

ORIGINAL RESEARCH

Phenotypic matching by spot pattern potentially mediates female giraffe social associations

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Keywords

Coat pattern geometry; giraffe; social network analysis; assortative mixing; dyadic tie strength; bond strength; social associations; intraspecific communication.

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Abstract

Animal color pattern is a phenotypic trait that may mediate assortative mixing (also known as homophily), whereby similar looking individuals have stronger social associations. Masai giraffe (*Giraffa camelopardalis tippelskirchi*) coat spot patterns show high variation and some spot traits appear to be heritable. Giraffes also have high visual acuity, which may facilitate intraspecific communication and recognition based on spot patterns. Giraffe groupings are dynamic, merging and splitting throughout the day, but females form long-term associations. We predicted that adult female giraffes show stronger associations with other females that have similar spot pattern traits. We quantified the spot pattern characteristics of 399 adult female Masai giraffes and determined the pattern similarity among pairs (dyads) in their social network. We then tested for an association between coat pattern similarity (spot size, shape, and orientation) and dyadic association strength, and quantified assortative mixing. The strength of social associations was positively correlated with similarity in spot shape. Our results are compatible with assortativity by coat patterns that are similar between mother and offspring, potentially reflecting an effect of relatedness on both pattern similarity and female social associations. These results offer evidence that spot pattern could function as a visual cue for intraspecific communication and kin or individual recognition in a fission-fusion species.

Introduction

As with most phenotypic traits, animal color patterns—a term that encompasses the coloration, brightness, and geometry of patches—are shaped by natural and sexual selection (Cott & Huxley, 1940). Investigating potential adaptive functions of color patterns enables a deeper understanding of their evolution. Three of the most prominent hypotheses explaining the adaptive functions of color patterns are the following: (1) parasite and predator evasion (Cott & Huxley, 1940; Endler, 1978; Kavaliers & Choleris, 2018) including camouflage or concealment (Stoner et al., 2003); (2) physiological regulation, such as thermoregulation (Cobb & Cobb, 2019; Ruxton, 2002) or water balance (Schwalm et al., 1977); and (3) communication between species (such as aposematism) or within species, such as a signal of individual quality (Pérez-Rodríguez et al., 2017), attractiveness to potential mates (Caro, 2005), or for kin and individual recognition (Tibbetts & Dale, 2007; Ward et al., 2020). Color patterns are traits that can lead to the phenomenon of assortative mixing (also known as homophily; McPherson et al., 2001; Fu et al., 2012), where similar looking

individuals tend to have stronger associations with each other. This process is known to play an important role in different social aspects for many animal species, from mating to kin recognition (Leedale, Lachlan, et al., 2020; Penn & Frommen, 2010; Wilson & Dugatkin, 1997), which supports the hypothesis that color patterns could have a function in intraspecific communication.

Giraffes (*Giraffa camelopardalis*) have complex and variable coat color patterns whose origins and functions are still understudied. Each giraffe has a unique pattern of spots, which does not change from birth to death (Foster, 1966) and certain spot traits appear to be heritable from mother to offspring (Lee et al., 2018). The function of giraffe spots remains unclear but hypotheses include juvenile camouflage (Lee et al., 2018; Mitchell & Skinner, 2003), thermoregulation (Skinner & Smithers, 1990), and kin and individual recognition (Ishengoma et al., 2017; Tibbetts & Dale, 2007). Larger, irregularly shaped, and rounder spots were associated with higher calf survival, suggesting the spots might serve at least in part as camouflage (Lee et al., 2018), but the functions of giraffe coat patterns could also differ with life stage. Numerous studies have suggested that

giraffes have exceptional visual acuity among mammals based on the anatomy of their eye (Coimbra *et al.*, 2013; Mitchell *et al.*, 2013; Veilleux & Kirk, 2014) and adaptations of their visual genes (Ishengoma *et al.*, 2017; Liu *et al.*, 2021). Thus, intraspecific monitoring and communication among giraffes may be primarily visual (Bercovitch & Deacon, 2015; Kasozi & Montgomery, 2018). Ishengoma *et al.* (2017) noted that the characteristic coat color of giraffes is reddish-brown spots set within a network of white lines, and that their eye's particular sensitivity to red pigment should allow for easier discrimination among individual giraffes. No studies have investigated visual acuity in relation to giraffe communication (Kasozi & Montgomery, 2018). Our objective here is to examine whether giraffe coat color patterns are correlated with strength of social associations among adult females, and therefore potentially serve a function in intraspecific communication. This information can improve our understanding of proximate mechanisms that might mediate the formation and strength of social ties in species with complex social systems.

Giraffes have a multi-tiered social system based on fission–fusion dynamics, with temporary groupings that merge and split throughout the day (Bond *et al.*, 2019; Carter, Brand, *et al.*, 2013; Carter, Seddon, *et al.*, 2013; Hart *et al.*, 2019) embedded within larger, stable social communities (Bond, König, Lee, *et al.*, 2021; Bond, König, Ozgul, *et al.*, 2021; Lavista-Ferres *et al.*, 2021; VanderWaal, Wang, *et al.*, 2014). Female giraffes form communities via long-term associations with familiar, related females (Bercovitch & Berry, 2013; Bond, König, Lee, *et al.*, 2021; Bond, König, Ozgul, *et al.*, 2021; Bond, Lee, Ozgul, *et al.*, 2021; Carter, Brand, *et al.*, 2013; Carter, Seddon, *et al.*, 2013). Subadult and younger adult males disperse, form temporary bachelor herds, and establish a dominance hierarchy, while older adult males roam alone among female communities searching for receptive females (Bond *et al.*, 2019; Dagg & Foster, 1976; Knüsel *et al.*, 2019; Lavista-Ferres *et al.*, 2021; van der Jeugd & Prins, 2000), so social associations among males are less stable over time (Carter, Brand, *et al.*, 2013). Therefore sex differences between females and males in social connectedness, with females having higher social association scores but fewer affiliates than males (Lavista-Ferres *et al.*, 2021) follows from their life history strategies.

Sociability appears to be especially important for female giraffes. Adult females have higher survival rates when grouping with more other females (Bond, Lee, Farine, *et al.*, 2021), and they are known to provide care to nonoffspring in crèches (Muller & Harris, 2022). If we assume spot traits are heritable (Lee *et al.*, 2018) and more strongly socially associated females are more genetically related than expected (Bercovitch & Berry, 2013; Carter, Brand, *et al.*, 2013), then recognizing and grouping with similarly patterned females—who are likely to be relatives—might improve their direct and inclusive fitness (Hamilton, 1964). Alternatively, if females associate with relatives by other mechanisms such as familiarity (Bekoff, 1981), then a correlation between association and spot traits also may arise. We thus hypothesise that adult female giraffes show stronger associations with other females that have similar spot pattern traits.

Among giraffe subspecies (Muller *et al.*, 2018) or species (Petzold & Hassanin, 2020), Masai giraffes (*G. c. tippelskirchi* or *G. tippelskirchi*) show particularly high variation in spot patterns (Fig. 1). We generated a social network of 399 free-ranging adult female Masai giraffes inhabiting a large, unfenced landscape in Tanzania, and quantitatively measured 10 of their spot traits. We predicted a correlation between spot pattern similarity and association strength between pairs of females and tested this prediction by quantifying assortative mixing. Identifying assortative mixing would offer evidence that coat color patterns in giraffes potentially serve a kin and/or individual recognition function.

Materials and methods

Study area and data collection

We studied a population of individually recognized adult female Masai giraffes inhabiting the Tarangire Ecosystem of northern Tanzania (Lee *et al.*, 2016). We collected data on locations and membership of temporary group formations from 2012–2018, identified individuals by photographs of their spot patterns and created encounter histories, which we used to conduct social network analysis to quantify dyadic association strength. We then measured spot traits using the photographs and quantified assortative mixing by spot traits. The main vegetation communities in the Tarangire Ecosystem are *Vachellia* [formerly *Acacia*] *tortilis* parkland, *Vachellia-Commiphora* woodland, gall *V. drepanolobium* woodland, *Combretum-Dalbergia* woodland, and open grassland (Lamprey, 1963). Our 2200 km² study area was in the core of the Tarangire Ecosystem and spanned four administrative units: Tarangire National Park, Manyara Ranch Conservancy, and Lolkisale and Mto-wambu Game Controlled Areas (Fig. 2). None of the administrative units are fenced, and all are connected by movements of adult female giraffes (Lee & Bolger, 2017).

We identified individuals using non-invasive photographic mark-resighting techniques. We collected data during 42 independent, daytime, fixed-route transect driving survey events between January 2012 and October 2018, with two consecutive (replicate) survey events towards the end of each of northern Tanzania's three precipitation seasons per year (short rains, long rains, dry season). We used a Canon 40D camera body with a Canon Ultrasonic IS 100–400 mm lens to photograph and identify individual giraffes from their unique spot patterns. When we encountered a singleton or group of giraffes, we 'marked' or 'resighted' individuals by driving to within 150 m distance and photographing their right-side thoracic area at a perpendicular angle. We assessed a suite of physical characteristics, including body shape, relative length of the neck and legs, ossicone characteristics, and visual estimation of height, to categorize giraffes as adult females (Strauss *et al.*, 2015). Female giraffes in the wild typically first reproduce at 5–6 years of age (Lee & Strauss, 2016) but become sexually mature at about 3.5 years (Dagg, 1971). Therefore, we considered adult females to be ≥ 4 years of age (Lee & Strauss, 2016). Additionally, we recorded date, time of the day, and geographical coordinates of the sampled individual if singleton or of the approximate centre

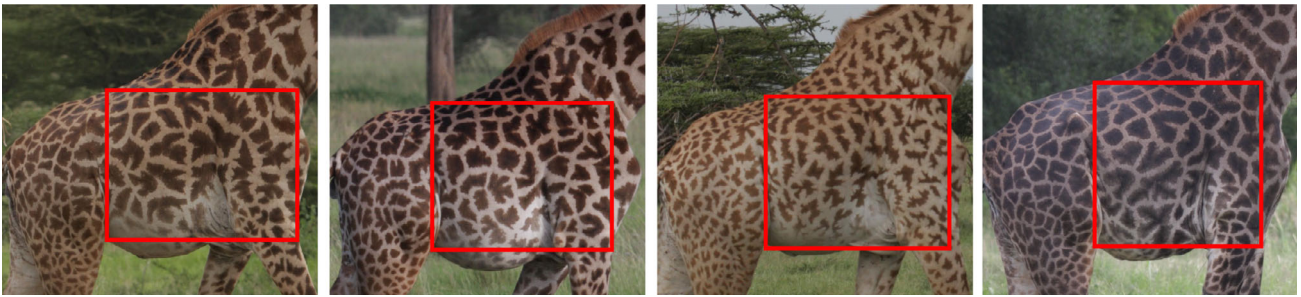


Figure 1 Region of interest selection on different individuals reveals the variety of spot pattern colors and geometries within one giraffe subspecies (or species).

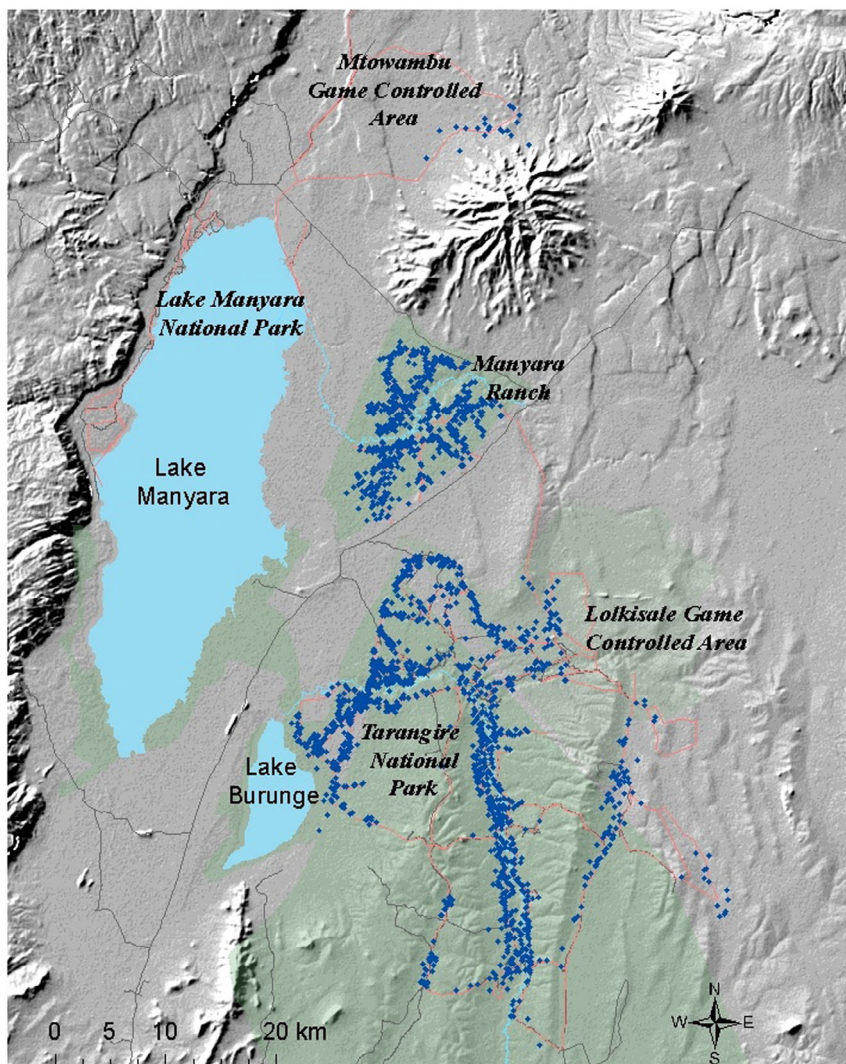


Figure 2 Locations of adult female Masai giraffes in the Tarangire Ecosystem study area (blue points). Red lines are tracks surveyed for giraffes from 2012 to 2018, and solid green polygons are protected areas (Tarangire National Park, Wildlife Management Areas, and Manyara Ranch).

of the group. To identify individuals, we used Wild-ID, a computer program that matches coat patterns from photographs with low error rates for giraffes (Bolger *et al.*, 2012). Photographic records were then converted into encounter histories and a group-by-individual database to enable the calculation of social networks and dyadic association strength.

Our dataset included all females first observed during the initial year of the study that (1) were adults the first time they were observed and (2) were detected at least 6 times over the course of the study to improve accuracy of social network analysis (Davis *et al.*, 2018). This resulted in a subset of 411 adult females. We used the gambit of the group to define associations, whereby individuals present together in the same group formation were considered associated during that survey (Whitehead & Dufault, 1999). A group formation was defined as one or more giraffes that were foraging or moving together, and with at least 500 m distance to the closest member of another group (Bond *et al.*, 2019; Carter, Brand, *et al.*, 2013; Carter, Seddon, *et al.*, 2013; VanderWaal, Wang, *et al.*, 2014). Giraffe groups were usually self-defining as the distances between individuals were substantially less within groups than between groups. We drove each road transect once per survey event. When an individual was detected on a different transect during the same survey, the individual was assigned to its first group formation of the survey event to avoid sampling biases and ensure independence of observations. We then selected only the individuals with at least one high-quality picture, accounting for resolution, angle, and absence of birds or plants interfering with the individual's coat pattern on the photograph, reducing the final dataset to 399 individuals.

Pattern extraction and quantification

Different properties of pattern geometry can be measured and used to quantify pattern variation. For pattern extraction and quantification, we used ImageJ (Schneider *et al.*, 2012) to analyse spot traits in our region of interest (ROI), a rectangular area fitting horizontally between the anterior edge of the rear leg and the chest, and vertically between the back and where the belly and hind leg meet (Fig. 1, electronic Appendix S1 (Figure S1)). We measured ten metrics within the ROI, excluding the spots that were split by the boundary of the ROI: (1) Nr = number of complete spots within the ROI; (2) $Area$ = spot area; (3) $Perim$ = spot perimeter; (4) $Angle$ = spot orientation (angle between major axis of the fitted ellipse and the x-axis of the image); (5) FD = Feret diameter (or maximum caliper, the longest distance between two points on the spot's boundary); (6) FA = angle of the Feret diameter against the x-axis of the image; (7) AR = aspect ratio, or ratio of width to height, between major and minor axis of the spot's fitted ellipse; (8) $Circ$ = circularity (quantifies the closeness of the spot to a perfect circle, with a value of 1 indicating a perfect circle and smaller values indicating more elongated shapes); (9) $Round$ = roundness (inverse of the aspect ratio); and (10) $Solid$ = solidity (describes how smooth versus tortuous is the edge of a shape). All the measurements were done in *giraffe units* (GU, where 1 GU equals the height of the ROI in pixels) to account for differences in image resolution and animal size

and to compare individuals with a scale-invariant standard measurement. These same metrics and methods were also used by Lee *et al.* (2018) to measure mother and calf spot traits.

We quantified the total phenotypic variation of each spot trait's measurements for every focal female by calculating mean, standard deviation, and coefficient of variation (CV, ratio of the standard deviation to the mean) of all spots in that female's ROI (Table 1). Manual correction of some spot shapes might have increased error (electronic Appendix S1 (Figure S1)). We therefore tested the repeatability of measurements by comparing spot pattern trait measurement results from different images of the same individual. We used a repeatability measure (R) which describes the within-individual correlation among measurements of spot pattern (Nakagawa & Schielzeth, 2010). We carried out the repeatability analysis for 40 individuals with at least two high-quality images using the package *rptR* (Stoffel *et al.*, 2017) for R (R Core Team, 2020). To quantify variation and covariation among the traits and reduce the dimensionality of the large number of traits, we performed a principal component analysis (PCA) with the R packages *FactoMineR* (Lê *et al.*, 2008) and *factoextra* (Kassambara & Mundt, 2017). We then quantified assortative mixing by the principal components.

Social network analysis

A social network describes observed patterns of associations as a set of nodes—in our case, individual adult female giraffes—connected by edges representing the presence or absence of an association (binary) or how frequently they associate (weighted) (Farine & Whitehead, 2015). To investigate assortative mixing by spot traits in female Masai giraffes, we created a weighted social network over the study period using the simple ratio index of association between two individuals (dyad) (Farine & Whitehead, 2015) with the R package *asnipe* (Farine, 2013). The simple ratio index is the number of times individual *A* was seen with *B*, divided by the total number of times individual *A* was seen (Farine, 2013). The index ranges from 0, indicating that *A* and *B* were never seen together, to 1, indicating that *A* was always seen with *B*. This was based on the grouping events data, where all females belonging to the same group during a survey occasion were counted as socially associated.

Assortative mixing

To determine assortativity among females based on coat patterns, we measured weighted assortativity coefficients for continuous traits (r_c^w), which is derived from the Pearson correlation coefficient of degree between pairs of linked nodes (electronic Appendix S2) and ranges from 1 (completely assorted) to -1 (completely disassorted), where a completely random network has a r_c^w equal to 0 (Farine, 2014). The weighted assortativity index sums the weights so that an association of 0 does not contribute to the index value. Positive assortment means that similar nodes are more strongly associated than expected, whereas negative assortment indicates avoidance of similar nodes (Farine & Whitehead, 2015). We calculated the respective standard errors through jack-knife resampling (Farine, 2014). We carried out the analysis on the weighted social network, thus measuring

Table 1 Summary statistics for 10 spot traits from 399 Masai giraffes photographed in the Tarangire Ecosystem, Tanzania 2012–2018, including mean, standard deviation (sd), coefficient of variation (CV), repeatability (R), standard error of R (SE (R)), and the *P*-value of R (*P*-value (R))

	Area ^a	Perim ^b	FD ^b	Nr ^c	Round	Solid	Circ	AR	Angle ^d	FA ^d
Mean	0.02	0.66	0.19	33.8	0.61	0.82	0.48	1.81	83.2	85.3
sd	0.01	0.42	0.09	9.74	0.17	0.08	0.19	0.64	47.9	45.9
CV	0.50	0.63	0.49	0.29	0.28	0.10	0.40	0.35	0.58	0.55
R	0.78	0.81	0.78	0.79	0.85	0.92	0.89	0.87	0.81	0.91
SE (R)	0.13	0.18	0.14	0.13	0.21	0.05	0.18	0.11	0.11	0.04
<i>P</i> -value (R)	0.005	0.007	0.004	0.001	0.002	0.001	0.002	0.005	0.009	0.001

Repeatability analysis was conducted for a subset of 40 individuals. Measured coat pattern traits were: area, perimeter (Perim), Feret diameter (FD), number of spots (Nr), roundness (Round), solidity (Solid), circularity (Circ), aspect ratio (AR), angle (Ang), and Feret angle (FA).

^aGiraffe units (GU)².

^bGiraffe units (GU).

^cCount.

^dDegrees (°).

assortment by the spot pattern principal components based on the edge weight between an associated dyad. We determined statistical significance of the association strength based on coat pattern by constructing null models through pre-network permutations (Farine, 2017). Null models (i.e., creating random networks without the phenomena of interest) are useful for social network analysis because data about social interactions are not independent (Croft *et al.*, 2011). Constructing null models also controls for non-social factors that could drive aggregations of animals (Farine, 2017; VanderWaal, Atwill, *et al.*, 2014). We established the significance of the resulting r_c^w using pre-network permutations that randomized the observations of individuals between groups from the original data set (Farine, 2017). The distribution of r_c^w values from randomized networks was then compared with the observed network r_c^w value.

For the randomizations, we controlled for the temporal distance among individual detections by restricting swaps only within each survey occasion, which consists of two consecutive survey events ($n = 21$ occasions). We permuted the social network 1000 times, maintaining constant group size and thus individual gregariousness during each randomisation. In each permutation individuals were randomly swapped between groups and the network was recalculated (Whitehead, 2008). For each permuted network we measured the assortativity coefficient r_c^w and compared the distribution of the randomized assortativity coefficients to the observed value. For assortativity (positive r_c^w), the *P*-values (P_{rand}) were measured as the proportion of randomized r_c^w being larger than the observed values, and the case of disassortment (negative r_c^w) as the proportion of random r_c^w being more negative than the observed r_c^w . The analyses were run with the R package *assortnet* (Farine, 2014).

Results

We quantified 10 spot pattern traits for each focal adult female Masai giraffe in our study sample (Table 1, electronic Appendix S1 (Figure S2)). For 40 individuals with two different photographs analysed, we found strong repeatability (R) for almost every trait metric (Table 1). Most spot traits showed high variability, with relatively large coefficients of variation

(Table 1). Because some traits were measuring similar properties of the spots, some of the metrics were correlated (electronic Appendix S1 (Table S1)); therefore, we used PCA to eliminate collinearity between spot pattern traits. We found that the first three dimensions of the PCA together explained 82.6% of the overall data variance (Fig. 3, electronic Appendix S1 (Figure S3)). See Fig. 4 for coat patterns corresponding to low and high PC values. The first principal component (PC1) explained 38.4% of the overall variance among spot traits and included all size-related spot traits, area, perimeter, Feret diameter, and total number of spots, with larger PC1 values describing larger spots. The second dimension (PC2) explained 26.5% of the total data variance and was composed mainly of spot shape-related traits, namely circularity, solidity, roundness, and aspect ratio, with larger PC2 values describing rounder spots and smaller values coding for more elongated spots. The third dimension (PC3), which explained 17.7% of the total variance, included spot orientation-related traits, Feret angle and angle of the spots, with larger PC3 values corresponding to left-directed spots and smaller values to right-oriented spots.

Each focal female giraffe in our study sample was observed on average 14 times ± 5 sd (range 6–31) over the 7-year study. The mean dyadic association strength, measured by the simple ratio index, among all 399 females in the sample was 0.004 ± 0.019 sd (range 0–0.412), and among associated females was 0.059 ± 0.039 sd (range 0.019–0.412).

Female giraffes showed positive assortment by PC2 (observed $r_c^w = 0.0320$, permuted mean $r_c^w = 0.0116$, $P_{\text{restr.rand}} = 0.049$, Table 2, electronic Appendix S2 (Figure S4)), reflecting shape traits. Females did not mix assortatively based on the other two principal components, reflecting shape size and orientation (Table 2, electronic Appendix S2 (Figure S4)).

Discussion

Animal color pattern is a phenotypic trait that can lead to assortative mixing (Ward *et al.*, 2020). Here, we quantified spot traits of the coats of free-ranging adult female Masai giraffes in the Tarangire Ecosystem of Tanzania and confirmed that these traits were highly variable among individuals. We

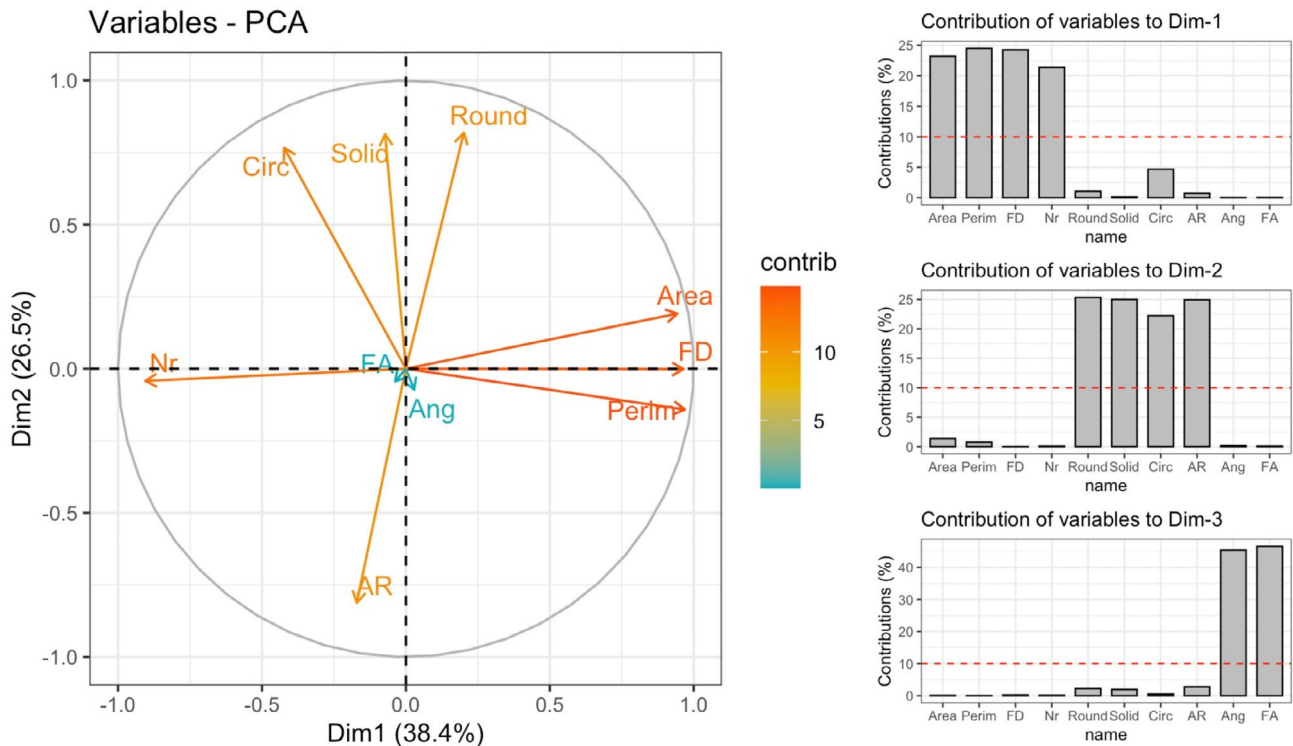


Figure 3 Contributions of 10 spot trait variables to the first three dimensions of the principal component analysis (PCA): PC1 (Dim1) explained 38.4% of the total variance and summarized mainly measurements of size-related traits: area, perimeter (Perim), Feret diameter (FD) and number of spots (Nr); PC2 (Dim2) explained 26.5% of the total variance and included mainly shape-related traits: aspect ratio (AR), circularity (Circ), solidity (Solid), and roundness (Round); Feret angle (FA) and angle were mainly explained by PC3 (Dim3).

showed that association strength between dyads was positively correlated with spot shape similarity (PC2), supporting our prediction of homophilic preference based on spot traits in this species. Previous research found that coat patterns do not change over life (Foster, 1966) and that aspects of giraffe spot shape are highly correlated between mother and offspring and are therefore likely heritable (Lee et al., 2018). Traits that are genetically based and developmentally stable, as well as highly variable, have potential to be useful cues for individual or kin recognition (Penn & Frommen, 2010; Tibbetts & Dale, 2007). Our results offer evidence that individual differences in spot patterns might facilitate conspecific recognition and therefore mediate social associations among female giraffes.

This study links together previously formulated hypotheses about the presence of stronger relationships among female kin, and the heritability of spot traits in giraffes. Based on social network analyses and genetics of individuals, Carter, Seddon, et al. (2013) found that female Angolan giraffes (*G. c. angolensis* or *G. giraffa*) showed preferred associations with females that were more related to them than by chance. Further, spot shape showed significant similarity between Masai giraffe mothers and their calves in the Tarangire Ecosystem (Lee et al., 2018). Female giraffes in our study preferred to associate with other females of similar spot shape, thus female giraffe associations may be based on kinship as reflected by spot traits which are heritable. The visual cue of coat spot

pattern may enable kin recognition in general, and potentially individual identification of familiar giraffes. To be clear, we do not suggest that giraffes are mathematically quantifying the shape of the individual spots of other giraffes they encounter, but it is possible that they are able to rapidly assess the general ‘gestalt’ of the patterns.

A recognition system among conspecifics has been suggested to involve the production of an external cue, the perception of the cue and formation of a recognition template by a receiver, and action taken by the receiver based on perceived similarity between the template and an encountered phenotype (Leedale, Li, & Hatchwell, 2020; Mateo, 2004; Sherman et al., 1997; Ward & Webster, 2016). Recognition can be based on vocal (Leedale, Lachlan, et al., 2020; Pfefferle et al., 2013), olfactory (Gerlach et al., 2008; Johnston & Bullock, 2001; Krause et al., 2012), or visual cues (Petrie et al., 1999). The recognition mechanism can be of genetic or environmental origin, and the matching of perceived phenotypes to a recognition template may be genetically encoded or learned (Greisser et al., 2015; Hatchwell et al., 2001). Studies of intraspecific communication based on color patterns in mammals are lacking, but mammalian coat patterns may be just such a visual recognition template, and might be particularly useful for social species with high fission–fusion grouping dynamics and high visual acuity, such as giraffes, in which grouping behaviour influences fitness (Bond, Lee, Farine, et al., 2021).

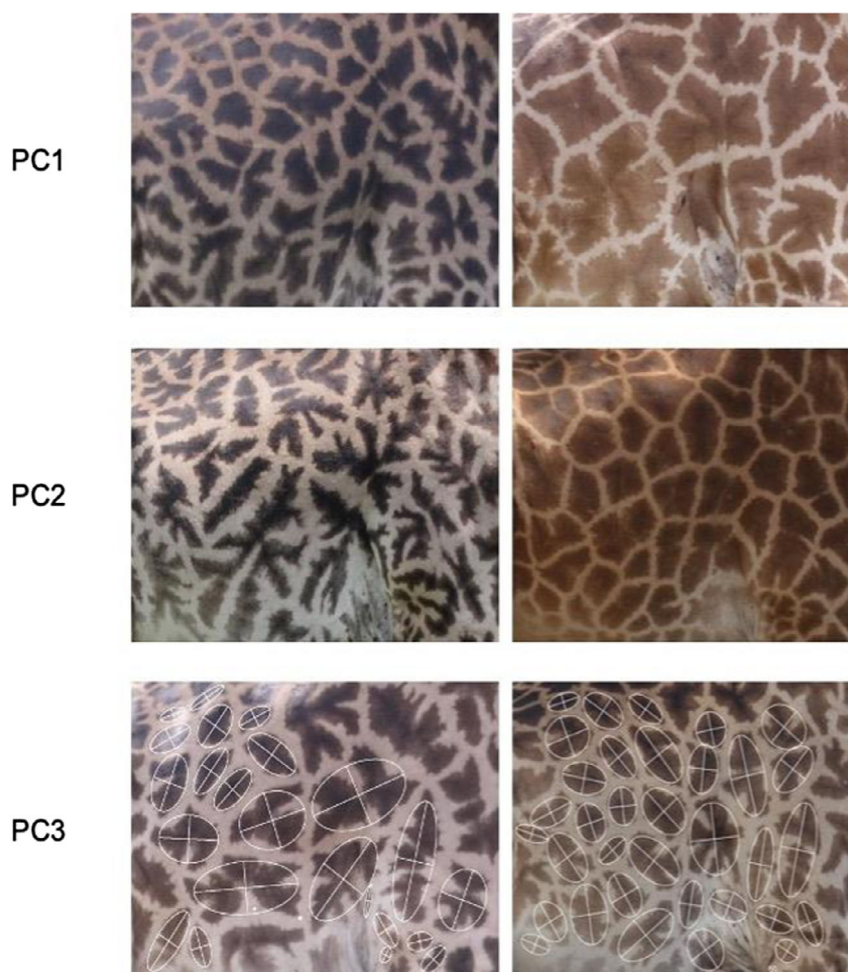


Figure 4 Differences of giraffe coat patterns based on PC dimensions. PC1 explained spot size variation: the minimum value of PC1 with smaller spots (left) compared with the maximum one with larger spots (right); PC2 explained spot shape variation: on the left is illustrated the coat pattern with the minimum PC2 value with more elongated spots against the maximum PC2 value with rounder spots (right); PC3 described spot orientation, with spots more oriented to the right (the major axis of the fitted ellipse) for minimal values of PC3 (left) compared to left-directed spots with maximal PC3 values (on the right).

Giraffes inhabit spatially and temporally dynamic savanna ecosystems, and their fission-fusion social system permits group formations to merge and split constantly in response to fluctuations in water or food resources (e.g., Holekamp *et al.*, 2012) or local risk of predation (e.g., Thaker *et al.*, 2010). Therefore, recognizing kin based on a phenotypic cue of spot pattern shape (i.e., the shape of reddish spots set within a network of white lines; Ishengoma *et al.*, 2017, see also Fig. 1), which might therefore constitute the giraffe's recognition template, could aid female giraffes who have split from their preferred associates and calves to regroup easily. Female giraffes that group with more other females have improved survival rates leading to higher reproductive success (Bond, Lee, Farine, *et al.*, 2021). Adult female giraffes cooperate in caring for young (Dagg & Foster, 1976; Leuthold, 1979) and occasionally allow allonursing (Bond & Lee, 2019). Giraffe calves form crèches accompanied by one or a few older

females, so mothers can range relatively far from their offspring to drink or forage (Dagg & Foster, 1976; Leuthold, 1979). If as we suspect, female Masai giraffes form stronger associations with familiar, related females to cooperatively raise young (which are also potentially recognizable by their spot traits), then such phenotypic recognition imparts inclusive fitness benefits as well (Cornwallis *et al.*, 2009; Hamilton, 1964).

We detected assortative mixing but the strength of homophily by spot traits was somewhat weak, as our assortativity coefficient values were positive but close to zero. This may reflect the highly fission–fusion nature of giraffe groups, and that females likely also associate with non-kin, for example when converging on spatially limited food or water sources especially during the dry season (Bond *et al.*, 2019). Furthermore, young female giraffes in our study area sometimes dispersed into different social groups than those into which they

Table 2 Assortativity coefficients and standard errors (SE) for giraffe spot traits in a weighted social network

Trait	Observed assortativity		Permuted assortativity (time restrictions)		$P_{\text{restr.rand}}$
	r_c^w	SE	Mean r_c^w	SD	
PC1	0.0037	0.0115	0.0116	0.0053	0.913
PC2	0.0320	0.0111	0.0116	0.0053	0.049
PC3	-0.0164	0.0106	0.0116	0.0052	0.354

P -values were calculated using permutations with temporal restrictions. The permuted assortativity coefficients were presented as the mean r_c^w value of the 1000 permuted networks, and P -values were the proportion of assortativity coefficients from the permuted networks that were greater or less than the observed coefficient. Significant results are bolded.

were born, although they dispersed far less often than young males (Bond, Lee, Ozgul, *et al.*, 2021). Such social dispersal would also lead to females associating with non-kin.

In this study, we extracted and quantified giraffe coat pattern traits from photographs with high measurement repeatability, following the framework previously used by Lee *et al.* (2018), and identified three primary categories of spot measurements, including size, shape, and orientation. We confirmed that digital image processing using software such as ImageJ can objectively and reliably quantify spot traits and categorize individuals into general phenotypic groups. This methodology could be applied to studies that compare spot traits among subspecies (or species) of giraffes across Africa, and contribute to the current taxonomic debate (Bercovitch *et al.*, 2017). Such additional research could also quantify and explore within-subspecies (or species) variation in spot traits.

We conclude that free-ranging adult female giraffes show positive assortative mixing by spot pattern traits, with spot shape appearing to be the basis of the assortative mixing. We suspect that homophily based on spot shape reflects kinship, since Carter, Seddon, *et al.* (2013) found relatedness to be positively correlated with association strength among female giraffes, and Lee *et al.* (2018) found spot size and shape were likely inherited from mother giraffe to offspring. Our study links and advances these previous findings by confirming that spot traits were individually variable among females and were correlated with the strength of their social associations. Our study also provides a reliable and quantitative methodology to apply towards future research testing whether related females indeed have similar spot pattern traits. Overall, we posit that size and shape of spot color patterns in giraffes may function at least in part as concealment, following Lee *et al.* (2018), while the shape of spot patterns may facilitate individual recognition and potentially signal relatedness.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Pattern extraction and quantification.

Appendix S2. Assortative mixing.

Figure S1. ROI selection and processing for pattern geometry quantification: (a) ROI defined with the “belly-method”; (b) ROI cutoff from the starting image; (c) thresholded image; red circles indicate some “errors” that have to be manually corrected to reflect the real pattern; (d) hand-corrected image that will be used to extract the pattern geometry measures.

Figure S2. The histograms show the distribution of each spot trait: area (Area), perimeter (Perim), number of spots (Spots), solidity (Solid) and aspect ratio (AR) deviate from normality (Shapiro–Wilk test, $P < 0.05$); angle (Angle), roundness (Round), circularity (Circ) and feret angle (FA) follow a normal distribution (Shapiro–Wilk test, $P > 0.05$).

Figure S3. Screeplot of the principal component analysis (PCA): the graph shows for each dimension the percentage of explained variance; the first three dimensions together explained the 82.5% of the total variance.

Figure S4. Comparison between observed and randomized distribution of the assortativity coefficient (r_c^w) with time restrictions: in each graph the red lines show the observed assortativity coefficient; the second principal component dimension showed a significant positive r_c^w (Prand < 0.05).

Table S1. Correlation matrix for the 10 spot traits from 399 individual adult female giraffes: area, perimeter (Perim) and Feret diameter (FD) were positively correlated; number of spots (Nr) was negatively correlated to area, perimeter and maximum calliper; aspect ratio (AR) and roundness (Round) are also negatively correlated; circularity (Circ), Feret angle (FA), and angle did not show significant correlation with other spot traits.