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**Rafiq, K, Jordan, NR, Meloro, C, Wilson, AM, Hayward, MW, Wich, SA and McNutt, JW (2020) Scent-marking strategies of a solitary carnivore: boundary and road scent marking in the leopard. *Animal Behaviour*, 161. pp. 115-126. ISSN 0003-3472**

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1 **Scent marking strategies of a solitary carnivore: boundary**  
2 **and road scent marking in the leopard**

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19

20 **Abstract**

21 Scent marking, where individuals deposit signals on objects in the environment, is a  
22 common form of chemical signalling in mammals and is thought to play a critical role in  
23 maintaining social organisation within wide-ranging, spatially-dispersed populations.  
24 Senders, however, can incur scent marking costs through mark production, time  
25 investment in patrolling and depositing/maintaining mark sites, and increased risk of  
26 detection by predators and prey. To mitigate these costs, senders can adapt spatial  
27 patterns of scent marking to increase the probabilities of their scent marking being  
28 encountered by intended receivers. Relatively little, however, is known of the spatial  
29 scent marking placements of many wide-ranging carnivore species, with most studies  
30 focussing on scent mark form and function. Here, we use detailed observational data  
31 collected from over seven years of following individual leopards and high-resolution GPS  
32 radio collar data to investigate the spatial placements of scent marks within a leopard  
33 population in northern Botswana. We found that male leopards within our study area  
34 exhibited a boundary scent marking strategy by showing higher investments in the  
35 maintenance of marking sites in peripheral areas of their home range. We also found  
36 that leopards scent marked over four times as frequently and investigated over three  
37 times as frequently when travelling on roads than when travelling along natural routes,  
38 suggesting that roads may function as key locations for olfactory information. Compared  
39 to leopards from less productive ecosystems, such as the Kalahari, our results (1) suggest  
40 that leopards can be highly flexible in their marking strategies, with strategies impacted  
41 by the surrounding environment, and (2) provide evidence that human-modifications of  
42 the environment now play an important role in facilitating social cohesion within this  
43 solitary carnivore.

44 **Keywords:** communication, leopards, olfaction, *Panthera pardus*, road ecology, scent  
45 marking, territory maintenance

## 46 **Introduction**

47 Scent marking, where individuals deposit signals on objects in the environment, is a  
48 common form of chemical signalling in mammals and is thought to have functional roles  
49 in territoriality and mate acquisition (Gosling and Roberts, 2001; Macdonald and  
50 Loveridge, 2010). Whilst the roles of scent marks appear relatively consistent across  
51 species, scent marking behaviours vary between and within populations, and across  
52 landscapes (Allen et al., 2016a). Senders incur scent marking costs through mark  
53 production, time investment in patrolling and depositing/maintaining mark sites, and  
54 increased risk of detection by predators and prey (Gosling and Roberts, 2001; Hayward  
55 and Hayward, 2010; Hughes et al., 2012). To mitigate these costs, senders must make  
56 decisions on the optimal placements of scent marks. For example, they can select for  
57 areas or objects that increase the probabilities of signals being encountered by intended  
58 receivers, as is seen in Eurasian lynx (*Lynx lynx*), which increase scent marking frequencies  
59 along routes frequently travelled by conspecifics (Allen, Hočevár, de Groot, & Krofel,  
60 2017; Krofel, Hočevár, & Allen, 2017).

61 Even though it can be difficult to ascribe actual function to scent marks without  
62 considering the responses of receivers, the spatial placements of marks can provide  
63 insights into the optimal scent marking strategies for communication (Gosling and  
64 Roberts, 2001). The spatial marking strategies employed by senders are likely dependent  
65 on the interplay between a number of factors, including resource distributions (Zhou et  
66 al., 2015), home range sizes (Gorman & Mills, 1984), and movement patterns of  
67 conspecifics (Krofel et al., 2017). As such, the placements of scent marks can vary  
68 considerably across closely related species and also within different populations of the

69 same species. Gorman and Mills (1984), for example, found that the spatial marking  
70 strategies of hyaena species varied between ecosystems. Within highly productive  
71 landscapes, home ranges were small and scent marks were concentrated along borders:  
72 in less productive landscapes, home ranges were larger and hinterland scent marking  
73 strategies, i.e. where signals are concentrated within central home range areas, were  
74 favoured and more economical than border strategies (Gorman & Mills, 1984).

75 Human modifications to the environment may also impact scent mark placements by  
76 altering the space use of intended receivers (Wilmers et al., 2013). Roads, in particular,  
77 may promote scent marking efficiency by increasing scent mark encounter rates if they  
78 are preferentially used by intended receivers. In some species, for example, roads  
79 channel individual movements because of their positive effects on prey encounter  
80 probabilities (Fahrig & Rytwinski, 2009) and landscape permeability (Abrahms et al.,  
81 2016). However, although roads are thought to promote scent marking efficiency for  
82 some species (Krofel et al., 2017), results have been mixed, and other species have shown  
83 similar marking rates when travelling on roads and natural routes (Zub et al., 2003). This  
84 suggests that marking on roads carries costs. Disturbance from road traffic may, for  
85 example, decrease the persistence of scent marks and thus reduce the overall benefits of  
86 road marking. Additionally, it seems likely that dominant competitors will preferentially  
87 use roads, while inferior competitors will avoid them (Hayward et al., 2015; Mahon,  
88 Banks, & Dickman, 1998), thereby challenging the ubiquity of individual species  
89 responses. Thus, there likely exists a trade-off in the use of roads for scent marking, and  
90 its resolution is likely to be species and context dependent (Zimmermann, Nelson,  
91 Wabakken, Sand, & Liberg, 2014).

92 Scent marking is widespread amongst felids and plays a critical role in maintaining social  
93 organisation within their wide-ranging, spatially-dispersed populations (Sunquist &

94 Sunquist, 2002). However, relatively little is known of the spatial scent marking  
95 placements of many wide-ranging felid species, with most studies focussing on scent  
96 mark form and function (Allen et al., 2017), and relatively little is known of the impact of  
97 roads on the scent marking behaviours of felids (but see Krofel et al., 2017).

98 Leopards (*Panthera pardus*) are solitary large felids, present in a range of habitats across  
99 Africa and Asia (Jacobson et al., 2016), including areas with high levels of human-  
100 development (Odden, Athreya, Rattan, & Linnell, 2014). They show intrasexual  
101 territoriality, with range overlap varying between populations, and scent marking  
102 behaviours that are thought to play a key role in helping them to maintain territories and  
103 find reproductive partners (Bailey, 2005; Mizutani & Jewell, 1998). Scent marks are  
104 placed throughout territories by both sexes and the observed scent marking behaviours  
105 are consistent with those seen in other felids (Allen, Wittmer, Setiawan, Jaffe, & Marshall,  
106 2016; Bailey, 2005). Little is known, however, of how leopard scent marking placements  
107 change across ranges and the adaptive significance of such placements, and much of what  
108 we do know is based on the identification of scent sites *post-hoc* during spoor tracking  
109 surveys (Bothma, 2004; Jenny, 1996; but see Bailey, 2005). Thus, the results are typically  
110 biased to easily detectable scent mark types across substrates that leopards can be  
111 tracked along, and they are incapable of incorporating information on relative placements  
112 within home ranges into the analyses.

113 Here, we use detailed observational data, collected over seven years of following  
114 individual leopards, i.e. focal follows, and high-resolution GPS radio collar data to  
115 investigate the scent marking behaviours of leopards in northern Botswana. We used  
116 data from GPS radio collars to delineate male leopard home ranges and data collected  
117 from focal follows to investigate scent marking frequencies across different substrates  
118 and areas of the home range. We also took advantage of the high-resolution data

119 provided by the radio collars to investigate scent mark revisitation times, which we  
120 considered to be a proxy of the time elapsed before scent marks at sites were  
121 replenished. Data from two leopard radio collars with spatio-temporal overlap were used  
122 in a case-study into the scent marking behaviours of neighbouring competitors within  
123 overlapping areas of their home ranges. We assumed that, as with many other felids,  
124 leopard scent marks have a functional role in territoriality (Macdonald and Loveridge,  
125 2010), and so we generated a series of hypotheses to consider the spatio-temporal scent  
126 marking strategies used by leopards. We hypothesised that scent marking behaviours  
127 would occur most frequently and sites would be revisited more quickly within the  
128 boundary areas of home ranges rather than the central areas, i.e. that leopards would  
129 display a boundary scent marking strategy, since these are the areas where scent marks  
130 are most likely to be encountered by intruders (Gosling and Roberts, 2001). We also  
131 predicted that roads would impact scent marking behaviours. Specifically, we  
132 hypothesised that leopards would scent mark at higher frequencies on roads and would  
133 replenish these signals more often because of the potential of roads to channel  
134 conspecific movements (e.g. Krofel et al, 2017).

## 135 **Methods**

### 136 Study site

137 This study was carried out in northern Botswana in the south-eastern region of the  
138 Okavango Delta over an area of ~520 km<sup>2</sup>. The study landscape was a heterogeneous mix  
139 of habitat types, dominated by regions of mopane and acacia-dominated mixed  
140 woodlands (Broekhuis, Cozzi, Valeix, McNutt, & Macdonald, 2013). The study area  
141 included Moremi Game Reserve and adjacent wildlife management areas that were  
142 primarily used for photographic tourism. There were between one and three safari  
143 lodges operating within the area throughout the study period, each typically running

144 game drives twice daily, and one permanent research camp, operated by the Botswana  
145 Predator Conservation Trust (BPCT). As a result, there was a well maintained network of  
146 roads that dissected our study site (Figure 1), which remained relatively consistent  
147 throughout the study period, and many resident large carnivores were habituated to  
148 vehicles.

#### 149 Behavioural observations

150 Scent marking data were collected by researchers from the BPCT during focal follows  
151 from October 2011 until December 2017. Data were collected on 12 leopards (eight  
152 males and four females) that were sufficiently habituated to directly observe without  
153 eliciting any obvious changes in behaviour. Leopards were individually distinguishable by  
154 their unique rosette pelage patterns (Grey, Kent, & Hill, 2013) and were located through a  
155 combination of spoor tracking and radio telemetry. Behavioural observations were  
156 carried out from within research vehicles at distances of 10–50 m and 20–100 m from  
157 leopards when individuals were resting and moving, respectively. Upon locating leopards,  
158 sessions were initiated regardless of whether leopards were inactive or active, and in this  
159 way, samples were not biased to the collection of active behaviours, such as scent  
160 marking. Data were recorded using a critical incident sampling protocol (Altmann, 1974)  
161 and collected on Palm TX units using Pendragon Forms (from 2011 until 2015) and on  
162 Android smartphones using the KoboToolbox application (from 2015 - 2017). When  
163 leopards scent marked or investigated substrates, the mark type (Table 1), substrate  
164 marked, and leopard identity were all recorded alongside GPS coordinates.

165 From 2015–2017, each marking site within the focal session was also assigned a unique ID  
166 so that combinations of behaviours at the same site could be identified, and the track  
167 type (road or natural) that the leopard was travelling along was recorded. Scent marks  
168 placed on the same substrate and within 0.5 m of other marks were defined as occurring



169 at existing scent marking sites, i.e. counter marking. Roads were defined as routes whose  
170 regular use by ground vehicles resulted in semi-permanent vehicle signatures on the  
171 landscape. Spatio-temporal GPS coordinates were also collected during transitions  
172 between leopard behavioural states that were of interest to the wider BPCT project, for  
173 example, during transitions between inactive and active behaviours.

#### 174 Radio collars

175 Across our study period, we fitted seven male leopards with GPS radio collars developed  
176 by the Royal Veterinary College, University of London (A. M. Wilson et al., 2013).  
177 Leopards were immobilised by a Botswana-registered veterinarian using a drug cocktail  
178 including two or more of ketamine (50 - 200 mg), metomidine (2 - 5 mg), xylazine (225 -  
179 250 mg) and Zoletil (6 - 250 mg). Whilst individuals were immobilised, radio collars were  
180 fitted and vital signs monitored for signs of stress. Reversal drugs, atipamezole (3 - 24  
181 mg) or yohimbine (3 mg), were administered after immobilisation work was complete,  
182 and researchers within a vehicle remained with recovering individuals until their  
183 movement coordination returned to pre-immobilisation levels. Radio collars were fitted  
184 with GPS-inertial measurement units that allowed collars to switch between different  
185 sampling frequencies depending on the leopard's activity. GPS sampling frequencies  
186 switched between three states: five fixes per second during periods of high acceleration,  
187 one fix per five minutes during other periods of locomotion, and one fix per hour during  
188 periods of inactivity (A. M. Wilson et al., 2013). Radio collar GPS fixes were accurate to  
189 within 10 m (Wilson et al., 2013; supplementary information). Radio collars were fitted  
190 with mechanical (SIRTRACK) or bio-degradable material drop-off units, and all leopards  
191 were monitored until radio collars dropped-off. Radio collars weighed ~340 g and so  
192 represented < 2% of carnivore body masses. Radio collared individuals were visited at  
193 least every two to three weeks (with longer delays between visits due to logistical

194 challenges, e.g. vehicle failures, field conditions) to check animal welfare, download radio  
195 collar data *via* radio link, and to collect scent marking data. No ill-effects of radio collars  
196 were observed on animal welfare. We were able to collect scent marking data during  
197 focal follows for four of the seven radio collared leopards.

198 All field work was approved by the Department of Wildlife and National Parks (DWNP),  
199 Botswana, (permit no: EWT 8 / 36 / 4 xxxv (31)). Darting and radio collaring activities  
200 were approved by DWNP and darting reports submitted to the department after every  
201 leopard capture.

#### 202 Home range, boundary, and overlap area classifications

203 Kernel utilisation distributions (KUDs) for radio collared leopards were estimated using a  
204 bivariate distribution and plug-in bandwidth selection ( $H_{\text{plug-in}}$ ) in the R environment for  
205 statistics (R Core Team, 2018) using the packages *ks* (Duong, 2017) and *adehabitatHR*  
206 (Calenge, 2006). The 95% isopleths were extracted from KUDs and used to represent  
207 individual leopard home ranges. Boundary areas were then visualised by filling holes  
208 within 95% polygons and shrinking outer home range boundaries by 1,000 m using the  
209 *rgeos* package (Bivand & Rundel, 2017). The 1,000 m definition was based on preliminary  
210 analysis showing that, on average, leopard home ranges overlapped with neighbours by  
211 1,040 m ( $\pm 283.46$ , standard error). Scent marks within 1,000 m of outer boundaries were  
212 thus defined as occurring within boundary areas and those over 1,000 m from boundaries  
213 were defined as within central areas. For one pair of neighbouring leopards (CHK &  
214 GSE\_12), we also had scent marking and GPS data from each individual over the same  
215 sampling period. We defined the overlapping area of their 95% isopleths as the 'overlap  
216 area' and refer to it as such throughout the remainder of this paper.

#### 217 Road marking classification

218 A geospatial vector file of the main roads within the study area, georeferenced manually  
219 by driving roads, was provided by the BPCT. Visual inspection of the map showed that it  
220 contained most roads within the area; however, some roads were intermittently present  
221 throughout the study period and so were under-represented within the map. Leopard  
222 focal session tracks were reconstructed from spatio-temporal coordinates and leopards  
223 were defined as travelling on roads when two or more consecutive timestamps were  
224 within 15 m of the nearest road. The 15 m threshold was used to account for GPS  
225 positional errors and was validated by matching the classification from this threshold  
226 against recorded track types of our 2015–2017 data subset. The threshold classification  
227 method had 92% accuracy with classifications derived from focal sessions, with visual  
228 inspection suggesting that the majority of discrepancies were due to under-  
229 representation of roads within the geospatial vector file. Thus, we assumed our 15 m  
230 threshold robust enough to assign track types to all pre-2015 data.

### 231 Revisitation times to scent marks

232 We calculated scent mark revisitation times for radio collared leopards by linearly  
233 interpolating leopard GPS fixes at one second intervals, creating a 15 m buffer, i.e. 15 m  
234 radius, around scent marking site coordinates, and identifying how long after scent  
235 deposition or after their previous visit, leopards took to return to the site. This 15 m  
236 buffer was chosen to account for GPS sampling errors. Where there were multiple scent  
237 marks deposited at the same GPS coordinates within the same session, we used only one  
238 scent mark to avoid pseudoreplication. We could not account for non-linear leopard  
239 movements between raw fixes. Thus, although there were scent marking sites where we  
240 had no revisits, we cannot conclusively say that individuals did not return to them during  
241 our sampling period. To account for this, we removed those sites that were not revisited  
242 from our dataset and calculated the mean revisitation times to the remaining scent

243 marks. The visitation times of CHK and GSE\_12 to one another's scent marks within the  
244 overlap area were also calculated using the methods above.

#### 245 Statistical analyses

246 We calculated distances that leopards travelled during focal sessions from reconstructed  
247 tracks, which we then used to represent scent marking and investigating behaviours as  
248 frequencies (per km). We excluded from our analyses sessions with tracks shorter than  
249 100 m to avoid inflated frequencies resulting from short follow distances. For example, if  
250 a leopard marked four times over a 20 m distance, this would have given an inflated  
251 marking frequency of 200 scent marks/km. For each session, we segmented  
252 reconstructed tracks into periods of travel on roads and natural routes, and for those  
253 individuals with GPS data. We further segmented these tracks into periods of travel  
254 within boundary and central areas of home ranges. Scent mark counts and distances  
255 travelled for different segments of the same road/natural routes and boundary/central  
256 areas combinations within each focal session were then summed to give overall values for  
257 each unique combination for that session.

258 Due to the data being non-normal and resilient to any change in the distribution after any  
259 kind of transformation, we employed the Mann-Whitney U test to compare frequencies  
260 between marking and investigating behaviours and to compare behaviour frequencies  
261 between sexes.

262 To investigate behaviour frequencies within different home range areas and on different  
263 track types, we used a series of linear mixed-effects models using the *nlme* R package  
264 (Pinheiro, Bates, DebRoy, & Sakar, 2016). Separate models were fitted for overall scent  
265 marking and investigating behaviours and for each of the top two scent marking  
266 behaviours. We used counts as responses and an offset of log (track length) was included

267 within models to account for variation in the distances leopards were observed between  
268 focal sessions. Leopard identity was included within models as a random effect to control  
269 for repeated sampling of the same individuals. To account for unequal variances across  
270 leopards and increasing variance with track length, we specified and included variance  
271 structures for leopard identity and session distance in the models as weights (Zuur 2009).  
272 Linear mixed-effects models were also used to model revisitation times and also included  
273 leopard identity as a random effect. Models looking at scent marking and investigating  
274 behaviours across different track types used the full scent marking dataset, i.e. included  
275 both male and female leopards, and included track type as an explanatory variable.  
276 Leopard sex was not included as a fixed effect because sex was encoded for within our  
277 leopard identity random effect. A data subset that included only the leopards which we  
278 had GPS data for, i.e. only male leopards, was then used to investigate behaviour  
279 frequencies across boundary and central areas and scent mark revisitation times. These  
280 models included track type, location within the home range, and an interaction between  
281 track type and location as fixed effects. Models investigating behaviour frequencies and  
282 revisitation times within the CHK-GSE\_12 dyad used a subset of the GPS and scent  
283 marking datasets for CHK and GSE\_12 that were collected over the same time period.  
284 Location (with three levels: boundary, central, and overlap area) was included as an  
285 explanatory variable within these models but track type was not included because of  
286 limited sample sizes.

287 An information theoretic approach was used to rank all combinations of models derived  
288 from the global model using Akaike's information criterion corrected for small sample  
289 sizes (AIC<sub>c</sub>) (Burnham & Anderson, 2002). Models within six AIC<sub>c</sub> units of the highest  
290 ranked were retained within a candidate model subset (Richards, Whittingham, &  
291 Stephens, 2011). A model averaging approach was then applied to identify model

292 parameters with a significant impact on the response, i.e. model averaged parameters  
293 which were shown to have confidence intervals excluding zero (Grueber, Nakagawa,  
294 Laws, & Jamieson, 2011). Candidate models from which model parameters were  
295 estimated are included as supplementary tables (Tables A1– A4). Throughout the results,  
296 we report model predicted means with standard errors.

## 297 **Results**

### 298 Overview

299 We collected data over 786 hours of leopard observations that took place over 491 focal  
300 sessions, on eight male and four female leopards, during which leopards were followed  
301 for over 143.22 km. We recorded 894 occurrences of scent marking behaviours and 663  
302 occurrences of investigating behaviours by five male and two female leopards. The  
303 majority of focal follow hours occurred in the early evenings (42%,  $N = 330$ ) and early  
304 mornings (26%,  $N = 204$ ) during peak leopard activity and when light conditions aided  
305 data collection (Hubel et al., 2018), followed by data collected during the day (24%,  $N =$   
306 189) and night (8%,  $N = 63$ ).

307 Scraping and spraying were the most common scent marking types – accounting for 83%  
308 ( $N = 746$ ) of all scent marks observed (Table 2). Leopards were documented scent  
309 marking or investigating nine main substrate types, with grasses, shrubs, and trees  
310 accounting for approximately 90% of all marking sites (Figure 2). Most scrape ( $N = 301$ )  
311 scent marks were on grass (81%) and most sprays were on shrubs (52%,  $N = 195$ ) and  
312 trees (34%,  $N = 127$ ).

313

314 Leopards visibly investigated 65% ( $N = 244$ ) of scent marking sites from our 2015–2017  
315 subset before scent marks were deposited, but 82% of all substrates that were  
316 investigated ( $N = 299$ ) went on to be scent marked ( $N = 244$ ). Approximately 57% ( $N =$   
317 213) of deposit sites had only one scent marking behaviour carried out at them. Scraping  
318 and squat urinating commonly occurred at the same marking sites, with most scrapes,  
319 from our 2015-2017 subset, (84%,  $N = 131$ ) involving leopards squat urine counter  
320 marking on the site after scraping.

321 Overall, we documented that leopards scent marked ( $7.82 \pm 0.73$  marks/km) more  
322 frequently than they investigated substrates ( $5.93 \pm 0.64$  investigations/km) (Mann-  
323 Whitney U test:  $N_{1,2} = 148$ ,  $U = 12\,582$ ,  $P < 0.05$ ). Since other felid studies have primarily  
324 focussed on scrape and spray marks, we also calculated marking frequencies for these  
325 behaviours and found that frequencies did not significantly differ between the two scent  
326 mark types (Mann-Whitney U test:  $N_{1,2} = 148$ ,  $U = 11\,060$ ,  $P = 0.881$ ) (Table 2). Scent  
327 marking frequencies between the sexes were similar for overall scent marking (Mann-  
328 Whitney U test:  $N_{\sigma} = 128$ ,  $N_{\varphi} = 20$ ,  $U = 1574$ ,  $P = 0.098$ ), scraping (Mann-Whitney U test:  
329  $N_{\sigma} = 128$ ,  $N_{\varphi} = 20$ ,  $U = 1588$ ,  $P = 0.075$ ), and spraying (Mann-Whitney U test:  $N_{\sigma} = 128$ ,  $N_{\varphi} =$   
330 20,  $U = 1321$ ,  $P = 0.816$ ) behaviours. There was also no significant difference in  
331 investigating frequencies between the sexes (Mann-Whitney U test:  $N_{\sigma} = 128$ ,  $N_{\varphi} = 20$ ,  $U =$   
332 1478,  $P = 0.262$ ).

### 333 Scent marking on roads

334 Leopards scent marked and investigated over three times as frequently when travelling  
335 on roads than natural routes (Table 3; Figure 3). Scrape marks followed the same pattern,  
336 with higher marking frequencies on roads than natural routes, but sprays were deposited  
337 at similar frequencies across both track types (Table 3; Figure 3).

### 338 Scent marking across home ranges

339 Male leopards scent marked in both boundary and central areas of their home ranges and  
340 did so at similar frequencies (Table 4; Figures 4-5). Although there was no overall effect of  
341 location within the home range on investigation frequencies, there was an interaction  
342 between location and track type (Table 4). Specifically, although investigating behaviour  
343 frequencies when travelling on roads appeared similar in boundary and central areas,  
344 leopards investigated more frequently within boundary than central areas along natural  
345 routes (Figure 6). This appears, however, to be a reflection of the relatively small  
346 standard errors associated with travel along natural routes relative to those associated  
347 with travel along roads. No interaction effect between location and track type was  
348 detected on scent marking frequencies.

349 When analysing the CHK-GSE\_12 dyad, we found that a leopard's location within its home  
350 range, i.e. within the central, boundary, or overlap area, had no impact on scent marking  
351 frequencies (Table 5). There was, however, an impact of location on investigating, with  
352 leopards investigating at higher frequencies in boundary than overlap or central areas of  
353 their home range (Table 5; Figure 7).

### 354 Revisitation times

355 On average, leopards revisited scent marks  $29.55 \pm 0.54$  days ( $N = 215$ ) after they were  
356 deposited. Although revisitation times to scent marks deposited on roads ( $N = 128$ ;  $28.64$   
357  $\pm 0.49$  days) were statistically significantly shorter than on natural routes ( $N = 87$ ;  $30.90 \pm$   
358  $1.12$  days) (Table 6), the scale of the change suggested little biological significance. There  
359 was an interaction between track type and home range location (boundary vs. central) on  
360 revisitation times to scent marks (Table 6; Figure 8). Specifically, revisitation times  
361 remained similar for scent marks deposited on natural routes in central and boundary



362 areas of home ranges but decreased by 51% when travelling on roads in boundary than  
363 central areas (Figure 8).

364 When analysing the CHK-GSE\_12 dataset, we found that leopard revisitation times to  
365 their own scent marks were lower in overlap areas ( $N = 31$ ;  $17.73 \pm 2.53$  days) than in  
366 central ( $N = 120$ ;  $40.89 \pm 3.86$  days) and boundary home range areas ( $N = 55$ ;  $35.98 \pm 4.46$   
367 days) (Table 6). It took on average  $25.36 \pm 3.51$  days ( $N = 33$ ) for leopards to encounter  
368 scent marks left in the overlap area by their neighbour.

### 369 **Discussion**

370 Leopards within our study area adapt their scent marking and investigating behaviours  
371 based on the location within their home range and on the medium upon which they are  
372 travelling. Although scent marking frequencies remain consistent across home ranges,  
373 male leopards exhibit a boundary scent marking strategy by revisiting boundary scent  
374 marking sites on roads more quickly than scent marks in central areas, presumably for  
375 scent site maintenance and investigation. Within our study area, higher investment in  
376 scent marking behaviours at boundaries, particularly within overlap areas, may have  
377 increased scent marking efficiency and helped to establish social dominance by increasing  
378 the chances of active signals being encountered by neighbouring competitors. Scent  
379 marking within central areas may then primarily aid mate acquisition. We speculate that  
380 our male leopards may thus scent mark at similar rates throughout home ranges to  
381 facilitate finding a mate but prioritise maintaining marks at boundary locations because of  
382 the potential to lose both territory and long-term mating opportunities. Thus, scent  
383 marking at boundaries may facilitate the defence of central home range resources and  
384 reduce exploitation competition, e.g. for reproductive opportunities, within central home  
385 range areas (Peres, 1989). These results caution against ascribing function to scent marks  
386 purely from spatial distributions (Gosling and Roberts, 2001). Future studies would

387 benefit from considering the receivers of signals at different locations, e.g. by camera  
388 trapping scent sites (Allen et al., 2016b), and the olfactory information contained within  
389 scent marks, e.g. using scent presentation experiments (e.g. Allen et al., 2014; Jordan et  
390 al., 2010) or chemical profiles of scent marks (Vogt et al., 2016), in order to fully  
391 appreciate scent mark functions.

392 Male leopards scent marked at frequencies in overlap areas comparable to other areas of  
393 their home range but investigated their non-overlapping boundary areas at higher  
394 frequencies – although we acknowledge that our overlap areas for GSE and CHK in 2012  
395 were likely an underestimate given that not all leopards within our study system were  
396 radio collared. Regardless, such spatial patterns of scent marking investment may occur  
397 if neighbours and strangers compete for different resources and so represent different  
398 levels of threat to the investigator (Müller & Manser, 2007). In other words, male  
399 leopards may investigate at higher frequencies in non-overlapping boundary areas to  
400 identify dispersing males looking to establish new territories. That revisitation times to  
401 scent marks in the overlap area were lower than elsewhere in the home range also  
402 suggests an importance of maintaining familiarity with stable neighbours through  
403 regularly maintained scent marks. Whether there are community communication sites  
404 within these overlap areas that are used to communicate with conspecifics (e.g. Allen et  
405 al., 2014) and maintain familiarity is unknown. Camera trap placements at scent marking  
406 sites within areas where leopard home ranges overlap could be used to investigate this  
407 further.

408 Consistent with our predictions, male leopards had higher scent marking and  
409 investigating frequencies when travelling on roads than when travelling along natural  
410 routes, suggesting that roads may function as key locations for olfactory information.  
411 This may occur because roads increase mark encounter frequencies, perhaps by

412 channelling leopard movements and increasing landscape permeability, as they do for  
413 other species of large carnivores (e.g. Abrahms et al., 2016; Zimmermann et al., 2014).  
414 This has implications for the exploitation of these signals by unintended receivers as it  
415 increases mark encounter probabilities for all species that use roads as movement  
416 corridors (Hughes et al., 2012). Interspecific eavesdropping is particularly common  
417 amongst southern African carnivores, with some data suggesting higher occurrences of  
418 interspecific than intraspecific counter mark (Apps et al., 2019) and other data suggesting  
419 that hyaena latrines have functions as multi-species scent marking sites (Vitale, 2017).  
420 Leopards may thus incur costs from increased road marking if signals are encountered by  
421 unintended receivers that might alter their behaviours in ways that could be maladaptive  
422 to the sender. For example, predator odours can lead to increased vigilance of prey  
423 (Parsons et al., 2018; but see van der Meer et al., 2012). Further, although most human-  
424 traffic within the study area was diurnal and so occurred outside of peak leopard activity  
425 periods (Hubel, Golabek, Rafiq, McNutt, & Wilson, 2018), minimising direct disturbance to  
426 scent marking leopards, the costs (or benefits) of traffic on scent mark degradation are  
427 unknown. For example, it is unknown whether traffic can help distribute scent marks  
428 more widely, i.e. by capturing signals on tyres. Ultimately, the decision to scent mark on  
429 roads likely reflects a number of trade-offs in the costs, such as eavesdropping and mark  
430 disturbance, and benefits, such as increased conspecific encounter probabilities, of road  
431 marking.

432 Leopards in less productive landscapes, where population densities are lower and home  
433 range sizes larger, such as those within the Kalahari (Bothma, 2004; 2012), may adopt  
434 different spatial scent marking strategies, as is seen in hyaenas across different  
435 ecosystems (Gorman and Mills, 1984). This idea is supported by the observation that our  
436 leopard scent marking frequencies were comparable to leopards within tropical

437 rainforests (Jenny, 1996) but were approximately three times higher than leopards within  
438 the arid Kalahari (Bothma, 2004). Although each of these studies used different survey  
439 methods and focussed on different scent mark types (scrapes and sprays, respectively),  
440 comparisons with our results suggest that scent marking frequencies differ between  
441 landscapes for, at least, specific mark types. Leopard home ranges within the southern  
442 Kalahari were over five times larger than those within our study area (Bothma, 2004;  
443 Hubel et al., 2018). Thus, large territories may preclude efficient scent marking at home  
444 range boundaries so that Kalahari leopards adopt a hinterland marking strategy and  
445 perhaps reduce overall scent marking frequencies (Gorman & Mills, 1984) – this is  
446 additionally a likely optimal strategy given that leopards in arid areas occur at lower  
447 densities and so have a reduced likelihood of trespassing on conspecific territories  
448 (Kingdon, 2013). Additionally, the relative importance of scent marking behaviours in  
449 mediating encounters is likely to differ with habitat characteristics. As such, increased  
450 visibility within the Kalahari may mean that the role of vision in mediating encounters is  
451 more important than in closed habitats and so investment in scent marking behaviours is  
452 reduced. Given the lack of GPS data available for females, our results apply primarily to  
453 male leopards. Further, although we did not detect differences in scent marking  
454 frequencies between sexes, is it possible that female scent marking strategies may  
455 diverge from males, e.g. due to sexually dimorphic life-histories. For example, in other  
456 felids, females exhibit higher scent marking frequencies during oestrus to advertise sexual  
457 availability (Allen, Wittmer, & Wilmers, 2014; Logan & Sweanor, 2010), and it is possible  
458 that similar patterns may exist for leopards.

459 The seven main scent marking behaviours and three investigating behaviours we  
460 recorded are consistent with those documented in other solitary felids (Allen et al.,  
461 2016a; Smith et al., 1989; Vogt et al., 2014). We also recorded one occurrence of

462 leopards scraping with their front feet. To our knowledge this has not been documented  
463 in the literature on leopards elsewhere and there may be population specific occurrences  
464 of this behaviour, as seen in pumas (*Puma concolor*) (Harmsen et al., 2010; Allen et al.,  
465 2014). Leopard scrapes were created throughout territories and were often accompanied  
466 by urine or faeces, suggesting that scrapes may act as visual cues and aid the discovery of  
467 accompanying scent marks (Allen et al., 2014). Further, although we may have missed  
468 instances where urine was deposited alongside scrapes, we find it unlikely that we missed  
469 urination at all single deposit scrape sites. Indeed, it is widely referenced within the  
470 literature that urine is not present at all scrapes (e.g. (Allen, Yovovich, et al., 2016;  
471 Harmsen et al., 2010; Smith et al., 1989). Thus, given that not all scrapes were  
472 accompanied by secondary scent marks, scrapes may also be used to deposit marks from  
473 inter-digital glands on the feet (D. Wilson & Mittermeier, 2009). This suggests that  
474 scrapes and urine scent marks may have different roles in communication. Multiple scent  
475 marks at sites were then typically a combination of signals likely coming from  
476 subcutaneous or inter-digital glands, such as scrapes and rubs, and those occurring from  
477 bodily excretions, such as sprays and urine (Harmsen et al., 2010). Such combinations of  
478 scent marking behaviours may facilitate scent matching and aid mate selection (for  
479 review see Candolin, 2003).

480 In summary, we have shown that male leopards are highly flexible in their scent marking  
481 behaviours and that human-modifications of the environment can play a key role in  
482 facilitating information transfer within this solitary species. Leopards were shown to  
483 exhibit a boundary scent marking strategy by showing higher investments in the  
484 maintenance of boundary located scent marks. Roads appear to play a particularly  
485 important role in information transfer, likely functioning as key locations for olfactory  
486 information that increase scent marking efficiency and thus help to maintain social

487 cohesion. Our results also suggest a key role of familiarity in maintaining territoriality  
488 between neighbouring competitors and show that leopards exhibit higher frequencies of  
489 investigating behaviours in areas where they are conceivably most likely to encounter  
490 same-sex strangers. Thus we provide one of the few studies suggesting a *dear-enemy*  
491 effect in a solitary large carnivore, with most studies focussing on this effect within group-  
492 living species (Christensen & Radford, 2018). Our results also caution in using spatial data  
493 alone to infer scent marking strategies (Gosling and Roberts, 2001). As such, further  
494 research on the responses of receivers of scent marking signals and on the olfactory  
495 content of scent marks would provide further insights into the function of scent marking  
496 behaviours.

#### 497 **Acknowledgements**

498 We thank the Botswana government for providing permission to carry out this research  
499 under permit 'EWT 8 / 36 / 4 xxxv (31)'. We also thank A. Dheer, B. Abrahms, D.  
500 Kedikilwe, G. Gilfillan, R. Walker, J. Vitale, K. Golabek, R. Fuhrer, L. Gabanakemo, N.  
501 Keitumetse, M. Classe, E. Van Mourik, S. Lostrom, C. Bryce, C. Coco, B. Modise, H.  
502 Webster, S. Webster, and J. Mhongovoyo for contributing to the BPCT dataset and/or  
503 providing field assistance; D. Wells for assistance with analyses; and J. Lowe, S. Amos, and  
504 others for radio collar support. This work was funded by contributions from The Alice  
505 McCosh Trust, The Scottish International Education Trust, The Wilderness Wildlife Trust,  
506 The Explorers Club, Columbus Zoo, Ideas Wild, National Geographic, and Liverpool John  
507 Moores University. Collar development and deployment funding was provided by EPSRC  
508 (EP/H013016/1), BBSRC (BB/J018007/1) and ERC (323041).

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662

663 **Main tables**664 **Table 1: Description of the ten scent communication behaviours recorded during focal sessions.**

<u>Category</u>	<u>Behaviour</u>	<u>Description</u>
Scent marking	Scraping	Alternate raking of hind feet on substrate, may also include urinating over the substrate during or after the scraping.
Scent marking	Squat urinating	Squatting over substrate and urinating, in the absence of any scraping behaviour.
Scent marking	Spraying	Raising tail and spraying back urine onto substrate.
Scent marking	Rubbing	Rubbing face or body onto substrate. Can be done whilst leopard is standing or lying.
Scent marking	Defecating	Squatting over substrate and defecating.
Scent marking	Rolling	Lying and rolling several times on ground. Distinct from rubbing by back and forth rolling. May leave flattened vegetation.
Scent marking	Scratching	Using front claws to scratch substrate.
Investigating	Sniffing	Investigating by placing nose within 0.5 m of substrate and sniffing.
Investigating	Licking	Licking substrate (non-prey) or placing substrate within mouth and chewing for several seconds.
Investigating	Flehmening	Placing face within 0.5 m of substrate and inhaling scent whilst curling upper lip and exposing teeth. Facilitates mark investigation by vomeronasal organ.

665

666 **Table 2: Summary of the occurrence and mean frequencies (per km) ( $\pm$  SE) of the scent communication behaviours documented during the study.**  
667

<u>Category</u>	<u>Behaviour</u>	<u>Occurrence</u>	<u>Behaviour frequency (per km) (mean <math>\pm</math> SE)</u>
Scent marking	Scraping	371	3.42 $\pm$ 0.41
Scent marking	Spraying	375	2.96 $\pm$ 0.30
Scent marking	Rubbing	71	0.58 $\pm$ 0.15
Scent marking	Squat urinating	20	0.31 $\pm$ 0.15
Scent marking	Defecating	16	0.26 $\pm$ 0.09
Scent marking	Rolling	36	0.25 $\pm$ 0.07
Scent marking	Scratching	5	0.04 $\pm$ 0.02
Investigating	Sniffing	622	5.64 $\pm$ 0.58
Investigating	Licking	23	0.19 $\pm$ 0.08
Investigating	Flehmening	18	0.10 $\pm$ 0.08

668

669

670 **Table 3: Linear mixed-effects model ( $\Delta AIC_c < 6$ ) averaged parameters predicting scent communication**  
 671 **behaviour frequencies across track types.**

Parameters	Estimate	SE	Adj. SE	Confidence Intervals	
				Lower	Upper
<b>All marking</b>					
(Intercept)	1.075	0.141	-	0.796	1.355
Track type (road) *	0.703	0.219	-	0.271	1.137
<b>All investigating</b>					
(Intercept)	0.857	0.099	0.000	0.661	1.052
Track type (road) *	0.537	0.232	0.022	0.172	0.962
<b>Scraping</b>					
(Intercept)	0.533	0.092	-	0.095	0.828
Track type (road) *	0.669	0.169	-	0.337	1.000
<b>Spraying</b>					
(Intercept)	0.646	0.075	0.075	0.499	0.794
Track type (road)	0.054	0.119	0.120	-0.153	0.471

672 Within the parameters column, parameters are grouped by response variables for each model series (bold).

673 All models included leopard identity as a random effect. Candidate models can be found in Table A1. \*

674 denotes parameters with a significant impact on the response.

675

676 **Table 4: Linear mixed-effects model ( $\Delta AIC_c < 6$ ) averaged parameters predicting scent communication**

677 **behaviour frequencies across different home range locations and track types.**

Parameters	Estimate	SE	Adj. SE	Confidence Intervals	
				Lower	Upper
<b>All marking</b>					
(Intercept)	1.384	0.238	0.240	0.913	1.855
Track type (road)	0.320	0.289	0.290	-0.250	0.890
Track location (boundary)	0.046	0.147	0.150	-0.245	0.337
Track type (road) x track location (boundary)	0.024	0.163	0.165	-0.298	0.347
<b>All investigating</b>					
(Intercept)	0.959	0.163	0.165	0.635	1.283
Track location (boundary)	0.022	0.200	0.202	-0.436	0.497
Track type (road)	0.233	0.290	0.292	-0.327	0.832
Track type (road) x track location (boundary) *	0.668	0.653	0.656	0.192	2.035
<b>Scraping</b>					
(Intercept)	0.774	0.180	0.182	0.417	1.130
Track type (road) *	0.427	0.229	0.230	0.076	0.856
Track location (boundary)	0.018	0.107	0.108	-0.310	0.424
Track type (road) x track location (boundary)	0.044	0.186	0.187	-0.381	1.246
<b>Spraying</b>					
(Intercept)	0.739	0.136	0.137	0.470	1.009
Track location (boundary)	0.053	0.121	0.121	-0.163	0.483
Track type (road)	0.006	0.096	0.097	-0.342	0.389
Track type (road) x track location (boundary)	0.007	0.078	0.079	-0.525	1.043

678 Within the parameters column, parameters are grouped by response variables for each model series (bold).

679 All models included leopard identity as a random effect. Candidate models are presented in Table A2. \*

680 denotes parameters with a significant impact on the response.

681

682 **Table 5: Linear mixed-effects model ( $\Delta AIC_c < 6$ ) averaged parameters predicting CHK-GSE\_12 scent**  
 683 **communication behaviour frequencies across boundary, central, and overlap areas of home ranges.**

Parameters	Estimate	SE	Adj. SE	Confidence Intervals	
				Lower	Upper
<b>All marking</b>					
Intercept	2.868	0.781	0.800	1.306	4.431
Location (central)	0.177	0.298	0.304	-0.578	0.613
Location (boundary)	0.356	0.398	0.406	-0.760	0.831
<b>All investigating</b>					
(Intercept)	0.906	0.303	0.306	0.307	1.505
Location (central)	0.215	0.310	0.313	-0.138	1.070
Location (boundary) *	0.328	0.424	0.427	0.032	1.395
<b>All scraping</b>					
(Intercept)	1.343	0.485	0.495	0.373	2.312
Location (central)	-0.033	0.181	0.184	-1.304	0.629
Location (boundary)	-0.052	0.257	0.261	-1.831	0.762
<b>Spraying</b>					
(Intercept)	1.042	0.324	0.329	0.397	1.687
Location (central)	0.060	0.276	0.281	-0.814	1.431
Location (boundary)	0.182	0.474	0.478	-0.408	2.276

684 Within the parameters column, parameters are grouped by response variables for each model series (bold).

685 All models included leopard identity as a random effect. Candidate models are presented in Table A3. \*

686 denotes parameters with a significant impact on the response.

687 **Table 6: Linear mixed-effects model ( $\Delta AIC_c < 6$ ) averaged parameters predicting revisitation times across**  
 688 **different home range locations and track types.**

Parameters	Estimate	SE	Adj. SE	Confidence Intervals	
				Lower	Upper
<b>All leopard</b>					
(Intercept)	5.800	0.757	0.761	4.309	7.292
Location (boundary)	-0.104	0.645	0.648	-1.373	1.165
Track type (road) *	1.233	0.725	0.727	0.242	2.577
Track type (road) x Location (boundary) *	-1.769	1.144	1.147	-3.806	-0.624
<b>CHK-GSE_2012</b>					
(Intercept)	3.147	0.126	0.127	2.898	3.395
Location (overlap) *	-0.374	0.289	0.289	-0.909	-0.141
Location (boundary)	-0.110	0.171	0.172	-0.519	0.211

689 Within the parameters column, parameters are grouped by whether models were for all leopards or the

690 CHK-GSE\_12 subset (bold). All models included leopard identity as a random effect. Candidate models are

691 presented in Table A4.

692



693 **Appendix tables**

694 **Table A1: Summary of candidate models ( $\Delta AIC_c < 6$ ) of linear mixed-effects modelling output predicting**  
 695 **scent communication behaviour frequencies across track types.**

<b>Model</b>	<b>R<sup>2</sup></b>	<b>K</b>	<b>AICc</b>	<b><math>\Delta AIC_c</math></b>	<b>Wi</b>
<b>All marking</b>					
Track type	0.199	9	530.404	-	0.980
<b>All investigating</b>					
Track type	0.207	9	506.709	-	0.947
Null	0.161	8	512.463	5.754	0.053
<b>Scraping</b>					
Track type	0.187	9	567.454	-	0.997
<b>Spraying</b>					
Null	0.225	8	432.468	-	0.660
Track type	0.230	9	433.793	1.325	0.340

696 Within the model column, models are grouped by response variables for each model series (bold).

697

698 **Table A2: Summary of candidate models ( $\Delta AIC_c < 6$ ) of linear mixed-effects modelling output predicting**  
 699 **scent communication behaviour frequencies across different home range locations and track types.**

<b>Model</b>	<b>R<sup>2</sup></b>	<b>K</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>Wi</b>
<b>All marking</b>					
Track type	0.208	7	393.460	-	0.452
Null	0.180	6	394.908	1.447	0.219
Location + track type	0.213	8	395.214	1.754	0.188
Location	0.180	7	397.166	3.705	0.071
Location + track type + location x track type	0.216	9	397.204	3.743	0.070
<b>All investigating</b>					
Location + track type + location x track type	0.278	9	372.464	-	0.584
Track type	0.229	7	374.613	2.148	0.199
Location + track type	0.238	8	375.733	3.268	0.114
Null	0.198	6	376.530	4.066	0.076
Location	0.200	7	378.616	6.152	0.027
<b>Scraping</b>					
Track type	0.132	7	339.114	-	0.588
Location + track type	0.133	8	341.235	2.121	0.204
Location + track type + location x track type	0.141	9	342.662	3.548	0.100
Null	0.078	6	343.059	3.945	0.082
<b>Spraying</b>					
Null	0.197	6	328.654	-	0.504
Location	0.203	7	330.224	1.570	0.230
Track type	0.198	7	330.902	2.249	0.164
Location + track type	0.204	8	332.441	3.787	0.076
Location + track type	0.206	9	334.477	5.823	0.027

700 Within the model column, models are grouped by response variables for each model series (bold).

701

702 **Table A3: Summary of candidate models ( $\Delta AIC_c < 6$ ) of linear mixed-effects modelling output predicting**  
 703 **CHK-GSE\_12 scent communication behaviour frequencies across central, boundary, and overlap areas of**  
 704 **HR.**

<b>Model</b>	<b>R<sup>2</sup></b>	<b>K</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>Wi</b>
<b>All marking</b>					
Null model	0.540	4	363.071	-	0.910
Location	0.542	6	367.689	4.619	0.090
<b>All investigating</b>					
Null model	0.164	4	190.375	-	0.539
Location	0.226	6	190.691	0.316	0.461
<b>Scraping</b>					
Null model	0.527	4	284.114	-	0.902
Location	0.530	6	288.556	4.442	0.098
<b>Spraying</b>					
Null model	0.491	4	279.096	-	0.805
Location	0.507	6.00	281.930	2.834	0.195

705 Within the model column, models are grouped by response variables for each model series (bold).

706 **Table A4: Summary of candidate models ( $\Delta AIC_c < 6$ ) of linear mixed-effects modelling output predicting**  
 707 **revisitation times across different home range locations and track types.**

<b>Model</b>	<b>R<sup>2</sup></b>	<b>K</b>	<b>AICc</b>	<b><math>\Delta AICc</math></b>	<b>Wi</b>
<b>All leopard</b>					
Location + track type + location x track type	0.051	6	1063.604	-	0.766
Location	0.015	4	1067.305	3.701	0.120
Location + track type	0.020	5	1068.316	4.712	0.073
<b>CHK-GSE_2012</b>					
Location	0.073	6	631.808	-	0.712
Null	0.044	4	633.618	1.810	0.288

708 Within the model column, models are grouped by whether models were for all leopards or the CHK-GSE\_12  
 709 subset (bold).

710

711 **Main figure legends**

712 **Figure 1:** Map of the core study area (right) and its location within Botswana (left). Roads are shown  
713 within the core area as solid black lines. The core study area map was created using Google satellite  
714 imagery obtained within the QGIS *OpenLayers* Plugin (Kalberer & Walker, 2018).

715 **Figure 2:** Summary of the percentage of scent marking and investigating behaviours carried out on  
716 different substrate types.

717 **Figure 3:** Mean ( $\pm$  SE) leopard investigating and scent marking frequencies (per km) when travelling along  
718 roads and natural routes. \* denotes behaviours where there is an impact of track type on behaviour  
719 frequency (i.e. the track type model parameter's confidence intervals exclude 0).

720 **Figure 4:** Mean ( $\pm$  SE) male leopard investigating and scent marking frequencies (per km) when travelling  
721 along boundary and central areas of home ranges. \* denotes behaviours where there is an impact of  
722 home range area on behaviour frequency (i.e. the area model parameter's (or it's interaction's)  
723 confidence intervals exclude 0).

724 **Figure 5:** Distribution of male leopard follows (white circles) and scent communication behaviours (blue  
725 circles) across boundary (dark grey) and central (light grey) areas of leopard home ranges for individuals  
726 with GPS radio collars. Roads are depicted as black lines and leopard ID codes are provided in upper left  
727 corners of maps.

728 **Figure 6:** Mean ( $\pm$  SE) male leopard (a) investigating and (b) scent marking frequencies (per km) when  
729 travelling along road and natural routes in central and boundary home range areas.

730 **Figure 7:** Mean ( $\pm$ SE) scent marking and investigating frequencies (per km) for CHK and GSE\_12 when  
731 travelling across boundary (non-overlap), central, and overlap areas of their home ranges. \* denotes  
732 behaviours where there is an impact of home range location on behaviour frequencies (i.e. at least one of  
733 the location model parameter's confidence intervals exclude 0).

734 **Figure 8:** Mean number of days that it took leopards to return to scent marks in different areas of their  
735 home range and across different track types. Standard errors are  $\geq 0.07$  and  $\leq 0.68$  and so are not visible  
736 as error bars, but instead, they are presented alongside each plotted mean.