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Seeing spots: Measuring, quantifying heritability, and assessing fitness consequences of coat pattern traits in a wild population of giraffes (*Giraffa camelopardalis*)

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Abstract

Little is known about the heritability and fitness consequences of polymorphic variation in mammalian coat pattern traits in wild populations. Understanding the evolution of coat patterns requires reliably measuring traits, quantifying heritability of the traits, and identifying the fitness consequences of specific phenotypes. Giraffe coat markings are highly variable and it has been hypothesized that variation in coat patterns most likely affects fitness by camouflaging neonates against predators. We quantified spot pattern traits of wild Masai giraffes using image analysis software, determined whether spot pattern traits were heritable, and assessed whether variation in heritable spot pattern traits was related to fitness as measured by juvenile survival. The methods we described comprise a framework for objective quantification of mammalian coat pattern traits based on photographic coat pattern data, and spot trait measurements from individuals could be used as input to a cluster analysis for taxonomic or other group classifications. We demonstrated that characteristics of giraffe coat spot shape and color are heritable. We did not find evidence for fitness consequences of variation in spot traits on juvenile survival, suggesting that spot traits are currently not under strong directional or stabilizing selection for neonate camouflage in our study population. This may be due to either reduced predation pressure in the study area, or because spot variation may be more relevant to other components of fitness, such as adult survival or fecundity.

Keywords: adaptation, coat pattern, heritability, microevolution, natural selection, phenotypic selection, quantitative genetics

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Introduction

Coats of various colors and patterns are found on many mammal species and these phenotypic traits are hypothesized to play adaptive roles in predator and parasite evasion, thermoregulation, and social communication (Cott 1940; Searle 1968; Waage 1981; Skinner and Smithers 1990; Ortolani and Caro 1996). Pigmentation biology has played a prominent role in the foundation of genetics and evolutionary biology with most work on mammals focused on a few starkly different mouse color morphs (Hoekstra 2006). Studies of the heritability and adaptation of complex animal skin patterns have largely concentrated on fishes with their multiple chromatophores (Kalesh 2004). Researchers have proposed mathematical models for pattern-formation mechanisms in animal markings (e.g. Murray 1981; Maini 1997, 2004; Garvie and Trenchea 2014), and hypothesized the genetic and developmental mechanisms for the markings (Mills and Patterson 2009; Eizirik et al. 2010), and although studies of heritability and adaptation in wild populations are becoming more prevalent (Kruuk et al. 2008), we are aware of none that investigated the heritability and fitness consequences of complex coat patterns in wild mammalian populations.

Understanding the evolution of a trait in a wild population requires measuring individual variation in the trait, quantifying heritability as the proportion of observed phenotypic variation of a trait that is passed from parent to offspring, and assessing the fitness consequences of phenotypic variation (Lande and Arnold 1983; Falconer and Mackay 1996). The fraction of variability in a phenotypic trait that is explained by genetic factors is the broad-sense heritability, which can be estimated as the resemblance of the offspring to its parents (Falconer and Mackay 1996; Roff 1997). Fitness of a trait can be assessed in many ways, but because neonatal mortality is generally higher than any other age class (Lee and

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Strauss 2017), one of the most direct measurements of phenotypic fitness is juvenile survival (Paterson et al. 1998).

Giraffe (*Giraffa camelopardalis*) skin pigmentation is uniformly dark grey (Dimond and Montagna 1976), but the spots that make up their coat markings are highly variable in color, roundness, and perimeter tortuousness, and this variation has been used to classify subspecies (Lydekker 1904), and to reliably identify individuals (Foster 1966; Bolger et al. 2012). The variation in coat patterns and colors is consistent with the idea that these markings are polymorphic, and if heritable they may be adaptive (Lydekker 1904; Mitchell and Skinner 2003). Dagg (1968) first presented evidence from a small zoo population that the shape, number, area, and color of spots in giraffe coat patterns may be heritable, but analysis of spot traits in wild giraffes, and tools for objectively measuring spot characteristics have been lacking. It has been hypothesized that giraffe coat patterns evolved to camouflage neonates whose primary defense against predation is concealment (Langman 1977; Mitchell and Skinner 2003); thus the most likely fitness effects from variation in coat patterns should be variation in juvenile survival. Alternative hypotheses about the adaptive value of giraffe coat markings include thermoregulation (Skinner and Smithers 1990), and facilitation of individual recognition (*sensu* Tibbetts and Dale 2007) and kin recognition (*sensu* Beecher 1982; Tang-Martinez 2001) in this social species with good visual sensory perception (Dagg 2014; VanderWaal et al. 2014).

The spot patterns of Masai giraffes (*G. c. tippelskirchii*) are particularly diverse among giraffe populations, and the patterns of some Masai giraffes bear strong similarities to other giraffe subspecies such as South African (*G. c. giraffa*), Rothschild's (*G. c. rothschildi*), and reticulated (*G. c. reticulata*) giraffes (Dagg 1968, **Fig 1**). Indeed, some Masai giraffes have spots that are almost indistinguishable from those of reticulated giraffes, the most distinctively marked subspecies with spots that are nearly round with very smooth edges (low

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tortuousness). Quantifying heritability and fitness consequences of phenotypic variation in coat pattern traits of giraffes will both inform systematics for this species and contribute to the understanding of the evolution of mammalian coat patterns. Our purpose in this study was to 1) objectively quantify the spot pattern traits of wild Masai giraffes in northern Tanzania using image analysis software, 2) determine whether spot pattern traits were heritable, and 3) determine whether variation in heritable spot pattern traits was related to fitness as measured by juvenile survival.

Fig 1. Representative images of spot patterns of mother-calf pairs. The blue rectangle shows the area analysed using ImageJ to characterize spot pattern traits.

Methods

Field data collection

This study used data from individually identified, wild, free-ranging Masai giraffes in a 1700 km^2 sampled area within a 4400 km^2 region of the Tarangire Ecosystem, northern Tanzania, East Africa. We collected data during systematic road transect sampling for photographic capture-mark-recapture (PCMR). We conducted 26 daytime surveys for giraffe PCMR data between January 2012 and February 2016. We sampled giraffes three times per year around 1 February, 1 June, and 1 October near the end of every precipitation season (short rains, long rains, and dry, respectively) by driving a network of fixed-route transects on single-lane dirt tracks in the study area. We surveyed according to a robust design sampling framework (Pollock 1982; Kendall et al. 1995) with three occasions per year separated by a 4-month interval. Each sampling occasion was composed of two sampling events during

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which we surveyed all road transects in the study area with only a few days interval between events (4.3 years × 3 occasions year^{-1} × 2 events occasion^{-1} = 26 survey events).

During PCMR sampling events, the entire study area was surveyed and a sample of individuals were encountered and either "sighted" or "resighted" by slowly approaching and photographing the animal's right side at a perpendicular angle (Canon 40D and Rebel T2i cameras with Canon Ultrasonic IS 100 – 400 mm lens, Canon U.S.A., Inc., One Canon Park, Melville, New York, 11747, USA). We identified individual giraffes using their unique and unchanging coat patterns (Foster 1966) with the aid of pattern-recognition software Wild-ID (Bolger et al. 2012). We attempted to photograph every giraffe encountered, and recorded sex and age class based on physical characteristics. We categorized giraffes into four age classes: neonate calf (0 – 3 months old), older calf (4 – 11 months old), subadult (1 – 3 years old for females, 1 – 6 years old for males), or adult (> 3 years for females, > 6 years for males) using a suite of physical characteristics (Strauss et al. 2015), and size measured with photogrammetry (Lee et al. 2016a).

Quantification of spot patterns

We analysed spot traits of each animal within the shoulder and rib area by cropping all images to a rectangle that fit horizontally between the anterior edge of the rear leg and the chest, and vertically between the back and where the skin folded beneath the posterior edge of the foreleg (**Fig 1**). We quantified spot characteristics of each animal's pattern using the Color Histogram and Analyze Particles procedures in Program ImageJ (Schneider et al 2012). For color analysis, we used the entire analysis rectangle and full-color photos. For spot measurements we analysed 8-bit greyscale images that we converted to bicolor (black and white) using the Enhance Contrast and Threshold commands. To account for differences in image resolution and animal size, we set the measurement unit of each image equal to the

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number of pixels in the height of the analysis rectangle. Therefore all measurements are in giraffe units (GU), where 1 GU = height of the analysis rectangle (**Fig 1**). We analysed particles (spots) of all sizes, but excluded particles cut off by the edge of the analysis rectangle to avoid the influence of incomplete spots. We also excluded spots whose area was < 0.00001 GU^2 to eliminate the influence of speckles.

We characterized each animal's spot pattern traits within the analysis rectangle using the following twelve metrics: number of spots; mean spot size (area); mean spot perimeter; mean angle between the primary axis of an ellipse fit over the spot and the x-axis of the image; mean circularity ($4\pi \times [Area] / [Perimeter]^2$ with a value of 1.0 indicating a perfect circle and smaller values indicating an increasingly elongated shape); mean maximum caliper (the longest distance between any two points along the spot boundary, also known as Feret diameter); mean Feret angle (the angle [0 to 180 degrees] of the maximum caliper); mean aspect ratio (of the spot's fitted ellipse); mean roundness ($4 \times [Area]\pi \times [Major\ axis]^2$ or the inverse of *aspect ratio*); mean solidity ([*Area*] / [*Convex area*], also called tortuousness); mean shade ([65536 × r] + [256 × g] + [b] using RGB values from color histogram); and mode shade.

Heritability of spot traits

Parent-offspring (PO) regression is one of the traditional quantitative genetics tools used to measure heritability (Falconer and Mackay 1996). PO regression compares phenotypic trait values in parents to those same trait values in their offspring, with the slope of the linear regression line between the mean parent phenotype and the mean offspring phenotype providing an estimate of the heritability of the trait. We assumed phenotypic correlations provided a sufficiently accurate estimate of genetic correlation (Cheverud 1988; Kruuk et al 2008). Advantages and disadvantages of the parent-offspring method to estimate

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heritability compared to other methods such as the half-sibling method or the animal model have been elucidated (Åkesson et al. 2008; de Villemereuil et al. 2017), with the primary advantage when studying wild populations being that PO regression requires less information about family structure and only one offspring per individual (Roff 1997; Lynch & Walsh 1998). While PO regression can have low statistical power when estimating heritability due to environmental effects shared by related individuals (Wilson et al. 2010), this methodology was the most appropriate for our study design because we were unable to identify fathers, and our sample did not include any maternal siblings or half-siblings.

We identified mother-calf pairs by observing extended suckling behavior. Wild female giraffes very rarely suckle a calf that is not their own (Pratt and Anderson 1979). We examined all identification photographs for individuals in known mother-calf pairs, and selected the best-quality photograph for each animal based on focus, clarity, perpendicularity to the camera, and unobstructed view of the torso. For comparing mothers with calves, we selected a photograph of the calf at >9 months of age, because calf fur of younger animals is longer and obscures the spot edges. We found 31 known mother-calf pairs with high-quality photographs of both animals. For comparison of spot characteristics between known mother-calf pairs, we created a set of random cow-calf pairs using the same photographs by assigning a random mother to each calf (without replacement and without pairing a mother with her own calf).

We predicted spot pattern traits of a calf would be correlated with those of its mother but not with a random cow. We tested this prediction for each spot characteristic using simple linear regressions of calf values versus mother values, and calf values versus random cow values. We performed statistical operations using the lm function in R (R Core Development Team 2013).

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Fitness of spot patterns from juvenile survival

We assembled encounter histories for all calves first observed as neonates for survival analysis. For each calf we selected the best-quality youngest age photograph based on focus, clarity, perpendicularity to the camera, and unobstructed view of the torso. For our survival analysis, we used spot traits from the youngest-age photograph available in order to minimize age-related bias in photographs of animals that survived and those that died young.

We analysed survival using two methods: logistic regression and capture-mark-recapture. For the first analysis, we determined whether heritable spot traits were related to first season survival with logistic regression between survivors and those that were never sighted again and presumed to have died, using the glm function in R (R Core Development Team 2013). Based on our results from the PO regression method to quantify the heritability of spot traits, we examined linear and quadratic relationships of circularity and solidity (tortuousness) on juvenile survival to determine whether directional or stabilizing selection was occurring.

For the second survival analysis we estimated neonate survival during their first season of life as a function of individual spot traits using Program MARK to analyse 258 complete capture-mark-recapture encounter histories of giraffes first sighted as neonates (White and Burnham 1999). We analysed our encounter histories using Pollock's Robust Design models to estimate age-specific survival (Pollock 1982; Kendall et al. 1995), with and without spot covariates, and ranked models using AICc following Burnham and Anderson (2002). We determined significance of spot trait covariates if the confidence interval of the beta coefficient did not include zero, and/or if the covariate model was ranked higher than the null model without any covariate.

Results

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We were able to quantify twelve spot traits using ImageJ, and found the traits with greatest individual heterogeneity as measured by the coefficient of variation (CV) were the number and area of spots (negatively correlated traits), and mode shade (**Table 1**). Traits with the least individual variation were solidity and roundness (**Table 1**).

We found no spot pattern traits that had significant PO regression coefficients between calves and random cows, but two characters, circularity and solidity (tortuousness) (**Fig 2**) were significantly correlated between calves and their mothers indicating heritability (**Table 1**). The color characteristic of mode shade was nearly significant in the PO regression (**Table 1**).

Fig 2. Representative spot outlines from Masai giraffes in northern Tanzania and their corresponding circularity and solidity values. Ranges of spot values from 213 calves are given in parentheses.

Our logistic regression survival analysis of spot traits between 161 neonates that survived their first season and 87 that were never resighted after their first season and presumed dead found no significant effects of traits on survival. Our survival analysis of 258 calves first encountered as neonates using Program MARK indicated there was little evidence that individual covariates of spot traits significantly affected survival during the first season of life, but model selection uncertainty was high (**Table 2**). No covariates had significant beta coefficients, and the top-ranked model of survival was the null model with no spot covariates.

Discussion

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Our photographic capture-recapture dataset spanning multiple years enabled us to objectively quantify coat pattern traits of wild giraffes using image analysis software, and demonstrate that giraffe coat pattern traits of spot shape and color are heritable from mother to calf. The methods we described should serve as a framework for objective quantification of mammalian coat pattern traits, and could also be useful for taxonomic classifications based on photographic coat pattern data. We did not find strong evidence for fitness consequences of individual variation in heritable spot traits on juvenile survival suggesting that spot pattern traits are currently not under strong directional or stabilizing selection in our study population.

One possible explanation for the lack of juvenile survival effects from spot variation is the recent reduction in large predator density in our study area (Packer et al. 2011; Bauer et al 2015). If the function of the coat markings is to provide anti-predation camouflage, reduced predator densities due to trophy hunting and pastoralist retaliatory killings of predators may have alleviated predation pressure on giraffe calves sufficiently to remove the selection pressure for certain spot traits (Lichtenfeld 2005; Lee et al. 2016b). Alternatively, the possibility remains that spot traits may serve adaptive functions such as thermoregulation and/or social communication (Skinner and Smithers 1990; VanderWaal et al. 2014), and thus may demonstrate associations with other fitness traits, such as survivorship in older age classes or fecundity. Individual recognition, kin recognition, and inbreeding avoidance could also play a role in the adaptation of spot patterns for individual recognition in giraffes (Sherman et al. 1997; Tang-Martinez 2001; Tibbetts and Dale 2007).

Masai giraffe spot patterns are particularly diverse among giraffe populations, and there are spot patterns in northern Tanzania that bear strong similarities to other giraffe subspecies or species elsewhere in Africa (Dagg 1968, **Fig 1**). Two recent genetic analyses of giraffe taxonomy both placed Masai giraffes as their own species (Brown et al. 2007;

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Fennessy et al. 2016), but the lack of quantitative tools to objectively analyze coat patterns for taxonomic classification may underlie some of the confusion that currently exists in giraffe systematics (Bercovitch et al. 2017). We expect the application of image analysis to giraffe coat patterns will provide a new, robust dataset to address taxonomic hypotheses. We hope the framework we have described using imageJ to quantify spot characteristics will prove useful to future efforts at quantifying animal markings, and suggest the resultant trait measurements could be useful in a formal cluster analysis to classify subspecies or other groups based on variation in markings (Kaufman and Rousseeuw 2009).

Our analyses highlighted two aspects of giraffe spots that were most heritable and which may have adaptive significance. Circularity describes how close the spot is to a perfect circle, and solidity describes how smooth and entire versus tortuous, ruffled, lobed, or incised the edges are. These two characteristics could form the basis for quantifying spot patterns of giraffes across Africa, and gives field workers a new quantitative lexicon for describing spots (**Fig 2**). It is interesting to consider that the roundness and smooth versus rough edge traits we found heritable in giraffe spots brings to mind the smooth and wrinkled peas of Mendel (Bateson and Mendel 1913) and the rough/smooth bacterial colonies in Griffith's (1928) discovery of the "transforming principle" which led to the discovery that DNA was the heritable material (Avery et al. 1944). Our mode shade measurement was a crude metric, but even this rough approximation of spot color showed evidence of heritability. Color is greatly affected by lighting conditions, and we suggest standardization of photographic methods to control for lighting if color is to be analyzed in future studies.

Mammalian patterned coats are hypothesized to be formed by two distinct processes: a spatially oriented developmental mechanism that creates a species-specific pattern of skin cell differentiation and a pigmentation-oriented mechanism that uses information from the pre-established spatial pattern to regulate the synthesis of melanin (Eizirik et al. 2010). The

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giraffe skin has more extensive pigmentation and wider distribution of melanocytes than most other animals (Dimond and Montagna 1976). Future work on the genetics of coat patterns will hopefully shed light upon the mechanisms of coat pattern variation.

Our study revealed that several spot traits are heritable and, hence, able to respond to selection in giraffe populations. However, given that these spot characteristic traits did not significantly affect neonatal survival, the main theory for the adaptive nature of giraffe spots (Langman 1977; Mitchell and Skinner 2003), we conclude that spot patterns are currently of minor importance for the adaptation of our study population, although this may be a recent consequence of fewer predators. Other aspects of spot variation may prove to be more relevant to fitness, such as social effects of individual recognition or kin recognition, or thermoregulation, and deserve further investigation.

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Table 1. Summary statistics for parent-offspring regressions of spot traits of Masai giraffes in northern Tanzania. Mean trait values, SD (standard deviation), and CV (coefficient of variation), PO slope coefficients (heritability), F-statistics, P values, and r-squared values are provided. Significantly heritable traits are in bold.

	Number	Area	Perimeter	Angle	**Circularity**	Maximum Caliper	Feret Angle	Aspect Ratio	Roundness	**Solidity**	Mean Shade	Mode Shade
mean	18.9	0.04	0.99	87.96	0.51	0.29	88.2	1.69	0.63	0.84	7799280	6924050
SD	7.5	0.01	0.25	15.39	0.08	0.06	14.5	0.15	0.04	0.04	1985064	3930565
CV	0.40	0.39	0.25	0.17	0.15	0.19	0.16	0.09	0.06	0.05	0.25	0.57
PO Slope coefficient	0.20	0.20	0.27	0.04	**0.52**	0.21	-0.15	0.19	0.08	**0.53**	0.16	0.44
$F_{1,29}$	0.76	0.87	2.27	0.04	**9.97**	1.01	0.91	1.11	0.19	**9.73**	0.55	4.16
P value	0.39	0.36	0.14	0.84	**0.004**	0.32	0.35	0.30	0.66	**0.004**	0.47	0.051
r squared	0.03	0.03	0.07	0	**0.26**	0.03	0.03	0.04	0.010	**0.25**	0.02	0.13

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Table 2. Model selection results for giraffe calf survival as a function of spot trait covariates. No covariate model had a significant beta coefficient, and the top-ranked model was the null model of no covariate effects. Full model in all cases was {S(A + …) g”(.) g’(.) p(t) c(t)} with covariate structure in survival.

Covariate Model	ΔAICc	W	k
Null	0	0.20	38
Number of spots	0.61	0.15	39
Aspect Ratio	1.22	0.11	39
Roundness	1.53	0.09	39
Solidity	1.73	0.08	39
Max Caliper	1.87	0.08	39
Circularity	2.03	0.07	39
Area	2.18	0.07	39
Feret Angle	2.29	0.06	39
Perimeter	2.96	0.05	39

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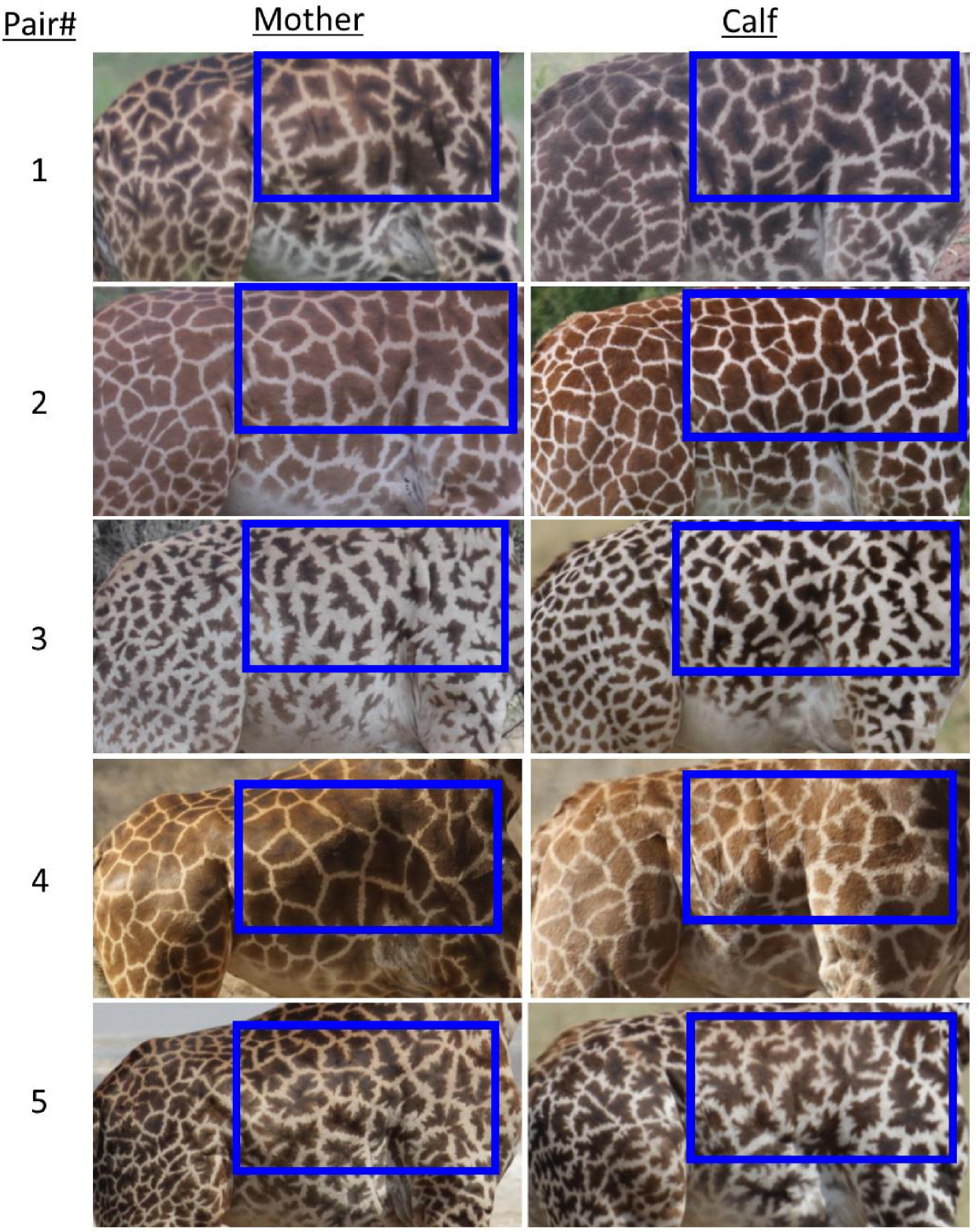
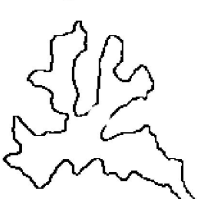
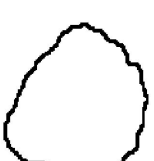

Pair#
Mother
Calf
1
2
3
4
5

Shape	Circularity (0.10—0.94)	Solidity (0.52—0.96)
	0.15	0.66
	0.13	0.60
	0.88	0.94
	0.37	0.76
	0.40	0.83
	0.57	0.82
	0.44	0.76
	0.12	0.53
	0.83	0.96