A novel technique for artificial pack formation in African wild dogs using odour familiarity

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INTRODUCTION

Reintroductions are recognized tools for species recovery. However, operations are costly, difficult to implement, and failures are common and not always understood. Their success for group-living species depends on the mimicry of natural processes that promote social integration. Due to fragmented landscapes, human mediated (i.e. artificial) group formation is often required.

African wild dogs (*Lycaon pictus*) are Endangered (Woodroffe & Sillero-Zubiri, 2012) and, in South Africa, the Kruger National Park and surrounds hold the only viable, contiguous population. Wild dogs are cooperative breeders, living in packs of 2–30. At 1–2 years old, same-sex subordinates disperse in search of unrelated, opposite-sex groups with which to form a pack (McNutt, 1996). This dispersal is intentionally mimicked in the managed metapopulation (Davies-Mostert, Mills & Macdonald, 2009), where human intervention is necessary to create gene flow by implementing translocations of individuals between distant sites. The managed metapopulation has been successful, with an increase from 17 individuals in 1998 to 202 in 2005, and a mean annual population growth rate of 1.08; higher than most unmanaged populations (Davies-Mostert, Mills & Macdonald, 2015).

Traditionally, wild dog artificial pack formation involves the use of an enclosure with two compartments sharing a common fence. This allows for some exposure – but limits direct physical contact – between opposite-sex groups (Potgieter, O'Riain & Davies-Mostert, 2015). An observer assesses the strength of social interactions between the two groups before opening a common gate to allow direct physical contact. However, double compartment enclosures are expensive to erect, and are not always feasible with limited resources. An additional drawback is the somewhat subjective assessment of social integration and potential aggression; misjudgements in these aspects can lead to injury or fatalities when groups containing unfamiliar individuals are joined (WAG-SA, 1998–2019). As a potential solution, field teams developed a technique of odour familiarity using single compartment enclosures.

Olfaction is an important mode of communication in wild dogs. Scent-marks demarcate territories (Parker, 2010) and provide information on dominance (Jordan, Apps, Golabek & McNutt, 2016). The odour familiarity technique involves rubbing the bodies of all individuals from two unrelated, opposite-sex groups with one another to mix their distinct odours. This technique has been used in the managed metapopulation four times previously, in conjunction with a single compartment enclosure, creating four stable packs (WAG-SA, 1998–2019). Despite its apparent effectiveness, there has been no formal assessment of its success. To address this, we performed behavioural observations during an enclosure period for two unrelated, opposite-sex groups of wild dogs, and describe the implementation and success of the technique. Decreasing resting distance to a partitioning fence and a decreasing frequency of hoo-calling (long-distance contact call; Robbins (2000)) predict success of social integration (Potgieter *et al.*, 2015) and, in free-roaming wild dogs, the strength of social bonds is also reflected in spatial resting patterns (McCreery, 2000). We hypothesized that, if the technique was successful in pack formation, individuals in the enclosure would 1) form an alpha pair (i.e. increase in over-marking), 2) decrease hoo-calling, 3) decrease negative interactions, and 4) increase frequency of mixed-sex resting groups over time.

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METHODS

The Kruger National Park (KNP) covers 19 485 km^2 in South Africa and contains the largest population of wild dogs in the country (~231 adults and yearlings February 2019; WAG-SA (1998–2019)). Between 1995 and 2000 the KNP population experienced a significant decline – notably in the north – from which it never recovered (Davies, 2000; Marnewick *et al*., 2014; Wilkinson, 1995). As a result, northern KNP was reclassified as recoverable wild dog range (RWCP & IUCN SSC, 2015).

Translocation

To reinforce the population, eight adult wild dogs from two unrelated groups (four males, four females) were translocated to northern KNP (Shingwedzi) on 31 July 2017. Individuals were placed into a 50 × 75 m single compartment enclosure with electrified 2 m high fence where they remained for 46 days (excluding arrival and release days). Each wild dog was transported in its own crate and awake for the duration of the journey. Upon arrival, each wild dog was given 1–2 mg of Fentanyl with 20–30 mg of Xylazine each to immobilize them for collar fitment (range of makes/models), allowing us to monitor pack composition and movements post-release. The wild dogs were fed an impala (*Aepyceros melampus*) carcass, and their water renewed, every third day.

Odour familiarity

While sedated, we rubbed the face, body, and anogenital region of each individual onto the same body parts of all other individuals. To do this, we placed the wild dogs into a single line and two people carried one wild dog at a time for rubbing onto others. Finally, we closely positioned the wild dogs together as one group and roused them with 10 mg of Naltrexone and 6.25 mg of Yohimbine. They slowly woke and, subsequently, individuals showed interest in each other. The activities in this study were part of the State Veterinary Services, South African National Parks, and Endangered Wildlife Trust disease and health survey (SANParks Project VSCHL1372 with addenda, SANParks Animal Use and Care Reference 013/16) and African wild dog introduction in northern Kruger National Park project (SANParks Project MARDG1481).

Behavioural observations

From 1 August–15 September 2017 we observed the wild dogs in the enclosure during active (first two hours after sunrise and last two hours before sunset, $n = 73$) and resting periods (one hour over midday, $n = 12$). We did not perform any observations from 4–10 September 2017 due to disease sampling by veterinarians that could have biased natural behaviours. One observer recorded behaviours from a vehicle outside the enclosure with access to the entire perimeter. During active periods, we performed focal observations, recording the frequency of hoo-calling and all social interactions. We identified 12 social interactions classified into two categories; positive and negative (described in Supplementary Table S1). During resting periods, we performed scan samples at 10-minute intervals, recording the composition of each resting group. We considered a group of individuals to be resting together if they were lying within two adult dog lengths (~180 cm; McCreery (2000)). As the enclosure contained several trees for shade, individuals were not forced to rest together. On day 14 a single female appeared outside the enclosure, remained at the site until the pack was released, and she then joined the pack. On feeding days, the carcass was given between 15:30 and 17:00 and feeding was included in $n = 8$ observation periods over the study period.

Statistical analyses

To investigate our hypotheses, we used a Mann-Kendall test (McLeod, 2011) to assess if there was a significant trend over time with regard to 1) over-marking rate, 2) hoo-calling rate, 3) proportion of negative interactions, and 4) the proportion of mixed-sex resting groups. We performed all statistical analyses and created figures in R v.1.1.463 (R Core Team, 2019).

RESULTS

Alpha pair

We observed over-marking between two individuals (one male always over-marked the same female). The trend of over-marking rate increased over time (tau = 0.32, $P = 0.01$, Fig. 1a) with a mean (±S.E.) rate of 0.27 ± 0.05/hour (range = 0–1). We observed mating between these two individuals on four occasions.

Hoo-calling

We recorded a mean hoo-calling rate of 0.18 ± 0.10/hour (range = 0–3.50). Hoo-calling began on

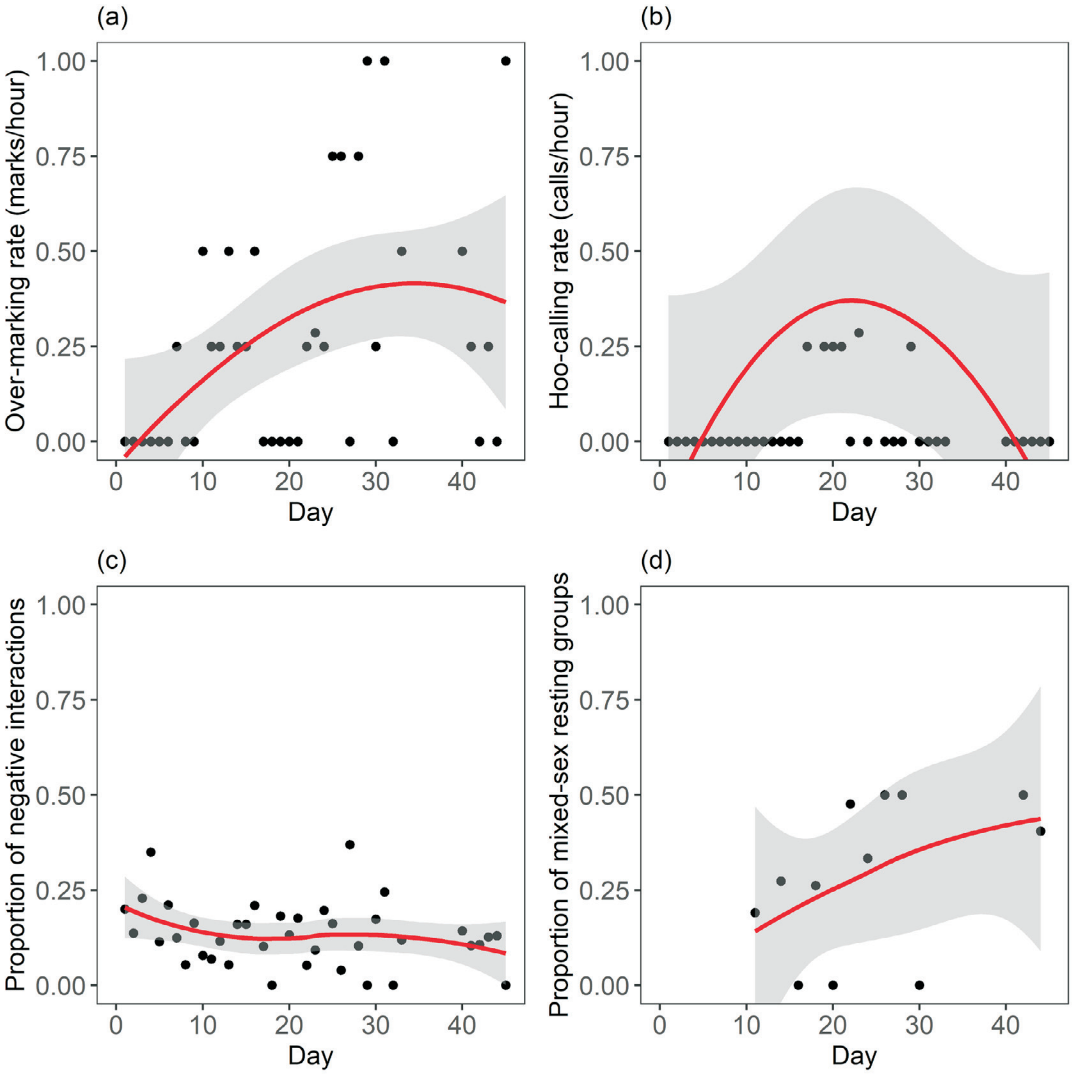

Fig. 1. The rate of (**a**) over-marking and (**b**) hoo-calling, and the proportion of (**c**) negative interactions and (**d**) mixed-sex resting groups observed throughout the enclosure period. Line represents the loess trend line and shaded region the 95% confidence interval.

day 17, shortly after the arrival of the female outside the enclosure on day 14, but subsequently decreased and did not occur again after day 29. Consequently, there was no significant trend in hoo-calling rate (tau = 0.06, P = 0.67, Fig. 1b).

Negative interactions

The mean proportion of negative interactions was 0.13 ± 0.01 (range = 0–0.37). Although the proportion of negative interactions shows a decreasing trend, this was not significant (tau = –0.17, P = 0.14, Fig. 1c).

Mixed-sex resting

The mean proportion of mixed-sex resting groups was 0.29 ± 0.06 (range = 0–0.50). Although the mean proportion of observed mixed-sex resting groups increased over time, the trend was not significant (tau = 0.38, P = 0.11, Fig. 1d).

Post-release

The pack of eight wild dogs was deemed to be socially integrated and released on 16 September 2017. On 5 May 2018, the pack was confirmed to be denning, with all nine individuals still alive and together. As such, we concluded that pack formation was successful both short- and long-term.

DISCUSSION

Over-marking is characteristic of dominant wild dogs, where alpha pairs scent-mark the same area consecutively to advertise their presence (Jordan *et al*., 2016). As over-marking began on day 5, our results suggest that an alpha pair was formed early in the enclosure period.

Hoo-calls are used to locate individuals separated while hunting or opposite-sex groups during dispersal (Robbins, 2000). At the beginning of the enclosure period, we did not observe hoo-calling; a positive sign of social integration (Potgieter *et al*., 2015). Because the hoo-call rate was likely initiated by the presence of the female outside the enclosure, and it decreased to zero shortly after her arrival, this likely represents the affiliation between her and the rest of the group within the enclosure. Additionally, as the same alpha pair remained, and other indicators of social integration were apparent, we do not believe that the presence of the female outside the enclosure hindered social integration. This is supported by the fact that the female ultimately joined the pack post-release.

Aggression is required to establish and maintain dominance hierarchies (Chase, Tovey, Spangler-Martin & Manfredonia, 2002), so negative interactions are natural for group-living species. We suggest that our results did not show a significant decline in negative interactions because it was initially low. We suggest, from this study and previous observations, that odour familiarity assists in reducing initial aggression between unfamiliar groups. As the hoo-calling rate was low and an alpha-pair formed early, this supports our conclusion. The neuropeptide oxytocin is produced during enjoyable events and has positive feedback on its own release (Chen & Sato, 2017). Furthermore, oxytocin promotes social bonding in domestic dogs (*Canis familiaris*) (Romero, Nagasawa, Mogi, Hasegawa & Kikusui, 2014). We suggest that this cycle of oxytocin production beings when individuals are allowed to physically interact and perform enjoyable behaviours. As such, this may impact initial aggression and assist in subsequent social integration. However, investigation into levels of oxytocin during bonding of wild dogs would be required to confirm this.

The occurrence of mixed-sex resting groups increased over time, but we suggest that the lack of significance is due to the small sample size (n = 12). In naturally forming packs, mixed-sex resting groups are indicative of stable pack formation (McCreery, 2000). Rewarding social behaviours, such as resting together or grooming, have positive effects on social bonding (Ågmo, Smith, Birnie & French, 2012). If this begins the oxytocin positive feedback loop (Chen & Sato, 2017), allowing individuals to perform such behaviours from the start may facilitate social integration. This is supported by Marneweck *et al*. (2019) who found that small wild dog groups with initial separation took longer to integrate.

Reproductive success of artificially formed wild dog packs increases with decreasing time spent in the enclosure (Marneweck *et al*., 2019), and a single compartment can increase the speed of social integration. In addition to holding positive benefits for welfare and success of the operation, decreased time within the enclosure also reduces management costs. We suggest that odour familiarity, in conjunction with single compartment enclosures, would benefit both animals and practitioners.

It is possible that confounding factors, such as the mere proximity when waking, had as much or more of an impact on success than odour familiarity. As such, we must caution our conclusions and urge further behavioural research into both artificial and natural pack formation to support existing data. We acknowledge that sample size is a limitation, but we believe our study provides an objective framework to implement odour familiarity in future pack formations, and provides a framework to measure success. We encourage its further use, and suggest that it may also be appropriate for the artificial formation of social groups of other species.

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Supplementary Table S1. Ethogram describing the social interactions between African wild dogs held in a temporary enclosure prior to their release into a new protected area. Negative interactions are denoted by (–) and positive interactions by (+).

Interaction	Description	Example
Aggression (–)	Behaviours including biting, erect tail, growling.	
Chasing (–)	Running after another individual running away. Or, lunging to keep individual away from food.	
Dominating (–)	A behaviour causing another individual to lay down and/or perform submissive behaviours.	
Drinking (+)	Drinking simultaneously out of the same water trough.	

Interaction	Description	Example
Feeding (+)	Eating at the carcass or a piece of carcass together.	
Greeting (+)	Approaching another individual and then licking, nuzzling, and/or sniffing.	
Lifting (+)	Lifting another individual with the head under the belly or between hind legs.	
Mating (+)	Mounting an opposite-sex individual.	
Over-marking (+)	Placing a scent-mark on top of another individual's scent-mark (can include a combination of urine, faeces, or glandular secretions).	
Playing (+)	Playful fights and rearing (i.e. an individual standing on their hind legs with forelegs on another individual).	

Interaction	Description	Example
Resting (+)	Laying less than two adult dog lengths (~180 cm) from another.	

Interaction	Description	Example
Submissive (+)	Behaviours including head and ears down with tail curved between the legs displayed towards another individual