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**DIRECT AND INDIRECT EFFECTS OF LINEAR
INFRASTRUCTURE ON LEOPARDS (*PANTHERA PARDUS*)
SPATIAL USE**

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Abstract

Transport infrastructures, particularly roads, are expanding globally, with potential impacts on wildlife, especially on felids that seem to be particularly affected by their negative effects. However, the effects of roads on their spatial behaviour have never been extensively evaluated, which limits our understanding on how roads affect their populations and which measures should be applied across their distribution range.

We analysed whether the spatial use of leopard *Panthera pardus* was affected directly by the presence of paved and unpaved roads and associated traffic disturbance or indirectly by the land uses that might be changed or associated to roads and whether they vary according to sex and period of the day. We compiled GPS locations from 36 leopards in seven regions of Africa and Asia and applied structural equation modelling to test all possible combinations of road types, period of the day and sex.

The findings revealed that paved and unpaved roads have both direct and indirect negative effects on leopards but varied by type of road, period of the day and sex. Interestingly, males seemed to be positively associated with unpaved roads at night. Our results suggest that paved roads might be a barrier to leopard movement across its range, which can be worsened by the absence of suitable habitat in its vicinity. These negative effects appear to be stronger on females which can lead to genetic isolation and, therefore, reduce genetic diversity in small populations.

This study provides an important step towards a more complete understanding of the effects of roads on leopards and how their magnitude varies by sex and time of the day. Measures to prevent or compensate habitat degradation when building or upgrading transport infrastructures should be mandatory. Reduction of traffic disturbance and underpasses should be implemented in areas with small populations of leopards.

Key words: SEM, paved and unpaved roads, land uses, intersexual differences, road ecology

Resumo alargado

Nos últimos anos tem-se vindo a assistir a uma grande expansão das infraestruturas de transporte a nível global. Por exemplo, as estradas geram impactos negativos na vida selvagem e nos ecossistemas, tais como efeitos de barreira, perda e degradação do habitat e aumento da mortalidade devido a colisão com veículos. Esta expansão irá continuar a ocorrer até mesmo em áreas sensíveis para a biodiversidade da América Latina, África e Ásia.

Um dos grupos de espécies particularmente afetados pelos efeitos negativos das estradas são os felinos, uma vez que, no geral, apresentam comportamentos territoriais e têm grandes territórios, que fazem com que tenham de percorrer longas distâncias, o que aumenta a probabilidade de encontrarem estradas e de as atravessarem, ficando vulneráveis à morte por atropelamento. As suas baixas densidades populacionais e reduzidas taxas de reprodução fazem com que as suas populações tenham dificuldades em recuperar destas mortes adicionais. Deste modo, várias espécies de felinos apresentam elevadas taxas de mortalidade devido a morte por colisão com veículos e são afetadas pelo efeito de barreira e pela degradação e fragmentação do habitat provocados pelas estradas.

Apesar deste conhecimento relativamente aos impactos negativos das estradas nos felinos, o efeito das estradas no comportamento espacial destas espécies nunca foi avaliado ao longo das suas áreas de distribuição geográfica. Para além disto, existem muito poucos estudos que abordam o modo como as estradas afetam os felinos tendo em conta o sexo dos indivíduos e o período do dia. Deste modo, o principal objetivo deste estudo foi analisar os efeitos diretos e indiretos através da alteração dos usos do solo das estradas no uso espacial dos leopardos *Panthera pardus* ao longo da sua ampla área de distribuição geográfica, tendo em conta o sexo dos indivíduos (fêmea ou macho), o período do dia (diurno ou noturno) e o tipo de estrada (pavimentada ou não pavimentada). O efeito direto refere-se ao efeito provocado pela estrada e a perturbação a esta associada (p. ex., do tráfego automóvel) no uso espacial dos indivíduos. Já o efeito indireto refere-se aos tipos de uso do solo que foram alterados ou que estão associados à estrada (p. ex.: urbanização, agricultura e desflorestação). O leopardo foi a espécie escolhida para este estudo por ser o felino que apresenta a maior área de distribuição e por estar globalmente classificado como Vulnerável com uma tendência populacional decrescente. Sabe-se que os impactos das estradas são uma das ameaças responsáveis por esta tendência, esperando-se construções de novas estradas, incluindo autoestradas, na sua área de distribuição.

Para realizar este estudo compilámos localizações de GPS de 36 leopardos provenientes de estudos anteriores, as quais se localizavam em sete regiões em África e na Ásia. Destas localizações, selecionámos duas por dia separadas por um período de 12h para assegurar um esforço de amostragem idêntico e para permitir a comparação entre o dia e a noite. Compilámos também dados espaciais relativamente aos usos do solo e às estradas presentes nas áreas de estudo e reclassificámo-los de acordo com as variáveis necessárias para o estudo (estradas – pavimentadas ou não pavimentadas; usos do solo – naturais (floresta fechada, floresta aberta, arbustos e vegetação herbácea) e humanizados (agricultura e áreas urbanas)). Criámos um buffer de 2000m em torno de todas as presenças, distância a partir da qual o número de ocorrências estabiliza, e gerámos pseudo-ausências dentro desta área. Para cada localização, tanto para as presenças como para as pseudo-ausências, calculámos a distância às estradas pavimentadas e não pavimentadas mais próximas e atribuímos o tipo de uso do solo em que se encontravam. Todo este trabalho de preparação dos dados para a análise estatística foi efetuado utilizando o software ArcGIS.

Avaliámos todas as oito combinações possíveis entre as variáveis disponíveis, isto é fêmea/macho, dia/noite e estrada pavimentada/não pavimentada. Recorrendo ao software R, aplicámos Modelos de Equações Estruturais (“SEM”, em inglês) que permitem calcular os efeitos diretos e indiretos e que facilitam a apresentação e interpretação dos resultados ao torná-los visualmente mais intuitivos. As regressões utilizadas no SEM foram Modelos Mistos Lineares Generalizados (GLMM), nas quais os indivíduos foram tidos em conta como efeitos aleatórios. No total, corremos oito SEM (correspondentes às oito combinações entre as variáveis) para as localizações que se encontravam até 2000 m de distância das estradas. Antes de correr os modelos, a multicolinearidade das variáveis foi testada e como estas não estavam fortemente correlacionadas entre si, todas as variáveis foram incluídas nos modelos.

Os resultados revelaram que o uso espacial dos leopardos aparenta ser afetado tanto direta como indiretamente pelas estradas e que estes efeitos variam de acordo com o tipo de estrada, sexo e período do dia. As estradas pavimentadas tiveram efeitos diretos negativos fortes em ambos os sexos durante o dia e nas fêmeas durante a noite, o que apoia a hipótese que os leopardos tendem a evitar áreas com elevada perturbação humana. O afastamento das estradas pavimentadas durante o dia poderá justificar-se por estas normalmente apresentarem uma maior perturbação durante o dia e os leopardos serem maioritariamente crepusculares ou noturnos. Ao contrário das fêmeas, os machos estiveram positivamente associados com as estradas não pavimentadas durante a noite, que poderá dever-se à utilização destas estradas para patrulharem os seus grandes territórios e reduzirem os custos energéticos. As fêmeas, por outro lado, parecem ser mais sensíveis a ambos os tipos de estradas, uma vez que estiveram negativamente associadas com as estradas pavimentadas e não pavimentadas durante a noite. Estas poderão ainda ter evitado a utilização das estradas não pavimentadas durante a noite precisamente para evitarem encontrarem machos e os possíveis confrontos que daí poderiam resultar, uma vez que estes felinos são territoriais e solitários e apresentam comportamentos agonísticos relativamente a conspécíficos. Para além dos efeitos diretos, as estradas pavimentadas aparentam ter efeitos indiretos devido à ausência de floresta fechada nas suas proximidades, que foi o uso do solo preferencial dos leopardos. Assim estas estradas parecem afetar negativamente a conectividade do habitat do leopardo, podendo representar um possível efeito de barreira, o qual será mais forte para as fêmeas.

Este estudo aponta para a importância de se adotarem medidas de conservação para reduzir os efeitos negativos das estradas nos leopardos, principalmente em áreas onde as populações sejam mais pequenas e estejam mais suscetíveis à fragmentação e ao isolamento. Estas medidas devem incluir o restauro e a preservação da vegetação próxima das estradas, a construção conjunta de passagens inferiores eficazes e vedações que assegurem a conectividade do habitat e medidas de limitação do tráfego automóvel em áreas protegidas. A perturbação humana também deve ser reduzida na proximidade de estradas em áreas importantes para a conservação do leopardo. Equipas multidisciplinares de universidades, agências de transporte e administração pública devem de agir em conjunto para que estas medidas sejam adotadas de forma eficaz.

Finalmente, é relevante que se realizem estudos futuros para aumentar ainda mais a nossa compreensão relativamente aos efeitos das estradas nesta espécie emblemática e ameaçada de extinção. Estes devem de incluir avaliações comparativas de densidades populacionais de leopardos, das taxas reprodutivas, das estruturas das populações e do fluxo genético em áreas com e sem estradas. Também seria relevante replicar esta análise com um número idêntico de machos e fêmeas, de modo a evitar enviesamentos, e adicionar outras variáveis que possam explicar os comportamentos observados, tais como a abundância de presas naturais.

Estudos deste tipo são relevantes nos dias de hoje, onde temos vindo e vamos continuar a assistir a uma expansão das infraestruturas de transporte. Aqui pudemos demonstrar pela primeira vez como os efeitos das estradas nos leopardos são um sistema complexo que depende de diversas variáveis. Estas análises são importantes para se encontrarem padrões gerais para uma espécie com uma distribuição tão abrangente, pois apresenta um estatuto de ameaça vulnerável e existe uma necessidade urgente de se adotarem medidas gerais de mitigação dos efeitos das estradas. Estas têm de facto efeitos diretos e indiretos negativos no uso espacial dos leopardos, podendo representar uma barreira ao seu movimento, com um efeito mais acentuado nas fêmeas, o que poderá ter implicações na dinâmica das populações de leopardos.

Palavras-chave: SEM, estradas pavimentadas e não pavimentadas, usos do solo, diferenças intersexuais, ecologia das estradas

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Abbreviations

GLMM – Generalized Linear Mixed Model

GPS – Global Positioning System

IUCN – International Union for Conservation of Nature

SEM – Structural Equation Model

TI – Transport Infrastructure

1 – Introduction

1.1 – State of the art

The transport infrastructure (TI) is present on nearly every terrestrial surface: the road network is estimated to extend to more than 20 million km and railways to more than 7 million km (Meijer et al., 2018; UNECE, 2022). Global road expansion of at least 25 million km is expected by 2050, mainly under the Belt and Road Initiative (Laurance et al., 2014), crossing many sensitive areas for biodiversity in Asia, Africa, and Latin America (Long et al., 2018; Moore, 2022). This is challenging for ecologically sustainable development in several countries as TI can increase mortality due to collision with vehicles and promote fragmentation and isolation of many populations with implications on their genetic diversity (see, e.g., Riley et al., 2014).

Felids seem to be particularly affected by the negative effects of TI, since they typically have large home ranges, low population densities, low reproductive rates, and exhibit territorial behaviour (Fahrig and Rytwinski, 2009). Their large territories lead them to travel long distances, increasing the likelihood of encountering roads and cross them. Furthermore, these traits lead to a more difficult recovery from additional deaths due to collision with vehicles. For example, collision is the main cause of mortality for European wildcat *Felis silvestris* and the second main cause for Asiatic cheetah *Acinonyx jubatus venaticus* (Bastianelli et al., 2021; Farhadinia et al., 2017). In India, 33 leopards *Panthera pardus* were killed by vehicles in less than five months (Pinjarkar, 2021), while in Iran, between 2000 and 2015, more than 26% of the non-natural mortality of Persian leopard *P. p. tulliana* was caused by road accidents (Naderi et al., 2018). In some regions of southern Africa, roads have been the main cause of accidental deaths of leopards (Swanepoel et al., 2015). Furthermore, roads can act as physical barriers to felids by shaping their movement and habitat selection, which can impact home ranges and their boundaries, as seen with bobcats *Lynx rufus* (Poessel et al., 2014). Leopards are known to avoid roads, especially those with high traffic volume (Thapa et al., 2021) or where there is a high probability of encountering humans (Ngoprasert et al., 2007; Van Cleave et al., 2018).

Nevertheless, the direct effects of roads, defined as the presence of the road and associated traffic disturbance, and the indirect effects of roads due to surrounding habitat degradation or urbanization, have not been fully evaluated for these species. Furthermore, the effects of sex or the time of day on the impact of roads on felids are poorly explored. However, a study with bobcats (Poessel et al., 2014) observed differences in responses from the two sexes toward roads. In this study, males crossed roads more often than females, which can have implications on gene flow and, therefore, on the viability of populations in the long term (Riley et al., 2014). This gap in knowledge hinders the creation of effective recommendations to mitigate the road effects on felids.

1.2 – Aim and objectives

The main goal of this study was to analyze whether the spatial use of leopards is affected by the presence of the road and associated traffic disturbance and/or by land uses that might be associated or changed by the presence of roads, and whether these vary according to sex and period of day. Although occupying a broad geographic range that spans several land use types across Africa and Asia, leopards are classified as Vulnerable by the International Union for Conservation of Nature (IUCN) with a decreasing population trend (Stein et al., 2020). Multiple threats are responsible for this trend, including habitat loss, fragmentation, human conflicts, and TI (Jacobson et al., 2016; Stein et al., 2020). Many highways and other roads are expected to be constructed or

upgraded in Southeast Asia and tropical Africa, which are part of the leopard distribution range (Stein et al., 2020; Zhai, 2021) and are likely to put further pressure on leopard populations (Farhadinia et al., 2019).

Several approaches have been used to analyze direct and indirect effects such as counterfactuals (Lange and Hansen, 2011), marginal structural models (Lange et al., 2012), and natural effect models (Vansteelandt et al., 2012). One of the most used approaches, especially in ecology, is the structural equation model (SEM) (e.g., Kim and Holt, 2012; Menéndez et al., 2007). This model links several variables in a single causal relationship which explores how they interact with each other in a path diagram. This representation of the interactions between variables allows for a more intuitive interpretation of direct and indirect effects (Lefcheck, 2020).

SEM was used to analyze the direct relationship between leopard occurrence and distance to roads and the indirect relationship between leopard occurrence and roads via neighboring land use types and to explore the variation due to type of road, time of the day, and sex. Information on leopard GPS-based movement in regions with roads across the species range was compiled and SEM was run to test all possible combinations of road types (paved and unpaved), period of the day (day and night) and sex (female and male). With this analysis, we intend to clarify the effects of roads on leopard spatial use and inform road planners and managers to avoid or prevent them through effective mitigation strategies.

2 – Methods

2.1 – Data compilation and preparation

We compiled GPS locations of 36 leopards from previous studies located in seven regions (Figure 2.1, Appendix A). From these data, 24 leopards were from Africa (Kenya and South Africa: African leopard *P. p. pardus*), while 12 were from Asia (Iran: Persian leopard *P. p. tulliana* and India: Indian leopard *P. p. fusca*) (Appendix A). Two thirds were males (24 males and 12 females) (Appendix B), and the great majority were adults ($n = 30$). Two leopards dispersed and established new home ranges, which was verified by calculating their Minimum Convex Polygon (MCP). In these cases, they were considered as two separate individuals (one with the original home range and the other with the new home range). Two GPS locations were selected per day with 12h apart for each individual to ensure the same effort among studies and to compare daytime with nighttime locations. A buffer of 2000 m was created around all locations of each individual to randomly generate the same number of pseudo-absence locations within the created area. We chose this distance since the number of occurrences tended to stabilize below 2000 m from roads (Appendix C).

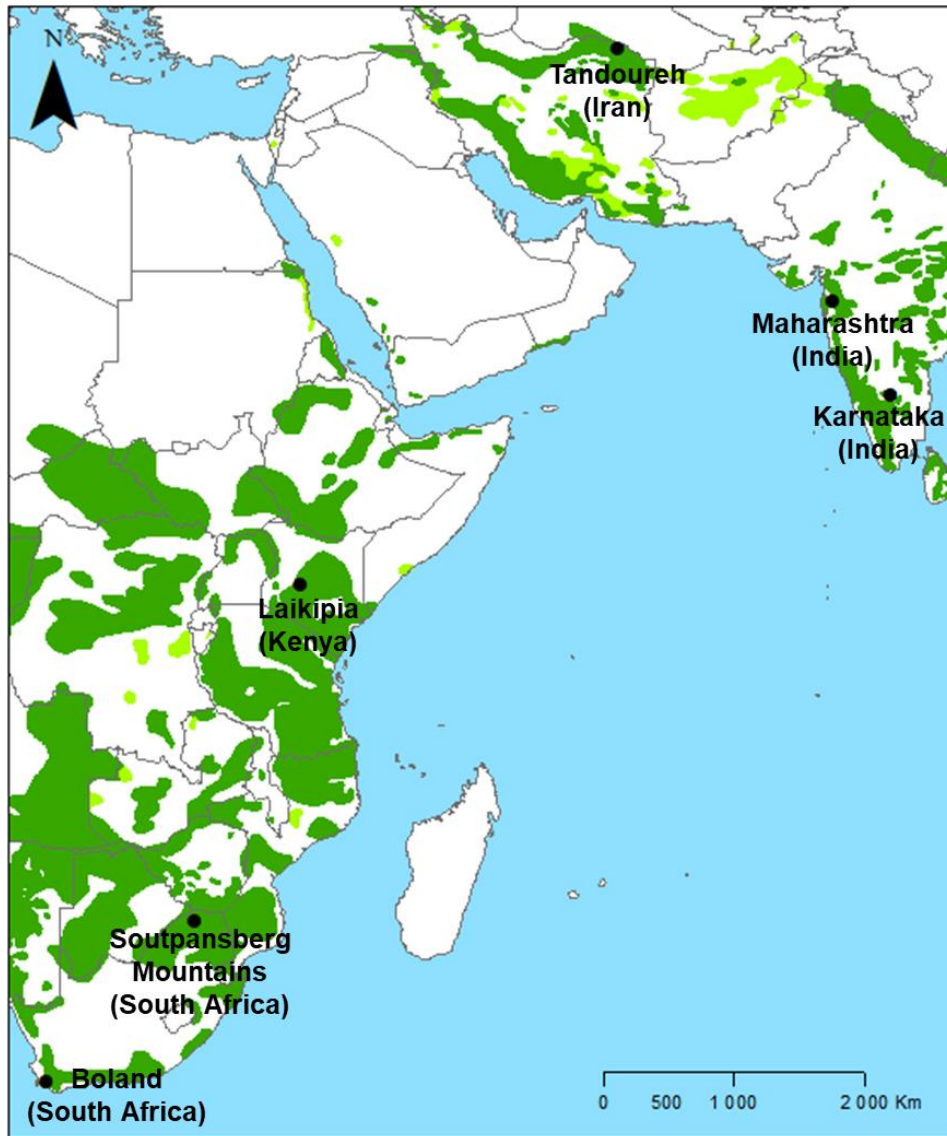


Figure 2.1. – Location of study areas and geographic range of the leopard (extant (dark green) and possibly extant resident (light green)).

The road network for each study area was obtained from different sources (Data Catalog, 2009; GIS English, 2018; ICPAC, 2017; OpenStreetMap Export, 2020). Roads were aggregated and reclassified in two road classes: paved and unpaved. In addition, the researchers that provided the leopard GPS-data gave a general estimation of the traffic volume by direct observation in each study area. Therefore, we assumed that unpaved and paved roads were proxies of low and high traffic volume, respectively, as there were obtained estimates of 0-20 vehicles per hour in unpaved roads and of 20-200 (or even more, in some cases) in paved roads (Appendix D). Any missing roads were georeferenced using ArcGIS (Esri Inc., 2019) with Google Earth as background.

Land use was obtained from Copernicus Global Land Cover with 100 m resolution (Buchhorn et al., 2020). As the leopard data was from different studies and sampling periods varied between 2009 and 2017 (Appendix B), we obtained land use maps that corresponded to each study area and period of survey. The available land uses were: 1 – closed forest (tree canopy of more than 70 %); 2 - open forest (15-70 % of trees and the rest being shrubs and grassland); 3 - shrubs

(woody perennial plants with less than 5 m tall); 4 - herbaceous vegetation (shrubs and tree cover being less than 10%); 5 - crops (temporary crops followed by a period of harvested and bare soil); and, 6 - urban areas (buildings and other humanized structures) (Buchhorn et al., 2020) (Appendix D). For each location (presence or pseudo-absence), the distance to the nearest unpaved and paved roads was calculated and assigned the land use type.

2.2 – Statistical analysis

We used SEM to analyse the direct and indirect effects of roads on leopards. In SEM, when a variable A has an effect on a variable B, and that variable B also as an effect on a variable C, B is called a mediating variable, since it is through B that A has an indirect effect on C (Chan, 2007). In this case, mediating variables are land use types through which roads have an indirect effect on leopards. We used piecewise SEM as it has no assumptions that are violated in ecological studies and allows consideration of linear regression extensions, such as random effects, unlike traditional SEM (Lefcheck, 2020).

We ran eight piecewise SEM, which are all possible combinations of road types (paved and unpaved), period of the day (day and night) and sex (female and male), for locations up to 2000 m from roads. The leopards' space use was causality linked with the other seven variables of interest: type of road and six land use types. Each SEM was composed by two types of generalized linear mixed models (GLMM) (e.g., Hosseini et al., 2011): 1) Each land use type (0/1) ~ distance to roads (m) and 2) leopard locations (1 - presence/0 - pseudo-absence) ~ distance to roads (m) + all land use types (0/1). Since piecewiseSEM does not support two random effects, these models were run with individuals and study areas as random effects, separately. As both models were not significantly different, we decided to only keep the random effect of individuals. Before running each model, we tested for multicollinearity between variables by performing Spearman rank correlation tests. As there were no high correlations (i.e., > 0.7), all variables were included (Appendix E). We ran these models in statistical software R (R Core Team, 2022) and used piecewiseSEM (Lefcheck, 2020) and lme4 packages (Bates et al., 2014).

3 – Results

3.1 – Number of locations

We obtained 10,200 presence and pseudo-absence locations for paved models, of which ~39% were presences (~46% were females and ~54% were males). We obtained 18,266 presence and pseudo-absence locations for the unpaved models of which ~53% were presences (~34% were females and ~66% were males) (Appendix B).

3.2 – Direct and indirect effects of roads at daytime

Paved roads negatively and directly affected both females and males (Figures 3.2a and 3.2b). Moreover, we found that paved roads can negatively and indirectly affect males (Figure 3.2b). Paved roads had a very strong positive relationship with urban areas, a strong positive relationship with crops, and a strong negative relationship with closed forest (Figures 3.2a and 3.2b). However, we found differences in the relationship between paved roads and land uses between sexes: for the female model, there was a significant positive relationship between paved roads and herbaceous vegetation, while for the male model there was a significant negative relationship between paved roads and herbaceous vegetation along with positive associations with open forest

and shrubs (Figure 3.2a and 3.2b). Contrary to females, males were significantly and positively associated with all types of natural land uses (closed and open forest, shrubs, and herbaceous vegetation) which suggest that paved roads may have indirect negative effects via closed forest and herbaceous vegetation and indirect positive effects via both open forest and shrubs on males (Figure 3.2b).

Unpaved roads seemed to have no direct effect on females or males but the relationship between roads and land uses and land uses with males suggest that indirect effects are present (Figure 3.2c and 3.2d). Unpaved roads were positively associated with shrubs and urban areas in both sexes (Figures 3.2c and 3.2d). For the male model, roads were also positively associated with crops and negatively associated with herbaceous vegetation (Figure 3.2d). As in paved models, females were not associated with any type of land use (Figure 3.2c) while males were positively associated with every natural land use type (Figure 3.2d). Thus, unpaved roads had both a positive indirect effect via shrubs and a negative indirect effect via herbaceous vegetation on males (Figure 3.2d). As for paved roads, males were more strongly associated to closed forest than any other land use type (Figure 3.2d).

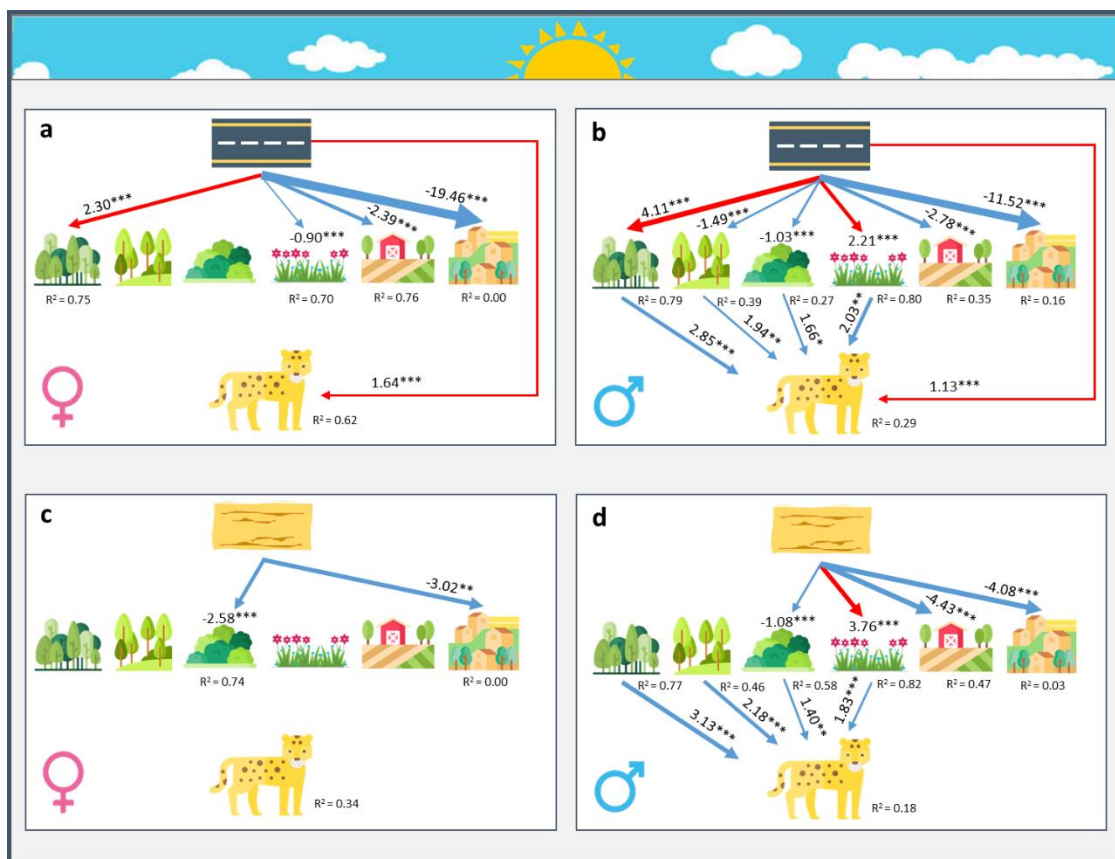


Figure 3.2 – Direct and indirect effects of roads on leopards at day. a – paved roads effects on females; b – paved roads effects on males; c – unpaved roads effects on females; d – unpaved roads effects on males. Path coefficients with the following levels of significance: * p-value < 0.05, ** p-value =< 0.01 and *** p-value =<0.001 and coefficient of determination (conditional R²). Land uses (from left to right): closed forest, open forest, shrubs, herbaceous vegetation, crops, and urban areas. Arrows: red, negative association; blue, positive association; the thickness of the arrows increases with the coefficient value.

3.3 – Direct and indirect effects of roads at night-time

Paved and unpaved roads had significant direct and negative effects on females (Figure 3.3a and 3.3c). Paved roads had a significant indirect and negative effect on males, while unpaved roads

seemed to have a positive direct effect on these individuals (Figures 3.3b and 3.3d). Paved roads were significantly and negatively associated with closed forest and strongly and positively associated with urban areas, followed by crops and open forest (Figures 3.3a and 3.3b). The females were positively and strongly associated with closed and open forest, while the males were less associated with closed forest and herbaceous vegetation. These observations suggest that paved roads may have a negative indirect effect through closed forest in both sexes, a positive indirect effect through open forests in females and a negative indirect effect through herbaceous vegetation in males.

Unpaved roads were negatively associated with closed forest and crops and positively associated with herbaceous vegetation for the female model (Figure 3.3c). For males, unpaved roads were negatively associated with herbaceous vegetation and positively associated with all other land use types except closed forest (Figure 3.3d). Females were positively associated with every land use type except crops (Figure 3.3c) whereas males were positively associated with both closed and open forest and herbaceous vegetation and negatively associated with urban areas (Figure 3.3d). Unpaved roads had a negative indirect effect through closed forests and a positive indirect effect through herbaceous vegetation in females and had indirect negative effects both through herbaceous vegetation and urban areas and an indirect positive effect through open forest on males (Figure 3.3c and 3.3d, Appendix F).

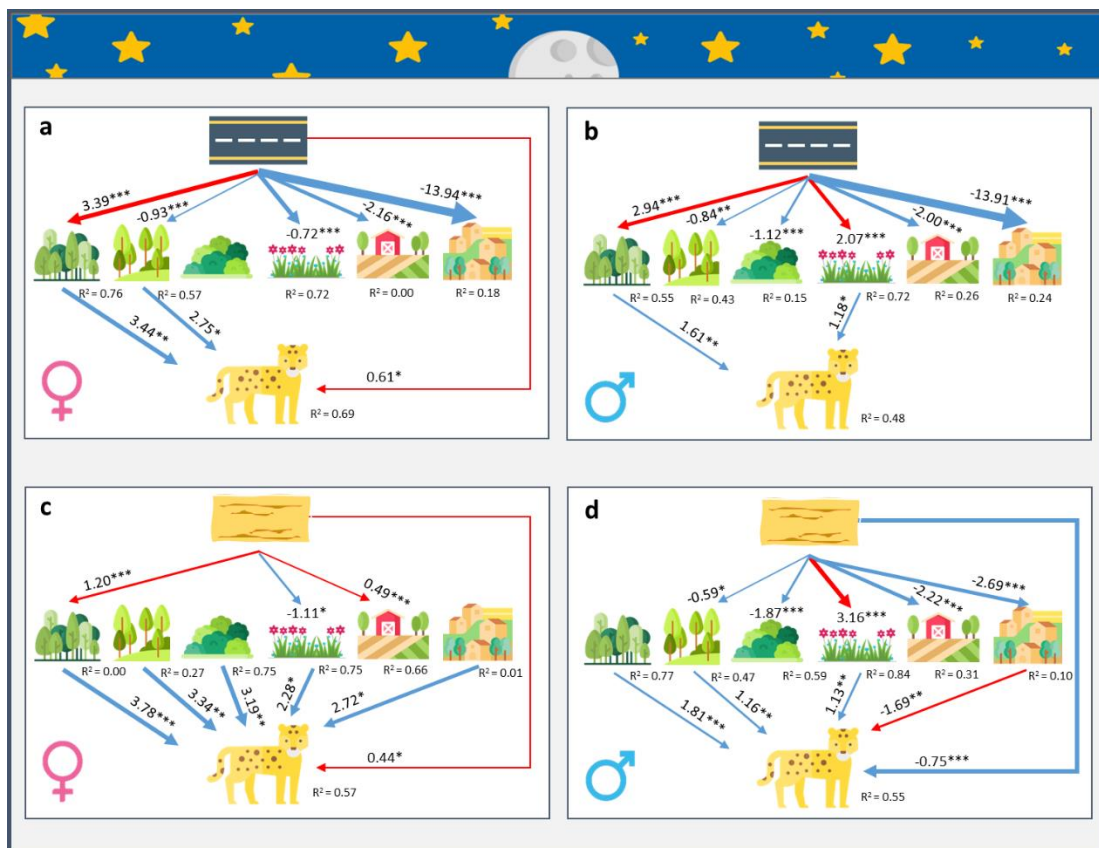


Figure 3.3 – Direct and indirect effects of roads on leopards at night. a – paved roads effects on females; b – paved roads effects on males; c – unpaved roads effects on females; d – unpaved roads effects on males. Path coefficients with the following levels of significance: * p-value < 0.05, ** p-value < 0.01 and *** p-value < 0.001 and coefficient of determination (conditional R²). Land uses (from left to right): closed forest, open forest, shrubs, herbaceous vegetation, crops, and urban areas. Arrows: red, negative association; blue, positive association; the thickness of the arrows increases with the coefficient value.

4 – Discussion

This study provides an important step towards a more complete understanding on how roads affect leopards by highlighting the role of direct and indirect effects of these infrastructures on leopard behaviour across its distribution range that vary regarding type of road, time of the day and sex. These results showed that females were more negatively and directly affected by paved and unpaved roads, while males seem to be positively associated with unpaved roads at night, but negatively and directly affected by paved roads during the day. Paved roads appeared to indirectly affect leopards by being negatively associated with closed forest, the land use type that is positively associated with both sexes.

The direct effects of paved roads on leopards support the hypothesis that leopards avoid high human disturbance (Kittle et al., 2018), especially at daytime when roads have higher traffic volumes, speeding vehicles, and roadside vegetation managements (Gleeson and Gleeson, 2012; Gunther et al., 2000; Taylor and Goldingay, 2004). When human disturbance is coupled with the crepuscular / nocturnal behaviour of leopards (Martins and Harris, 2013; Puls et al., 2021), they may tend to avoid paved roads during the day. Contrary to our results, Zaman et al. (2022) showed that some leopards become closer to secondary roads during the day. However, human movement was rare and one of the leopards' preferred prey, the roe deer *Capreolus capreolus*, showed a similar behaviour in relation to these roads. We also found that the direct effects of unpaved roads at night on leopards seem to be sex-specific, with males being positively associated with these roads. These results might be explained by leopard males having larger home ranges than females and having to patrol them more actively (Snider et al., 2021; Wilmers et al., 2017). At night, they may be forced to travel longer distances and therefore use unpaved roads to reduce energetic costs (Carter et al. 2015). Furthermore, as solitary (Mizutani and Jewell, 1998) and territorial (Norton and Henley, 1987) felids, leopards often exhibit agonistic behaviors when they encounter conspecifics (Roux and Skinner, 1989) which can lead to females, who are smaller and weaker (Bailey, 2005), not using unpaved roads to avoid encountering males (Gray and Prum, 2012).

Paved roads also had indirect effects on leopards. These roads were negatively associated with closed forest, having the strongest associations among all natural land uses. In some models, paved roads were positively associated with open forest and shrubs. These associations might have two possible explanations: paved roads are built in open areas, or they are built in forested areas which were converted into open forest and shrubby areas with less vegetation cover. For example, in Amazon (Brazil), the construction of roads has led to greater deforestation within 50 to 100 km of highways and promoted resources extraction and urban sprawl (Laurance et al., 2002; Barber et al. 2014). As expected, paved roads were positively associated with crops and urban areas (Laurance et al., 2009) suggesting that they promote the development of humanized landscapes that take advantage of roads for movement of goods and people. On the other hand, leopards were positively and strongly associated with closed forest (Mondal et al., 2022), which supports high densities of natural prey and abundant vegetation cover that provides refuge for rest and shelter from heat (Ario et al., 2022; Gebo et al., 2022; Liang et al., 2022; Périquet et al., 2022; Zeng et al., 2022). Due to the negative and positive associations with closed forest for paved roads and leopards, respectively, these roads appear to have a strong negative indirect effect on leopards. Since habitats of leopards are not associated with crops or urban areas (e.g., Kittle et al., 2018; Havmøller et al., 2019; Zeng et al., 2022), we would expect paved roads to have a negative indirect effect on leopards via these land use types, which was not observed in this study.

Although we found general and strong spatial patterns of direct and indirect effects of roads on leopards, caution is needed in interpreting some results: 1) Differences in the relationships between roads and land use types among models may be partially explained by the use of different subsets of the leopard locations for male and female models; 2) Strong associations of leopards with closed forest compared to open forest, as intermediate coverage is optimal for their hunting success (Atkinson et al., 2022), may be explained by how Copernicus Global Land Cover classified open forest (15-70% of trees), since less than 25% might not be enough cover for hunting by ambush or even produce enough prey biomass (Mondal et al., 2022); 3) Females were positively associated with urban areas at night for the unpaved model which might be due to lack of natural prey in some study areas as observed in other studies (Bista et al. 2022; Lamichhane et al., 2021); and 4) The lack of association between females and land uses during the day may be explained by other variables such as conspecifics, competitors, and human persecution, that were not analysed in this study (Pitman et al., 2013; Rouse et al., 2021; Tarugara et al., 2021).

Understanding general patterns of behaviour towards TI for this ecologically complex and wide-ranging species is crucial as it is globally threatened and there is an urgent need to find measures to mitigate the negative effects of roads which can be applied across its distribution range. This study provided comprehensive insights in the relationships between TI and leopards by highlighting the importance of preventing both direct and indirect negative effects of roads on leopards since many of them are frequently exposed to road networks and travel beyond protected areas (Devens et al., 2021; Dickman and Marker, 2005). Moreover, unpaved road effects are sex- and time-specific, which can affect patterns of social relations among individuals in a population with implications on the balance of demographic processes and genetic diversity, threatening the population viability in a long-term (Riley et al., 2014).

When upgrading or building a new road, design and planning must include mitigation of both direct and indirect effects, especially in areas with small and isolated leopard populations, which are more vulnerable to random demographic, genetic, or environmental events and therefore to local extinction (Jaeger et al., 2005). Traffic volume and speed should be reduced mainly during the night when individuals are more active. Human activities (e.g., logging, hunting, agriculture) and urban sprawl should be restricted in the proximity of roads (Zeng et al., 2022). Potential barrier effect should be prevented by improving habitat connectivity through forestation mainly between protected areas (Liang et al., 2022) and in areas with lower risk of conflict with humans, so that safe movement corridors are created (Ario et al., 2022). We also recommend that forest restoration should not occur immediately adjacent to roads to avoid attraction to roads and increase the risk of mortality due to collision with vehicles. Since underpasses have been proven to be successfully used by leopards, they should be placed in combination with fencing to guide individuals and their prey (Lala et al., 2022). Furthermore, to prevent possible human-leopard conflict escalation, as both humans and leopards use unpaved roads and there are reports of leopards attacking people on roads (e.g., Athreya et al., 2004), signs should be placed along unpaved roads to warn drivers and locals of possible encounters.

Further studies are needed to understand the implications of differential spatial responses towards roads, by evaluating leopard densities, population structure, reproductive rates, and gene flow in areas with and without roads (see, e.g., Boulanger & Stenhouse, 2014). It would also be relevant to replicate this analysis with an equal female/male ratio to prevent biases, and to add other variables, such as natural prey abundance, as it can be important to explain leopards' behaviour towards roads (Ario et al., 2022). Other interesting analysis would be to study the effects of roads

on leopard's natural prey and how it would indirectly affect leopard populations (Carter et al., 2022).

We expect that these findings will provide guidance for more ecologically sustainable road development. However, this goal can only be achieved if measures are effectively discussed by local stakeholders (e.g., universities, NGOs, road agencies, and public administration) to generate an integrated design and management plans in order to allow the coexistence of human and leopards and ensure the long-term viability of leopard populations.

4.1 – Conclusion

Roads have several negative impacts on wildlife and ecosystems that surround them, which are expected to worsen due to expected expansion of transport infrastructure. In this sense, studies on the direct and indirect effects of roads are of great relevance to our understanding on what is actually impacting species: the road itself or other effects associated to its presence. To my knowledge it is the first time a study offers a view on these effects on leopards' space use across its distribution range. We found that direct and indirect effects varied accordingly with type of road, period of the day, and sex: paved roads had more negative effects on leopards' spatial use than unpaved roads; females were more strongly, directly, and negatively affected by both type of roads, while males were positively associated to unpaved roads at night; paved roads indirectly affected leopards by being negatively associated to closed forest. More studies are needed to clarify the implications that these effects might have on the viability of populations. Finally, to reduce the identified negative effects of roads on leopards, we recommend several mitigation measures, which will only be effective if all local stakeholders are involved.

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Appendix A – Original references of GPS data, study area, individuals tracked and number of locations per individual in each study.

References	Study areas	Individual ID	N° of locations
Farhadinia et al. (2018)	Tadoureh (Iran)	F5_Iran	78
		M1_Borzou	318
		M2_Bardia	690
		M3_Borna	588
		M4_Tandoureh	420
		M6_Kaveh1	318
Van Cleave et al. (2018)	Laikipia (Kenya)	M6_Kaveh2	304
		CH	142
		HA	528
		KO	444
Snider et al. (2021)	Karnataka (India)	TA	180
		D031669	212
		5863	84
Odden et al. (2014)	Maharashtra (India)	5864	502
		5865	404
		5867	702
		58681	194
		58682	384
Wilmers et al. (2017)	Laikipia (Kenya)	Chumvi	136
		Ewaso	110
		Morani	86
		Mzee	122
Chilcott (2021)	Boland (South Africa)	BF8	336
		BM12	348
		BM17	228
		BM18	478
		BM21	18
		BM4	820
		BM5	684
Williams et al. (2017)	Soutpansberg Mountains (South Africa)	BM7	106
		Anni	32
		Bb	366
		Cc	118
		Drogo	236
		Jenny	386
		Michel	64
Omalley	14		
Pimms	382		

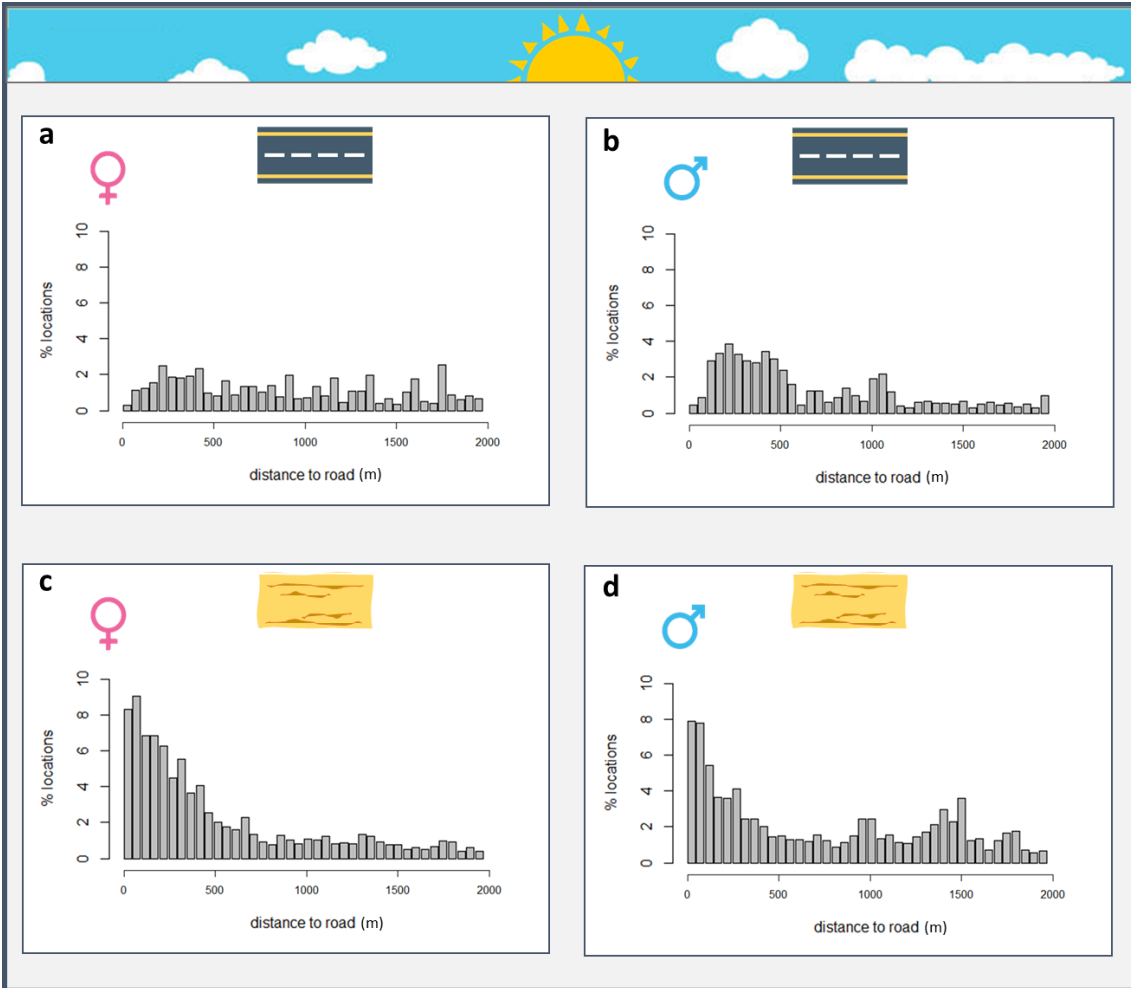
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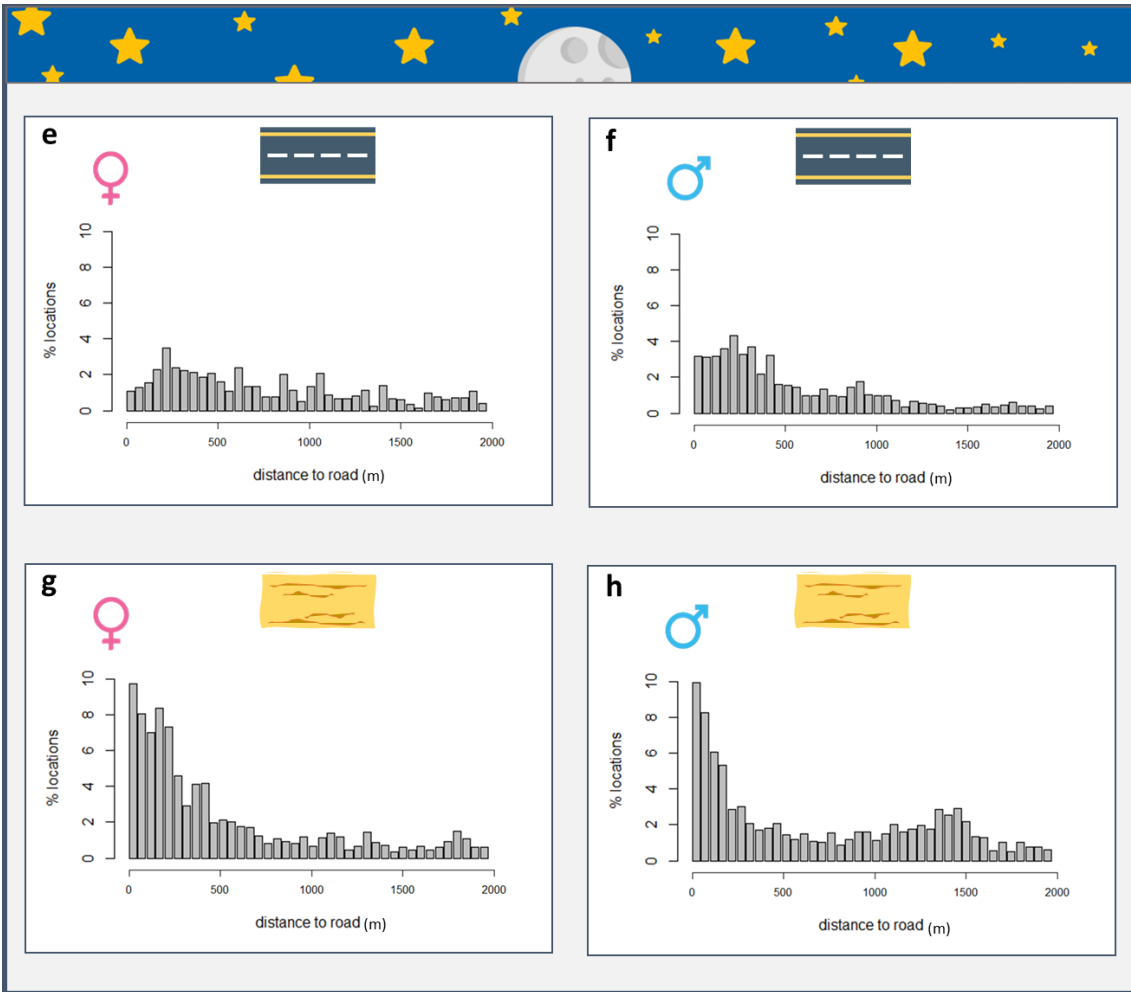
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Appendix B – Information on the number of individuals (total, male, and female) and locations (total, male, and female) for paved and unpaved roads and period of survey for each study area.

		Study areas							
		All study areas	Boland (South Africa)	Soutpansberg Mountains (South Africa)	Laikipia 1 (Kenya)	Laikipia 2 (Kenya)	Tandoureh (Iran)	Maharashtra (India)	Karnataka (India)
N° of individuals	total	36	8	8	4	4	6	5	1
	males	24	7	6	1	2	5	2	1
	females	12	1	2	3	2	1	3	0
N° of locations for paved models	total	4026	853	265	0	0	623	2073	212
	Presences males	2155	648	210	0	0	603	482	212
	females	1871	205	55	0	0	20	1591	0
	total	6174	913	529	1294	454	597	2182	205
	Absences males	2820	817	347	180	208	588	475	205
	females	3354	96	182	1114	246	9	1707	0
N° of locations for unpaved models	total	9744	2822	1190	1294	450	2383	1393	212
	Presences males	6469	2506	717	180	204	2321	329	212
	females	3275	316	473	1114	246	62	1064	0
	total	8522	2524	978	1294	454	2196	889	187
	Absences males	5835	2255	621	180	208	2143	241	187
	females	2687	269	357	1114	246	53	648	0
Period of survey		2009 -2017	2012-2014	2012 -2015	2014-2015	2014-2015	2014-2017	2009-2010	2014

Appendix C – Average percentage of locations per class of 50 meters of road distance of all study areas combining sex and type of roads at daytime and nighttime: (a) daytime, females, and paved roads; (b) daytime, males, and paved roads; (c) daytime, males, and unpaved roads; (d) daytime, males, and unpaved roads; (e) night-time, females, and paved roads; (f) night-time, males, and paved roads; (g) night-time, males, and unpaved roads; (h) night-time, males, and unpaved roads.

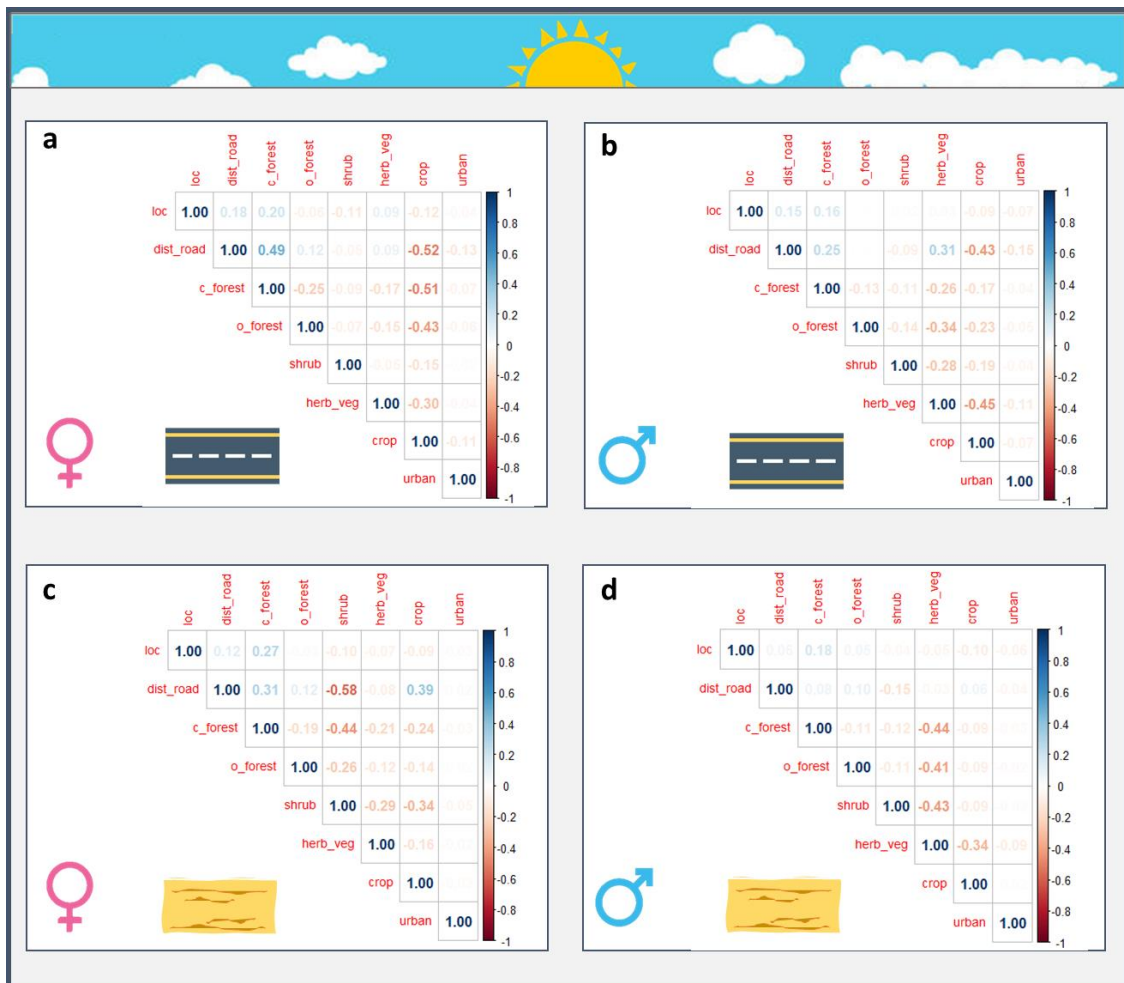


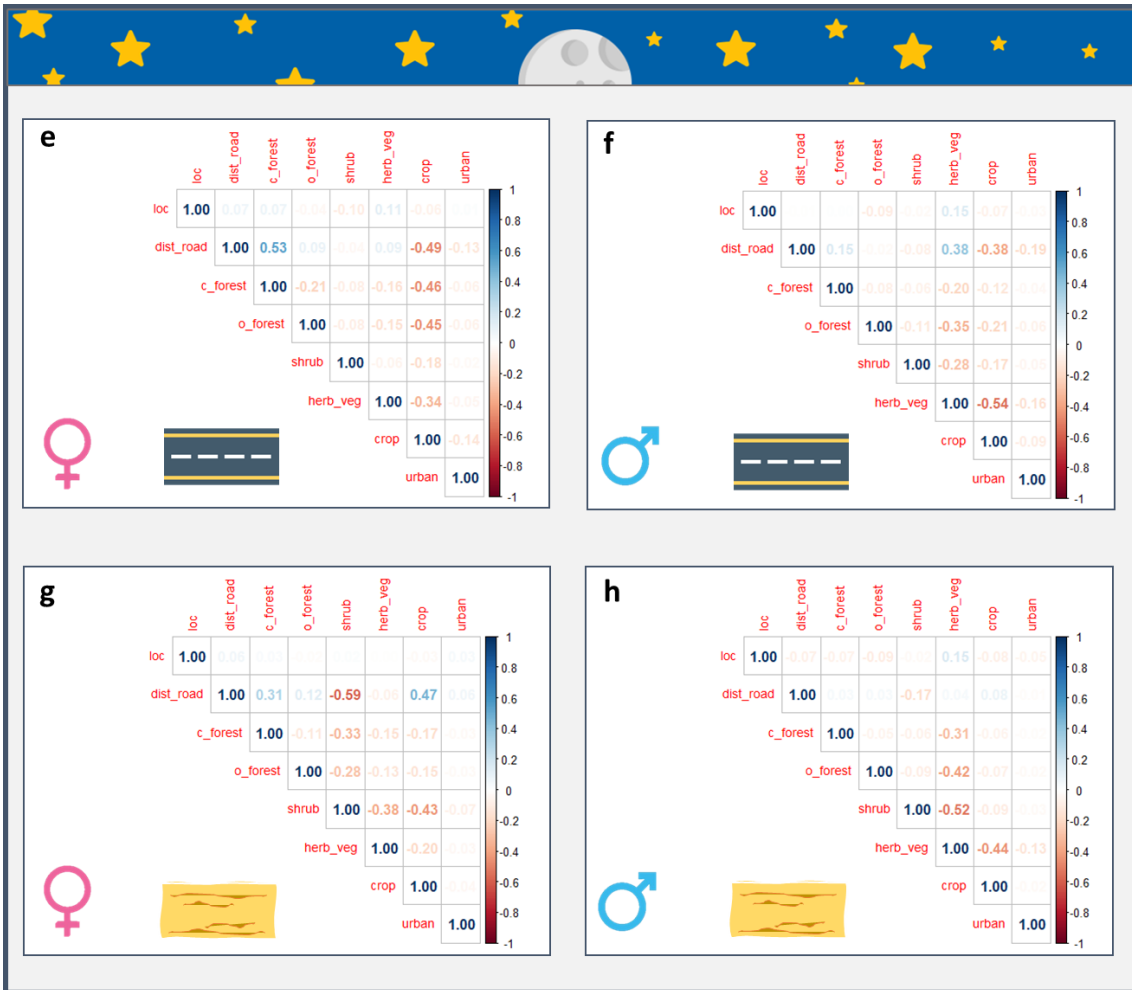


Appendix D – Variable name, description, type of data and units used in the SEM.

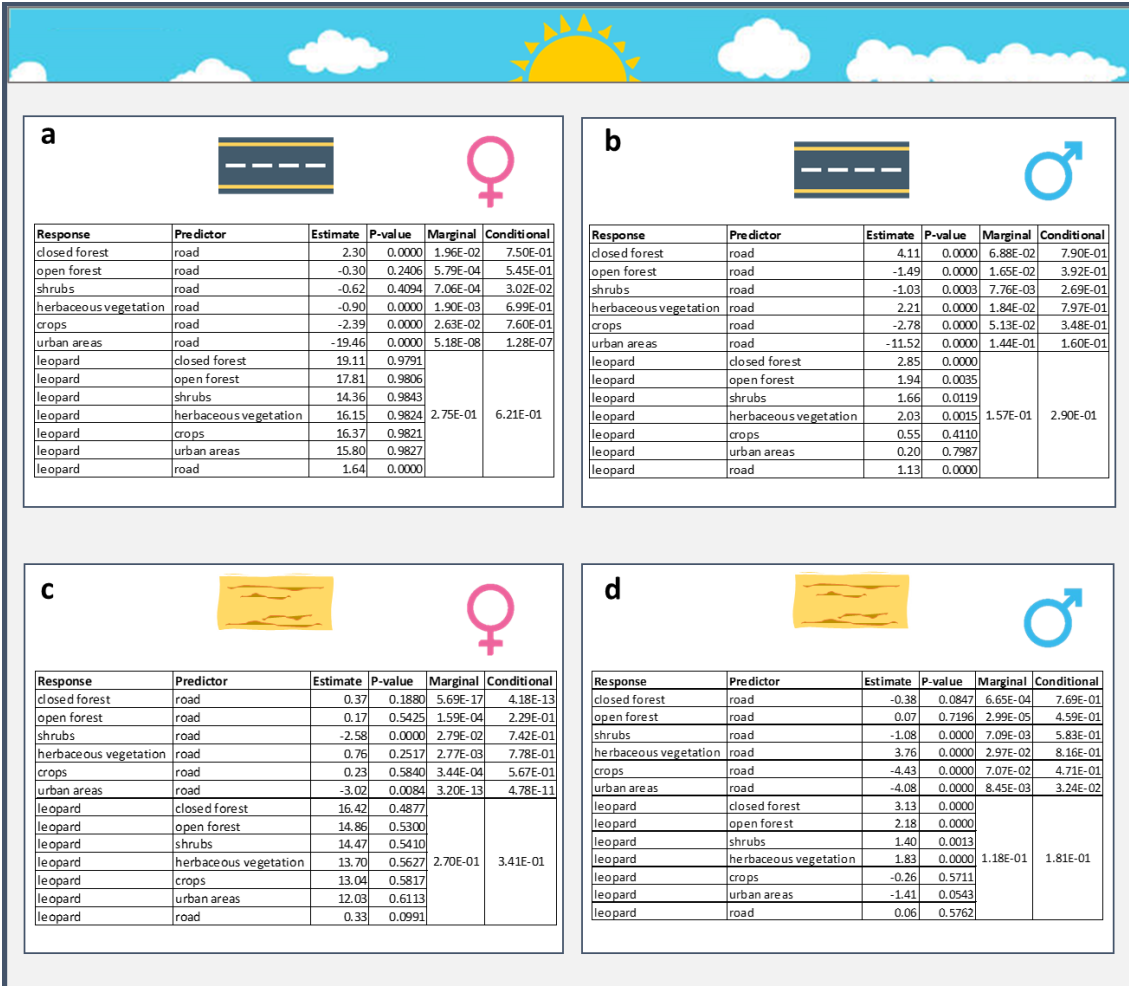
Variable	Description	Data	Units
Closed forest	Tree canopy of more than 70 %	Binary	0/1
Crops	Temporary crops followed by a period of harvested and bare soil	Binary	0/1
Herbaceous vegetation	Shrubs and trees cover being less than 10%	Binary	0/1
Leopard locations	Gps leopard presence locations and leopard pseudo-absences	Binary	0/1
Open forest	15-70 % of trees and the rest being shrubs and grassland	Binary	0/1
Paved road	Tarred road with mean traffic volume of 20-200 vehicles per hour	Continuous	m
Shrubs	Woody perennial plants with less than 5 m tall	Binary	0/1
Unpaved road	Dirt road with mean traffic volume of 0-20 vehicles per hour	Continuous	m
Urban areas	Buildings and other humanized structures	Binary	0/1

Appendix E – Spearman’s rank correlation results for each model taking into account time of the day, type of road and sex: (a) daytime, females, and paved roads; (b) daytime, males, and paved roads; (c) daytime, males, and unpaved roads; (d) daytime, males, and unpaved roads; (e) night-time, females, and paved roads; (f) night-time, males, and paved roads; (g) night-time, males, and unpaved roads; (h) night-time, males, and unpaved roads. Loc – leopard locations; dis_road – distance to roads; c_forest – closed forest; o_forest – open forest; shrub – shrubs; herb_veg – herbaceous vegetation; crop – crops; urban – urban areas.







Appendix F – Statistics of the structural equation model according to time of the day, sex, and type of road: (a) daytime, females, and paved roads; (b) daytime, males, and paved roads; (c) daytime, males, and unpaved roads; (d) daytime, males, and unpaved roads; (e) night-time, females, and paved roads; (f) night-time, males, and paved roads; (g) night-time, males, and unpaved roads; (h) night-time, males, and unpaved roads. Response (0/1), predictor (distance or 0/1), estimate, marginal R², and conditional R².







e



Response	Predictor	Estimate	P-value	Marginal	Conditional
closed forest	road	3.39	0.0000	4.33E-02	7.62E-01
open forest	road	-0.93	0.0007	4.91E-03	5.67E-01
shrubs	road	0.30	0.6621	1.63E-04	3.54E-02
herbaceous vegetation	road	-0.72	0.0000	1.15E-03	7.18E-01
crops	road	-2.16	0.0000	2.99E-08	3.64E-06
urban areas	road	-13.94	0.0000	1.58E-01	1.81E-01
leopard	closed forest	3.44	0.0040		
leopard	open forest	2.75	0.0211		
leopard	shrubs	0.63	0.6046		
leopard	herbaceous vegetation	2.26	0.0507	3.92E-02	6.87E-01
leopard	crops	1.98	0.0970		
leopard	urban areas	2.14	0.0840		
leopard	road	0.61	0.0150		

f



Response	Predictor	Estimate	P-value	Marginal	Conditional
closed forest	road	2.94	0.0000	4.06E-02	5.49E-01
open forest	road	-0.84	0.0077	5.26E-03	4.31E-01
shrubs	road	-1.12	0.0005	8.83E-03	1.54E-01
herbaceous vegetation	road	2.07	0.0000	2.46E-02	7.24E-01
crops	road	-2.00	0.0000	3.15E-02	2.64E-01
urban areas	road	-13.91	0.0000	2.23E-01	2.45E-01
leopard	closed forest	1.61	0.0058		
leopard	open forest	0.98	0.0631		
leopard	shrubs	0.65	0.2122		
leopard	herbaceous vegetation	1.18	0.0165	4.64E-02	4.84E-01
leopard	crops	-0.34	0.5205		
leopard	urban areas	-0.40	0.4919		
leopard	road	-0.27	0.1596		

g

Response	Predictor	Estimate	P-value	Marginal	Conditional
closed forest	road	1.20	0.0005	0.00E+00	0.00E+00
open forest	road	-0.49	0.1236	1.60E-03	2.71E-01
shrubs	road	-0.56	0.3362	1.74E-03	7.48E-01
herbaceous vegetation	road	-1.11	0.0357	6.49E-03	7.54E-01
crops	road	0.49	0.0000	1.40E-03	6.56E-01
urban areas	road	-1.59	0.0710	2.89E-05	7.73E-03
leopard	closed forest	3.78	0.0007		
leopard	open forest	3.34	0.0024		
leopard	shrubs	3.19	0.0034		
leopard	herbaceous vegetation	2.28	0.0318	4.38E-02	5.74E-01
leopard	crops	1.81	0.1018		
leopard	urban areas	2.72	0.0267		
leopard	road	0.44	0.0465		

h

Response	Predictor	Estimate	P-value	Marginal	Conditional
closed forest	road	0.30	0.3523	3.88E-04	7.74E-01
open forest	road	-0.59	0.0192	2.06E-03	4.71E-01
shrubs	road	-1.87	0.0000	2.05E-02	5.87E-01
herbaceous vegetation	road	3.16	0.0000	2.94E-02	8.39E-01
crops	road	-2.22	0.0000	2.26E-02	3.10E-01
urban areas	road	-2.69	0.0002	6.46E-03	9.77E-02
leopard	closed forest	1.81	0.0001		
leopard	open forest	1.16	0.0066		
leopard	shrubs	0.73	0.0792		
leopard	herbaceous vegetation	1.13	0.0043	3.55E-02	5.53E-01
leopard	crops	-0.62	0.1475		
leopard	urban areas	-1.69	0.0041		
leopard	road	-0.75	0.0000		