to identify individuals in the wild, using fecal samples collected non-invasively through systematic field surveys;
(c) provide density estimates and associated parameters derived from a set of SCR models, tailored to the type(s) of data commonly generated from non-invasive field surveys.

2. Materials and methods

2.1. Genome sequencing and SNP identification

We collected tissue samples from two captive dholes and one sample from a wild individual from the Western Ghats landscape in India and extracted DNA using the Qiagen DNeasy Blood & Tissue Kit (Catalog No.69504). Whole-genome libraries were prepared from the extracts using NEBNext Ultra II DNA Library Prep Kit (Catalog No. E7645S) and sequenced using 150 base-pair paired-end read chemistry on Illumina HiSeq X platform. Raw sequence reads were obtained for two additional samples from the NCBI SRA database (SRX4036090 and SRX4878898), trimmed and filtered for low quality using Trimmomatic (v0.36.0; Bolger et al., 2014), and mapped to a domestic dog reference genome (Hoeppner et al., 2014) using Bowtie2 (v2.3.0; Langmead and Salzberg, 2012). We sorted the mapped reads using SAMtools (v1.4; Li et al., 2009), filtered for PCR duplicates and indexed after adding read groups using Picard (v2.9.0; https://broadinstitute.github.io/picard/). Variants across samples were called using *freebayes* (Garrison and Marth, 2012) and filtered to include genotypes with minimum quality and depth of 30 and 10, respectively. The final variant call format (vcf) file included SNPs with minimum site-wide quality of 30, missing data (<20%), Hardy–Weinberg Equilibrium (variants with $p < 0.05$ removed) and a minimum allele count of 3. Additional details are provided in Supplementary File S1.

2.2. Designing SNPs

In the absence of a dhole reference genome, we identified potential primer sites by generating a 'makeshift' dhole genome in the following manner. Using the "doFasta 2" option in ANGSD (Korneliussen et al., 2014), we filtered for a minimum mapping quality of 30 and a site quality of 30 to generate a dhole fasta file from filtered sequencing data from a captive individual. We ran the "mPCRseq-dhole" program (https://github.com/rwtaylor/mpcrseq-dhole) to design a set of SNP primers for dholes. We improved upon the methods described in Natesh et al. (2019) by incorporating heuristics for primer success gleaned from literature, checks for pairwise primer-dimers, and primer specificity checks within the dhole genome and potential prey genomes. We used adapters from GTseq (Campbell et al., 2015), and designed the initial primers with Primer3 (Untergasser et al., 2012).

We obtained genome data (in fasta format) for co-occurring prey and predator species to check for and avoid overlap in sequences. For cases where the exact prey/predator species' genome was not available, we ensured representation by using a surrogate genome of closely related species from the same family (see Supplementary File S1). We selected

150 SNP primers based on exact matches to raw sequence data and SNP allele frequency to test with a different, independent set of fecal samples that had been genotyped with microsatellites (Supplementary File S1). After filtering the sequenced reads, we calculated the probability of identity (pID) – the probability that any two randomly selected individuals would have the same genotype – using GenAlEx (v6.5; Peakall and Smouse, 2006, 2012) to evaluate the confidence of individual identification.

2.3. Field surveys

We conducted field surveys in Wayanad Wildlife Sanctuary (Kerala, India), which extends across an area of 344 sq. km., separated into a north block (78 sq. km) and a south block (266 sq. km). Wayanad is contiguous with Nagarahole and Bandipur Tiger Reserves in Karnataka State and Mudumalai Tiger Reserve in Tamil Nadu State (Fig. 1). The sanctuary has a mixture of moist deciduous, dry deciduous and savannah woodland vegetation, and supports a wide assemblage of large mammals including the tiger *Panthera tigris*, leopard *Panthera pardus*, elephant *Elephas maximus*, gaur *Bos gaurus*, sambar *Rusa unicolor*, chital *Axis axis*, muntjac *Muntiacus muntjac* and wild boar *Sus scrofa*, besides dholes.

We surveyed along predetermined forest roads/trails, designed so as to maximize spatial coverage of the area (Fig. 1). Survey routes (10–15 km each) were divided into 100-m segments using a handheld GPS unit. Dhole scat samples were collected through systematic surveys in May–June 2019 by trained field surveyors. Each route was sampled between 1 and 6 times (each day treated as a sampling occasion), depending on logistical feasibility. Dhole scats were identified based on size, shape, location of deposition, and physical attributes such as scat piles in latrine sites (Andheria et al., 2007). Fresh dhole scats (scats deposited within 1–2 days in direct sunlight or 2–3 days under canopy shade) were collected using swabs (two separate swabs drawn for each piece of fecal matter) and stored in a lysis buffer solution (Longmire et al., 1997; Ramón-Laca et al., 2015). Geographic coordinates, condition of scats, and other descriptive details were entered onto datasheets by the surveyors. In each 100-m segment, we also recorded detections of old scats (3–7 days), track marks (usually 1–2 days old) and direct sightings; indirect signs that could not be unambiguously identified as belonging to dholes were not recorded.

2.4. Analysis: genetic methods

We extracted DNA from field samples using Qiagen DNeasy Blood & Tissue Kit (Catalog No.69504), adhering to the manufacturer's protocol but with some modifications. After adding 20 μl of proteinase K to 180 μl of the sample(s), the mixture was incubated overnight at 56 °C, and the DNA was then eluted in a final volume of 120 μl of AE buffer. Average DNA concentration was determined through quantitative polymerase chain reaction (qPCR) for a random subset (n = 46) of fecal sample extracts (additional details are in Supplementary File S1). Each qPCR constituted three replicates of a sample and two sets of negative controls, with no template, to test for contamination.

The library was prepared with the selected 150 SNP primers following protocols described in Natesh et al. (2019), with paired-end sequencing of 75 bp performed on an Illumina MiSeq platform. Variants were called once the sequenced data had been trimmed with a minimum phred score of 33 (each read has a base call accuracy of at least 99.9% and a minimum length of 30 base pairs), mapped with a minimum mapping quality score of 30 and sorted. We then subset the variant pool to retain only the focal SNPs filtered for genotype quality (>30), site quality (30), minimum depth (15) and missing data (<30%) using VCFtools (Danecek et al., 2011) and GATK (DePristo et al., 2011). Samples that performed poorly (>90% missing data) were removed from the analysis.

Individuals were identified using estimates of pairwise relatedness between samples, calculated using PLINK (version 1.9; Purcell et al., 2007). We first checked the relatedness scores for within-sample replicates, i.e., extracts from the two swab-draws from the same fecal sample. From relatedness estimates for within-sample replicates, we chose a soft threshold value of 0.80 (ideally, the two extracts should have had score of 1.00). Any pair of independent samples that had a relatedness value >0.8 were inferred to have come from the same individual. Each pair of fecal samples had up to 4 relatedness scores (two extracts for each sample). We carefully examined all matches with relatedness scores of 0.75 onwards to check for, assign and confirm individual ID assignments (additional details are in Supplementary File S1).

2.5. Estimating density using SCR models

To create the state-space, we first overlaid an array of 0.25 sq. km grid-cells (or pixels) across a large region encompassing a buffer of ~10

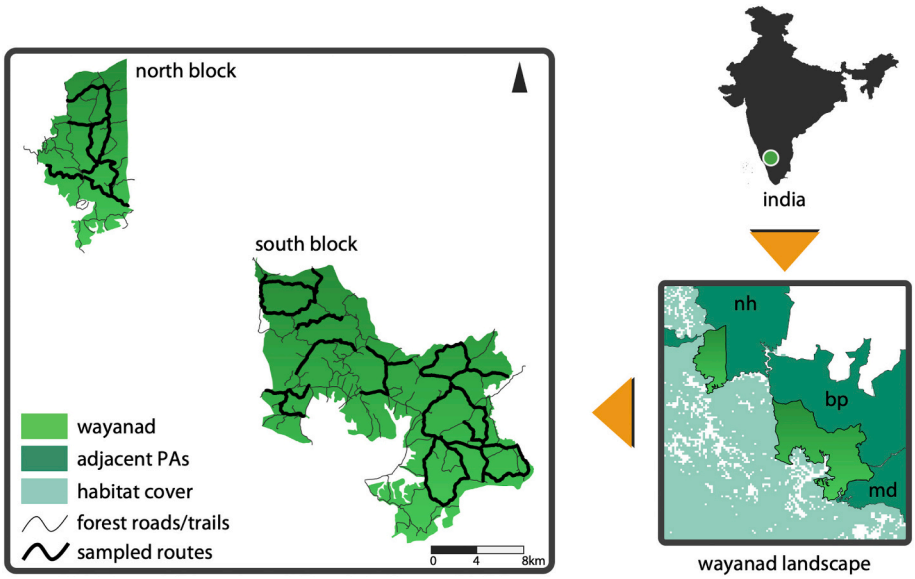

Fig. 1. Map of the study area showing Wayanad Wildlife Sanctuary in the State of Kerala, southern India. The sanctuary is embedded amidst a cluster of protected reserves (NH– Nagarahole, BP– Bandipur, MD– Mudumalai) and a non-protected habitat matrix with forests and agroforests.

km beyond the outer bounds of sampled routes.

Dholes are mostly restricted to forest habitats and sometimes use agroforest areas. We retained pixels with these two habitat types, and removed pixels that had open agriculture lands, large water bodies and large human settlements, i.e., habitats where potential activity centres cannot occur. The centroids of a subset of the cells that included sampled routes were treated as "detectors" (Fig. 2), and all detections of dholes within each of these sampled cells were associated with the corresponding detector. The classical SCR model treats space usage by individuals to follow a bivariate normal distribution, with expected number of encounters at each detector following a half-normal detection function (Royle et al., 2013). This classical model under a Bayesian framework estimates two key parameters: g0, the baseline detection probability at a detector device at an individual's activity center and σ, the scale parameter related to movement range of individuals. Density *D* is a derived parameter computed using g0 and σ, together with *N* the estimated number of individuals within the state-space (Royle et al., 2013).

Our field data included information on captures/recaptures of genetically marked individuals as well as unmarked detections– scats that could not be assigned individual identities through genetic methods, old scats (3–7 days old) that were deemed unfit for collection, track marks and direct sightings (Fig. 2). We adapted and built upon the Multiple Observation Process (MOP) model described by Tourani et al. (2020), which fully utilizes all sources of information to estimate the ecological state parameter (*N*), after parsing the sampling process into 'marked' and 'unmarked' detections to estimate p0– a shared detectability parameter. We implemented three variants of the model:

(i) *Model 1*: Classical SCR model using only data from marked individuals, and all model parameters estimated with uniform priors. This may be viewed as a reduced version of the MOP model, where detections arise from a single sampling process. For each individual, multiple detections at a detector location on a particular sampling occasion were collapsed into a binary format (1/0) to avoid issues with temporal autocorrelation.

(ii) *Model 2*: Standard MOP model using data on marked and unmarked detections, and model parameters estimated with uniform priors. This includes p0, a shared detectability parameter estimated from combining marked and unmarked observations, and an additional parameter, α, which is the probability that a successful detection is from a marked individual. In other words, α is the proportion of all detections for which we could assign individual identities (therefore, detectability for marked detections ≈ p0*α; detectability for unmarked detections ≈ p0*(1–α)). Unmarked detections at each detector for each sampling occasion were modelled as a Bernoulli process (see Tourani et al., 2020 for details).

(iii) *Model 3*: Model 2 with a partially informed prior distribution specified for σ, based on current knowledge of dhole home-range sizes (Srivathsa et al., 2017). We specified a normally distributed prior with a reasonably wide standard deviation (mean = 3500 m; SD = 1000; precision = 0.1e-5). Example code and data are provided in Supplementary File S2.

Model 3: Model 2 with a partially informed prior distribution specified for σ, based on current knowledge of dhole home-range sizes (Srivathsa et al., 2017). We specified a normally distributed prior with a reasonably wide standard deviation (mean = 3500 m; SD = 1000; precision = 0.1e-5). Example code and data are provided in Supplementary File S2.

We fit data to all the models under a Bayesian framework using Markov Chain Monte Carlo (MCMC) methods implemented in R v3.6.2 (R Core Team, 2017) and NIMBLE (de Valpine et al., 2017). The augmentation value *M* (maximum number of individuals that could potentially exist in the state-space) was set to 525, roughly 20 times the number of individuals detected and identified. We ran 20,000 MCMC iterations for each model, drawing posterior estimates from three chains with a burn-in of 2000 iterations, and the thinning rate set at 5. Convergence was assessed by examining trace plots and associated Gelman-Rubin diagnostic scores (Gelman and Rubin, 1992) for each parameter. We computed dhole abundance for the 'effective sampled area' (N_{esa}) summing grid-cell level estimates within a region demarcated by applying a buffer of $\sqrt{5.99} \times \sigma$ to the outer bounds of the sampled routes (see Royle et al., 2013 and Srivathsa et al., 2015 for details on effective sampled area calculations). We also present the abundance of dholes in Wayanad (N_{wy}) as a sum of grid-cell level estimates exclusively within the reserve's administrative boundary.

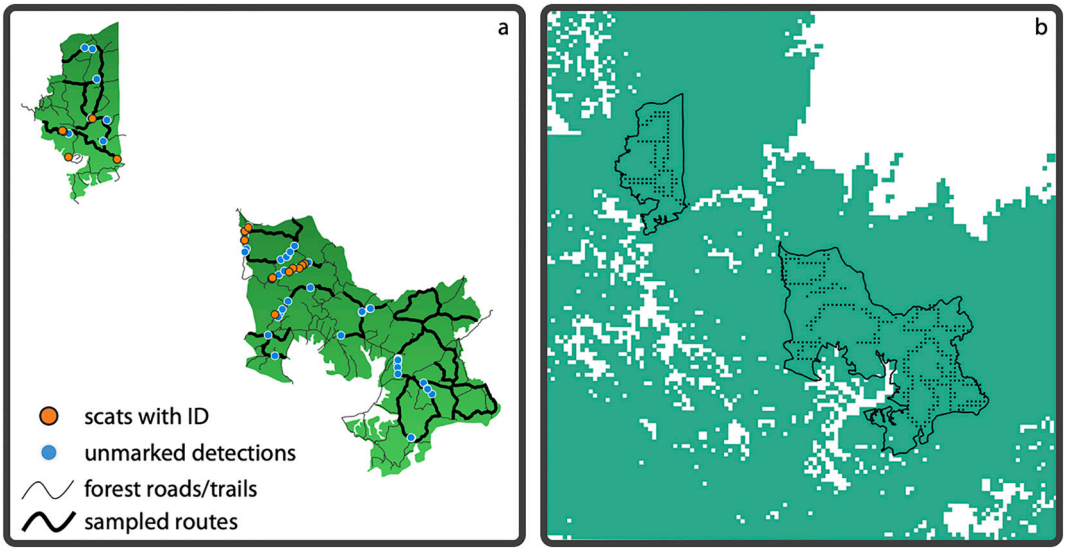

Fig. 2. Detections of dhole signs and detector grid array in Wayanad, 2019. (a) Spatial locations of scats from which individual dholes could be identified through genotyping, and all unmarked dhole detections– samples that could not be genotyped, old scats that were deemed unfit for collection, track sets and direct sightings; (b) State-space with a grid-cell array of 0.25 sq. km pixels. Colored areas represent dhole habitats, and black dots are detectors– centroids of pixels were traversed by sampled routes.

3. Results

Using the three tissue samples and two NCBI genomes, we identified a total of 351,397 SNPs and 607 primer pairs that constituted 344 unique target sites. The 607 primer pairs were then reduced to one primer pair per target site; 13 of these either matched multiple sites in the genome or did not have any matches. Upon testing the 150 SNP primer set with an independent set of fecal samples (Supplementary File S1), we obtained a final set of 75 SNPs across 18 individuals, for which estimated probability of misidentifying individuals was low (pID = 2.7E-18; pID_{sibs} = 1.0E-09).

For the field surveys, we invested a total of 741 km of walk effort to collect 114 fecal samples (228 sample extracts counting two replicate swab-draws per fecal piece). Of the 46 sample extracts randomly selected for qPCR, the median DNA concentration was 0.24 ng/μl (three extracts had extremely low concentration). After filtering the sequenced reads for sample extracts (n = 228), 50 extracts performed poorly (>90% missing data). The final vcf file consisted of 75 SNPs across 178 extracts; both swab-draws worked for 84 fecal samples and only one of the two draws worked for 10 fecal samples (genotyping success rate = 82.5%; Fig. 3). Average relatedness between pairs of within-sample replicates (n = 84) was 0.98 (range: 0.86–1). Using pairwise relatedness estimates across all samples at a soft threshold of 0.8 (Supplementary File S1), we identified 26 unique individuals, 24 of which were in 4 distinct spatial clusters, plausibly from 4 packs (deduced based on locations of captures/recaptures and field observations). The number of individuals detected ranged from 3 to 8 per spatial cluster.

The number of individual recaptures ranged from 2 to 12. The field surveys also generated 93 unmarked detections– 19 samples that could not be genotyped, 39 old scats that were deemed unfit for collection, 31 track sets and 4 direct sightings. Upon collapsing these data into binary format (1/0) for SCR analyses as described in the Methods section, 17 individuals had single captures, seven had two recaptures, two individuals had 3 and 4 recaptures each, and the number of unmarked detections was 72.

Parameter estimates and abundance values generated from Models 1, 2 and 3 are presented in Table 1. Gelman-Rubin diagnostic scores confirmed convergence of chains for all the estimated parameters (Supplementary File S3). Surface density maps in Fig. 4 depict grid-cell level posterior estimates across the state-space. Estimated mean densities ranged from 12 to 14.2 individuals per 100 sq. km across the three models. This translated to 64–69 individuals within the effective sampled area. The density estimate from Model 1 was slightly lower (Table 1) and the corresponding surface density map failed to project activity centres in locations that had evidence of dhole presence but no marked detections (Figs. 2 and 4). The similarity between parameter estimates in Models 2 and 3 suggested that, (i) dhole movement range during the sampling period was much lower than seasonal home range, and (ii) the data were adequately informative since the prior distribution specified did not alter the estimate of σ parameter (σ = 927 (SD ± 104) and 955 (SD ± 115) for Models 2 and 3, respectively).

4. Discussion

We present a strategic application of next-generation sequencing techniques– whole-genome resequencing and amplicon resequencing– for identifying individual dholes from non-invasively collected samples, and estimating density using advanced SCR models. Despite its utility, a combination of SNPs and SCR models has not been widely implemented till date (Arandjelovic and Vigilant, 2018). Our SNP–SCR approach, which relies on relatedness scores within and between samples as the basis for ascertaining individual identities, still represents a methodological improvement in generating population estimates while also offering ecologically informative insights for a species that is extremely difficult to observe or study in the wild.

4.1. Advancements over previous genetic methods

In an attempt to circumvent issues with misidentification of individuals, microsatellite-based studies typically gravitate towards using a smaller set of "high-quality" samples, and in the process discard many "low-quality" samples (von Thaden et al., 2017). In our study, discarded samples were few, and included only those that were extremely data-poor. A sufficiently large SNP set allowed for retaining samples at the cost of losing loci, but with the resulting loci still having sufficient power to identify individuals in the study population. Furthermore, our study relies on publicly available software programs to design primers and analyze data, capitalizes on information generated from next-generation sequencing tools, and allows for generating and readily comparing data across different laboratories (von Thaden et al., 2017, 2020). Besides the added benefits of data retention, the other advantage is of cost-effectiveness; multiplex PCR for genotyping has a substantially lower cost-per-sample and higher genotyping success rate as compared to other methods (Natesh et al., 2019; Eriksson et al., 2020). Our sequencing costs per sample replicate amount to $8, with one-time costs of primer designing at $10–25 per primer pair. In contrast, a standard microsatellite-based assessment entailing 12 microsatellite primers multiplexed at 4 loci would be ~$30 per sample (including 3 replicates), with a cost of $140 per fluorescently-labelled microsatellite primer pair. Our genotyping success rate of 82.5% was higher than microsatellite-based studies of dholes that have reported 24.5% (Iyengar et al., 2005) and 40.6% (Modi et al., 2019). However, we caution against the

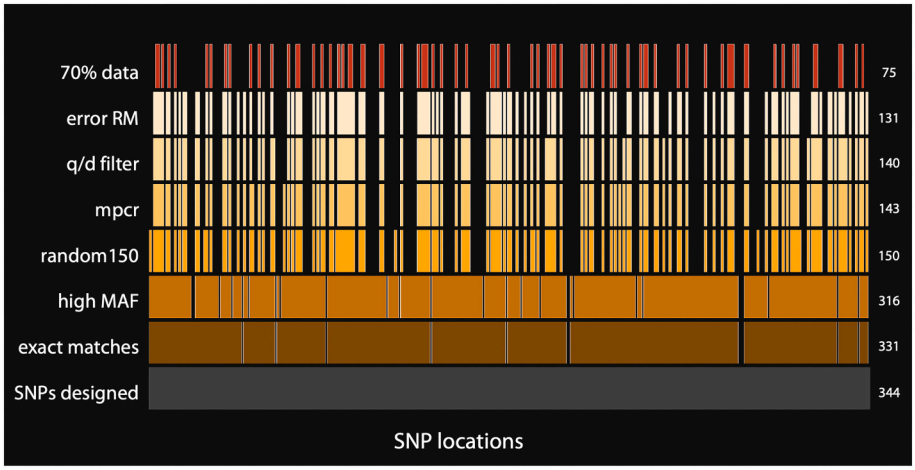

Fig. 3. Illustration of SNP filtering process using stepwise criteria. SNPs designed– 344 SNPs for which primer pairs were designed; exact matches– SNPs for which primer pairs had unique, exact matches to dhole; high MAF– SNPs with high minimum allele frequency; random150– SNPs for which 150 primer pairs were randomly selected; mpcr– primer pairs successfully amplified in multiplex PCR; q/d filter– filtered for genotype quality, site quality and depth; error RM– erroneous SNPs removed; 70% data– SNPs with at least 70% data. Numbers on the right indicate total SNPs retained in each step.

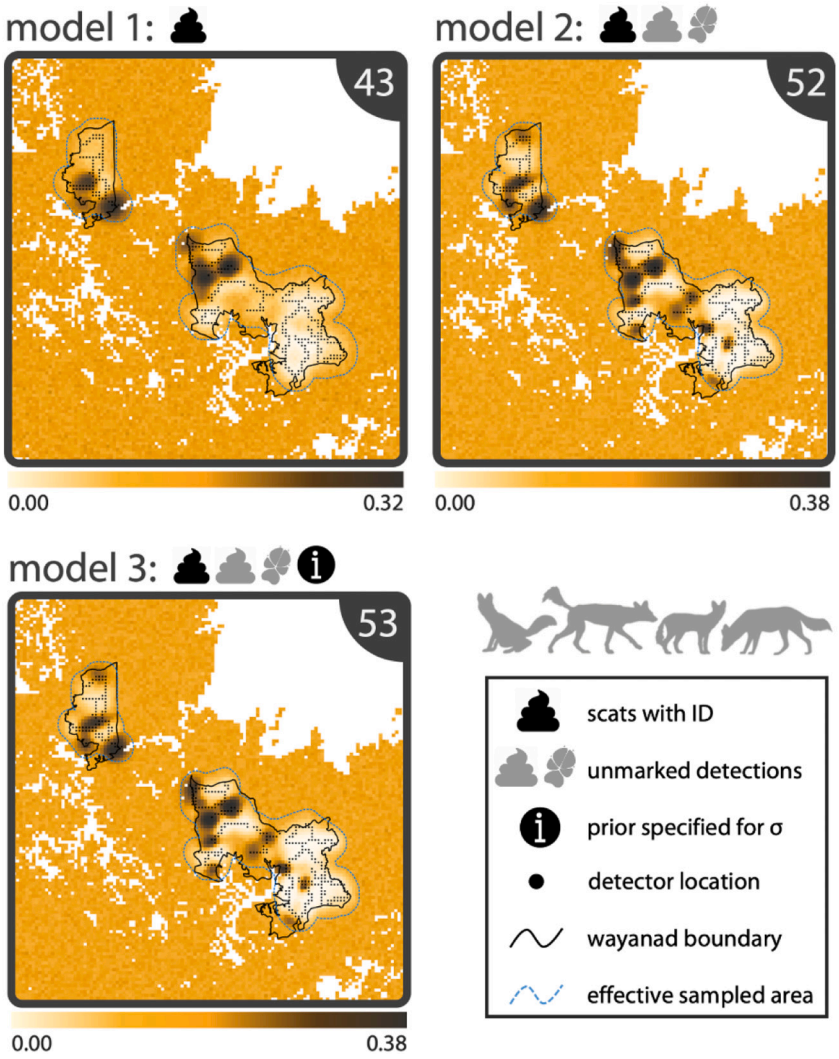

Fig. 4. Surface density maps from SCR models to estimate dhole densities in Wayanad landscape (2019). Pixel-level posterior estimates depict spatial patterns of dhole densities from Model 1 (only scats with ID), Model 2 (scats with ID and unmarked detections) and Model 3 (scats with ID, unmarked detections, and informative prior for σ). Numbers on the top-right in each map denote estimated dhole abundances within the administrative boundary of Wayanad from the corresponding model.

Table 1
Parameter estimates from SCR models fit to non-invasively collected dhole data (scats and signs) in Wayanad Wildlife Sanctuary (2019). Models 1, 2 and 3: g0 or p0– baseline detection probability, α– probability that a successful detection is from a marked individual, ψ– probability that an augmented individual was a real one, σ– scale parameter related to movement range and *D*– average density of dholes. Abundance parameters: *N* is the total number of dholes in the state-space, N_{esa} is the number of dholes within the effective sampled area and N_{wy} is the number of dholes exclusively within Wayanad administrative boundary.

	g0 (SD)	p0 (SD)	α (SD)	ψ (SD)	σ (SD)	*N* (SD)	N_{esa} (range)	N_{wy}	*D* (100 km^{-2})
Model 1	0.05 (0.02)	–	–	0.67 (0.15)	1121 (221)	350 (76)	64 (54–86)	43	12.0
Model 2	–	0.16 (0.04)	0.33 (0.04)	0.78 (0.12)	927 (104)	411 (62)	69 (60–80)	52	14.2
Model 3	–	0.15 (0.04)	0.33 (0.04)	0.78 (0.13)	955 (115)	409 (67)	69 (61–81)	53	14.1

direct comparison of genotyping rates across studies since sample conditions, number of primers assessed, variation in repeat motifs, and consensus genotype calling could affect success rates.

We note here that our methodology involved using relatedness scores to identify unique individuals, which is one among several potential methods (Galpern et al., 2012; Wang, 2016). This choice was based on a trade-off between being extremely conservative (e.g., allele-matching) versus optimizing the sample size and field protocols followed in our study. We strived to use a reasonable benchmark (relatedness scores of within-sample replicates) to determine a potential threshold for distinguishing individuals, but the relatedness scores may have been overestimated because of biased allele frequency estimates (S1 Fig. 1). Nonetheless, we recommend that scientists emulating methods presented here should adopt an approach that best suits their sampling process, data type and quality.

4.2. Field and analytical approaches

Field-based carnivore monitoring projects generally produce multiple types of data (camera trap photographs, geographic coordinates from telemetry, genetic samples, etc.). Ignoring ancillary data or failure to fully exploit all data sources could potentially produce biased

parameter estimates, or yield highly imprecise results with little ecological or conservation value (Ruprecht et al., 2020). Recent advancements over the classical SCR model have used a suite of data integration techniques in this endeavor (Gopalaswamy et al., 2012; Tenan et al., 2017; Murphy et al., 2018). By following a sampling protocol that involved (i) two swab-draws per sample and (ii) meticulous recording of all direct and indirect detections, our study redresses the twin issues of discarding samples because of genotyping uncertainty (Augustine et al., 2020), and discounting supplementary 'unmarked' data (Tourani et al., 2020). The three models we use are also representative of three scenarios— (a) when field data includes only detections of marked individuals, (b) field data also includes unmarked detections, and (c) data-based or field-based knowledge exists for defining priors distributions for parameters. We believe this treatment provides flexibility to scientists and managers dealing with subsets or combinations of data types, as is typical for studies of terrestrial carnivores or other similar species, particularly in the tropics.

4.3. Methodological considerations and caveats

We recognize a set of potential caveats and analytical considerations that are relevant to our study. First, since a dhole reference genome is not currently available, we used a domestic dog reference genome (as explained in Section 2.2 and Supplementary File S1). We anticipate future efforts in this regard would be invested in developing a dhole reference genome, and generating a larger set of sequences and identifying dhole-specific SNPs. Second, our model parameterization involved combining scats that could not be genotyped, old scats, tracks and direct sightings under a single category of "unmarked detections". In the original model formulation by Tourani et al. (2020), each survey type was associated with a separate baseline detection probability (p0) shared by identified and unidentified samples of that survey type. Due to small sample size, we were unable to estimate separate detection parameters for scats, tracks and sightings; this may have affected our estimates of detection probability and consequently, abundance, in the combined model. But given the large overlap in abundance estimates across all the models, this potential effect was negligible. Accounting for sample size considerations, we propose that future studies may explicitly model detection probabilities for each type of sign separately. This could also provide a comparison of the relative detectability of different types of detections. Third, incorporating and exploring the influence of ecological and anthropogenic covariates on expected spatial densities can provide useful insights on species–habitat relationships (Proffitt et al., 2015). Unfortunately, we could not implement covariate models because our field sampling was strictly within reserve boundary limits, with no spatial representation from outside the Protected Area which constitutes a sizable part of the state-space. Given that field conditions between these two zones are substantially different, we could not measure covariates that adequately reflected these patterns. Finally, we acknowledge that our approach may not meet the assumption that spatial locations and detections of individuals are fully independent. Dhole packs may contain 2 to 14 members, and on occasion, up to 25 individuals (Srivathsa et al., 2020c). SCR models are generally robust to non-independence of individuals' activity centres and capture probabilities (López-Bao et al., 2018), but this needs to be explicitly tested considering the aggregation and cohesion patterns in dhole packs (see Bischof et al., 2020).

4.4. Ecological insights and conservation implications

Wayanad, a relatively well-protected reserve, supports around 14 dholes per 100 sq. km, with ~50 individuals within the reserve boundary (around 7 packs of 6–8 members on average). Higher densities were clustered in drier, more rugged parts of the reserve and at lower elevations (Fig. 4). These conditions reflect mixed- to dry-deciduous forests which support higher densities of mid- to large-sized ungulate prey species (Kumar et al., 2021). Dhole density here is perhaps at the higher end of the spectrum, based on the typical range for large carnivore densities in tropical forest habitats. The only other attempt at estimating dhole density is from Thailand, where Ngoprasert et al. (2019) report 2.2–3 individuals per 100 sq. km; but we note that the aforementioned study used a count-based modelling framework (entailing certain strong assumptions and requirements) in a relatively low prey density area. Our results are also interesting in light of current tiger densities in Wayanad (11–12 per 100 sq. km; Jhala et al., 2015), consistent with findings by Karanth et al. (2017) that the two large predators can in fact co-exist at high densities under ideal conditions. Since the current IUCN assessment for dholes is based on expert opinions and surrogate population indices (Kamler et al., 2015), our study is a first step towards making quantitatively informed assessment of the species' status. We believe that the full potential of this approach would be realized with estimating demographic parameters and population trends, generated from multi-year data (Chandler and Clark, 2014). An intuitive progression hereon would be to combine spatial maps of annual changes in hotspots of anthropogenic threats to gauge their influence on fine-scale dhole densities. Replicating this effort across other reserves with varying sizes, prey densities, levels of protection and intensity of threats would provide a better understanding of the species' ecological requirements, and the true carrying capacities of protected reserves. Doing so can help recalibrate ecological expectations that are currently based on somewhat unreliable estimates on reserve size requirements (Woodroffe and Ginsberg, 1998), and set more realistic conservation targets.

5. Conclusion

Many well-intentioned monitoring programs unfortunately end up as failed opportunities in informing conservation policy and practice because of mismatches in aims, scope, resources, methodological techniques and implementation (Lindenmayer et al., 2013; Mascia et al., 2014; Hayward et al., 2015). Considered in context, we propose that the approach described is economically and logistically more feasible compared to other currently available methods, and may therefore be adopted as a standard protocol for conservation monitoring of dhole populations across critical conservation landscapes. We also foresee immense utility of our methods for studies of other ecologically important species which do not have natural pelage patterns, or those that are plagued by issues of data-deficiency, and insufficient funding and resources. Scaling up the methods presented here across spatially proximate or connected reserves (or habitat patches) could serve as a unified framework for understanding population ecology, population structures, landscape connectivity and metapopulation-level conservation requirements. This could bridge critical knowledge gaps for several imperiled species in dire need of incisive, science-based management efforts.

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CRediT authorship contribution statement

Arjun Srivathsa: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

Ryan G. Rodrigues: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Software; Validation; Writing - original draft; Writing - review & editing.

Kok Ben Toh: Formal analysis; Methodology; Resources; Software; Visualization; Writing - review & editing.

Arun Zachariah: Data curation; Resources; Writing - review & editing.

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Data Availability Statement

Data requests may be directed to the corresponding auhtor.

Declaration of competing interest

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

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