Sexual selection and endocrine profiles in wild South African giraffe (Giraffa camelopardalis giraffa)

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1 INTRODUCTION

Sexual selection involves mate choice and intrasexual competition (Darwin, 1871), an evolutionary mechanism that regulates reproductive success. Male-male competition in size dimorphic ungulates has been extensively studied (Andersson, 1994; Hirotani, 1989; Lent, 1965). Larger animals or individuals with larger weapons are more likely to win (Parker, 1974), hence have a greater chance to mate (Andersson, 1994).

Among many mammals, male reproductive tactics involve tracking female endocrine state, with escalated aggression and mating occurring when females are most likely to conceive (Andersson, 1994; Bercovitch, 1988; Clutton-Brock, 2016; Hirotani, 1989; Lent, 1965). Giraffe (*Giraffa camelopardalis*) bulls only mate guard, and copulate, when a female is in her fertile window and are therefore seldom observed copulating (Bercovitch et al., 2006). Various ungulates, including giraffe (Kondoh et al., 2017), use their vomeronasal organ to detect chemical cues indicating the reproductive status of the female (Halpern, 1987) . Male giraffes stimulate females to urinate by nuzzling their rumps, catch the urine in their mouths and transfer the chemicals to their vomeronasal organ by raising their head and upper lip (Dagg, 2014), also known as the flehmen response.

Giraffes live in a fission-fusion society (Bercovitch & Berry, 2012; Carter et al., 2013), with adult males often solitary, moving among female herds as a roaming reproductive strategy to assess female reproductive status (Bercovitch et al., 2006; Dagg, 2014). Giraffes do not display any visible signs of ovulation, but broadcast their reproductive status using chemical cues that males detect with flehmen (Kondoh et al., 2017). They have multiple ovulatory cycles prior to conception (Bercovitch et al., 2006), and they can conceive while lactating (Deacon et al., 2017).

Male sex steroid (Seeber et al., 2013; Wolf, Schaebs, et al., 2018) and glucocorticoid concentrations (Wolf, Bennett, et al., 2018) fluctuate as a function of the type of herd that they are in (e.g. presence of oestrous female and/or other males), their social activity within the herd, and their age. Adult giraffe bulls rarely fight, but fights have been observed near females that were likely in oestrus; however, the endocrine state of females was not determined, and no mating was observed afterwards (Brand, 2007). Our combination of behavioural and endocrinological data in this single-event case study demonstrates for the first time that sexual selection in giraffes, as in other size dimorphic mammals, is mediated by male aggressive reproductive tactics responding to female reproductive hormone levels.

2 METHODS

The study was conducted at Rooipoort Nature Reserve (28°36′59″S, 24°15′28″E), South Africa with giraffes, other ungulates and one large predator, the brown hyaena (*Hyaena brunnea*), freely roaming in 34,500 ha. The climate consists of cold dry winters and hot wet summers (Bezuidenhout, 2009). Six adult males and twenty-six adult females were present at the reserve. In this report, we include four adult males, categorised as 'older' (M5) or 'younger' (M1, M4, M7) using previously published criteria (Wolf, Bennett, et al., 2018; Wolf, Schaebs, et al., 2018), and two adult females: F19 (the inter-male aggression was directed at access to her) and F7 for establishing the reproductive status of F19.

Sexual activity and aggressive behaviour were observed on foot from dawn to dusk between 21 February (day 1) and 4 March 2018 (day 12). Continuous focal sampling was conducted on target subjects. In addition, we scanned the entire herd every 15 min and recorded ad libitum behaviours. Descriptions of activities rather than rates of behaviour were collected, because of the infrequency of both aggressive and sexual activity among giraffes.

Faecal samples were collected within 30 min of defecation following standardised procedures (Wolf, Bennett, et al., 2018; Wolf, Schaebs, et al., 2018) and were immediately stored on ice and frozen within 6 hr. Samples remained frozen until further processing at the Endocrine Research Laboratory, University of Pretoria, South Africa. Faecal steroids were extracted following established protocols (Möstl et al., 2002; Seeber et al., 2013; Wolf, Bennett, et al., 2018; Wolf, Schaebs, et al., 2018) and analysed for androgen (fAM) and glucocorticoid metabolite (fGCM) concentrations (males) and progesterone metabolite (fPM) concentrations (females). Immunoreactive fAM and fGCM concentrations were determined using enzyme-immunoassays (EIAs) previously established for giraffe (Bashaw et al., 2016; Wolf, Bennett, et al., 2018; Wolf, Schaebs, et al., 2018), utilising antibodies against 5α-androstane-3α-ol-17-one-HS (Palme & Möstl, 1993) and 5β-androstane-3α-ol-11-one-17-CMO-BSA (Möstl et al., 2002), respectively. Sensitivity at 90% binding was 24 ng/g dry faecal weight (DW) for the fAM and 1.2 ng/g DW for the fGCM EIA, respectively. Intra- and Inter-assay coefficients of variation (CV), determined by repeated measurements of low- and high-quality controls, were 4.49% and 5.47% as well as 4.24% and 5.31% (Intra-assay CV) and 6.02% and 7.30% as well as 5.50% and 9.33% (Inter-assay CV) for fAM and fGCM measurements, respectively. Immunoreactive fPM concentrations were determined using a progesterone EIA (Schwarzenberger et al., 1993). To biologically validate the EIA, we compared median postpartum fPM concentrations of female F7 (n = 3, median = 13.8, range 7.1–22.7) with fPM concentrations determined during late pregnancy (n = 5, median = 99.1, range 87.9–119.8), resulting in an overall 16.9 fold difference (Figure 1). The chosen EIA is therefore able to discriminate between fPM concentrations of different reproductive stages of female giraffe. Sensitivity of the assay at 90% binding was 19.2 ng/g DW. Intra-assay CV of low-

and high-quality controls was 5.69% and 6.53%, respectively, and inter-assay CV was 10.17% and 12.11%, respectively.

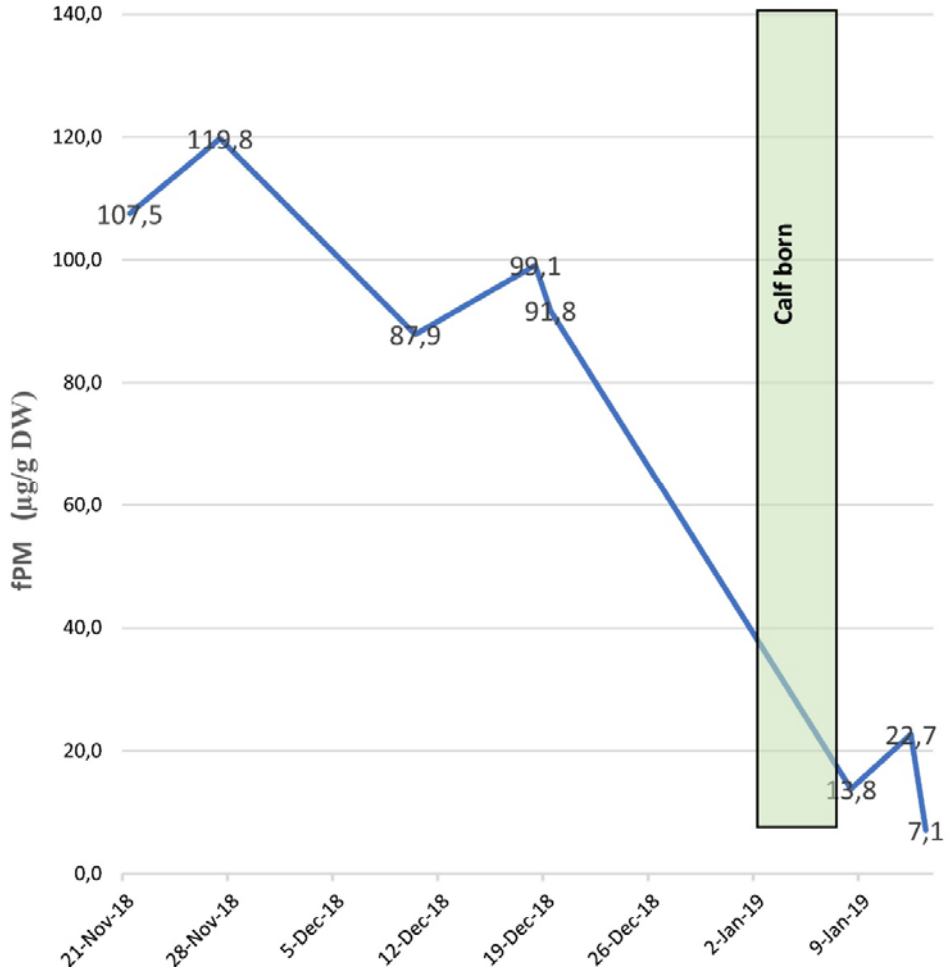

Figure 1. Hormonal responses of pregnancy of F7, showing pre- and postcalving concentrations determined using a faecal progestogen metabolite (fPM) EIA for biological validation of the assay. The calf was born in the first week of January, as marked in the graph

The gestation period of giraffes is approximately 15 months, with an interbirth interval close to 2 years (Bercovitch & Berry, 2009). F19 had a calf estimated to be 6 months old suggesting she should have 'normal' cycles again. There is a temporary increase in fPM concentrations just prior to ovulation in female giraffes (del Castillo et al., 2005). Although fPM concentrations during early pregnancy might overlap with highest fPM levels during the 'fertile window', the stages can be distinguished as males do not display sexual activity towards pregnant females (Bercovitch et al., 2006). Hence, we established the reproductive status of F19 as 'oestrus', given her fPM concentrations (see Results). The lag time between circulating and faecal endocrine concentrations is 1–2 days in giraffes (Bashaw et al., 2016), so our timeline reflects this offset of faecal hormone level and behaviour.

3 RESULTS AND DISCUSSION

On 21 February (day 1), we first observed M4 mate guarding and urine testing F19, which was followed by a flehmen response. We also observed F19 neck rubbing against M4's

hindquarters, an affiliative giraffe behaviour (see Table 1 for daily descriptions of behaviours). Progesterone levels of pregnant females are about 2 to 3 times higher than when they are cycling (del Castillo et al., 2005; Lueders et al., 2009). F7 had fPM concentrations of nearly 120 µg/g DW at the end of her pregnancy, while F19 had peak concentrations of slightly over 40 µg/g DW (Figure 2) between day 2 and either day 4 or 5 (an additional sample could not be collected on day 5). Given that giraffes have a fertile window of about 4 days (Bercovitch et al., 2006) with ovulation occurring approximately 2 days after progesterone levels peak (Bercovitch et al., 2006; Lueders et al., 2009), and that, in giraffes, a 1 to 2-day lag time exists between circulating hormone and faecal metabolite concentrations (Bashaw et al., 2016), we conclude that F19 most likely ovulated on day 5 or 6.

Table 1. Overview of behavioural observations of main subjects (F19, M4 and M5) and their faecal hormone metabolite concentrations

Day	ID	Hormone (µg/g DW)			Herd size	Behaviour	
		fPM	fAM	fGCM			
1	M4		11.92	1.592	7	Cofeeding, mate guarding and investigating (urine testing and flehmen) F19	
	M5					Not in herd	
	F19	19.60				Neck rubbing against M4s hindquarters multiple times throughout the day Left her calf with another adult female in the herd	
2	M4		12.40	1.592	7	Cofeeding, mate guarding and investigating F19	
	M5					Not in herd	
	F19	44.60				Neck rubbing against M4s hindquarters	
3	M4				5	Mate guarding and cofeeding F19	
	M5					Not in herd	
	F19					Cofeeding with M4	
4	M4		6.12	1.288	12	Not with F19in the morning, but cofeeding and mate guarding F19 in the afternoon (from 12:30 hr)	
	M5					Not in the herd	
	F19	41.33				Watching calves in the morning, joined adults in afternoon	
5	M4				12	Mate guarding, approaching, cofeeding and investigating F19	
	M5					Not in the herd	
	F19					Frequently neck rubbing against M4's hindquarters Cofeeding with M4	
6	M4		12.07	0.813	15	Morning: Mate guarding and cofeeding F19 Dominant gesture towards other males in the herd (M1 and M7) repeatedly chasing M1 and M7 away from F19, until they did not approach F19 anymore from 11:00 hr on Restricting F19 from approaching M1 and M7	Afternoon: Started and lost fight with M5 Sparring with M1 and M7 Got chased away from F19 by M5 Last approach to F19 at 13:23 hr Vigilant towards M5 and F19, stayed < 100 m away from F19 and M5 until 17:30 hr

Day	ID	Hormone (µg/g DW)			Herd size	Behaviour	
		fPM	fAM	fGCM			
							Bumping into and mounting M1 with an erect penis
	M5		5.06	1.490		Afternoon: Won fight with M4 Dominant gesture towards M4, chasing him Mate guarding F19 Mounting F19 frequently	
	F19	17.96				Morning: Majority of the time feeding (and cofeeding with M4) Escaping from M4	Afternoon: Cofeeding with M5 Tolerating affiliation from M5 Tolerating mating attempts and courtship M5
7	M4		21.23	1.123	16	Mate guarding F19 Keeping up with F19 who was cantering away from him	
	M5					In the herd based on GPS unit, but not seen	
	F19	4.50				Cantering away from males in the herd (M4 and M7)	
8					12	M4, M5 and F19 in the same herd, but not near each other Both males investigated other females	
9	M4				12	Investigating (urine testing and flehmen) females in the herd other than F19	
	M5		34.05	2.060		Investigating (urine testing and flehmen) females in the herd other than F19	
	F19					Predominantly feeding, no interaction with any males	
10	M4				Herd split up	Investigating (urine testing and flehmen) females in the herd other than F19	
	M5					Left to another herd	
	F19					Predominantly feeding, no interaction with any males	
11	M4					In herd with F19 but mate guarding and investigating other females	
	M5		38.26	1.933		Not in herd with F19 and mate guarding and investigating other females	
	F19					No interaction with any males	
12	M4					In herd with F19 but mate guarding and investigating other females	
	M5		10.27	1.601		Not in herd with F19 and mate guarding and investigating other females	
	F19					Predominantly feeding, no interaction with any males	

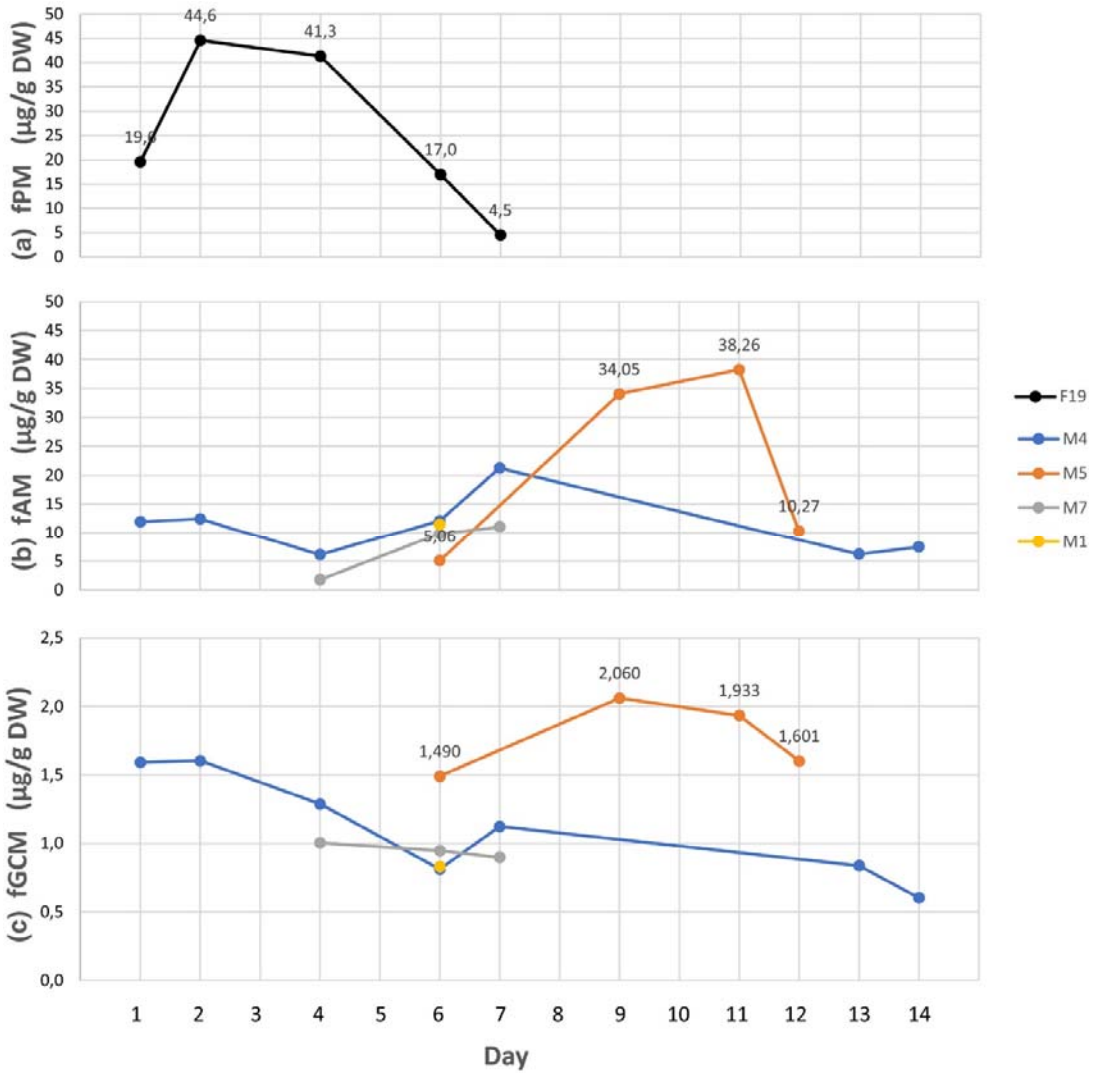

Figure 2. Hormonal responses of five giraffes to behavioural and social changes, as measured by (a) a faecal androgen metabolite (fAM), (b) faecal progestogen metabolite (fPM), and (c) faecal glucocorticoid metabolite (fGCM) EIA over a two-week period. With pre- and postsamples of the fight and mating event on day 6. Actual values of hormone metabolite concentrations are only added for the female and the 'winning' bull (M5)

On day 6 M5 joined the herd and approached F19. M4 began following M5 and started a fight, which lasted only 48 s. The physical confrontation involved both males hitting their heads against their opponent's torso until M4 left. Afterwards, M5 began mate guarding and cofeeding with F19, and several hours after the fight M5 started bumping into F19 and tapping against her hind legs using his front leg, a bull giraffe courtship activity. At 15:35, nearly 4 hr after the fight, M5 (which is the oldest bull at the reserve) mounted F19 with an erect penis and continued to mount her until observations were terminated at sunset (18.57 hr). Multiple mounting of females in their fertile window is common in giraffes (Bercovitch et al., 2006).

Our data from the wild conform with that obtained in captivity documenting that sexual activity occurs about 2 days after female progesterone levels peak (Bercovitch et al., 2006; Lueders et al., 2009). Males urine test both noncycling and cycling females but concentrate 81% of their sexual activity to females who are in their fertile window (Bercovitch

et al., 2006). We conclude that male fighting and sexual activity observed coincided with female ovulation.

Furthermore, our male endocrine data reveal an increase in both fAMs and fGCMs along with male sexual and aggressive behaviour. Sexually active bulls have fAM levels approximately four times higher than sexually inactive bulls, reaching about 30 µg/g DW (Seeber et al., 2013; Wolf, Schaebs, et al., 2018), as well as increased fGCM concentrations (Wolf, Bennett, et al., 2018). In our study, M4 attained a zenith in fAM concentrations on the day of actively chasing away other males and seriously fighting with M5, while the fAM levels of M5 were nearly seven times higher 3 days after fighting and mating. Of the 37 fGCM samples analysed only two samples were higher than 2 µg/g DW (mean = 1.14, *SD* = 0.45), with one of those M5's on the day after sexual activity.

In summary, male mate guarding and mating only occurred during the fertile window, while male genital inspection occurred with multiple females, including those not cycling. Both androgen and glucocorticoid concentrations increased in males following fighting and sexual activity.

Our study is the first to document, using the endocrine profile of females, that when adult bull giraffe fight, the object can be a female in oestrous. Such findings are expected among size dimorphic mammals, but no other study had observed giraffe bulls physically contesting access to a female whose endocrine profile indicates that she was within her fertile window. Our case report reveals that wild giraffe bulls are detecting the fertile window of cows and adjusting their behaviour in line with the probability of female ovulation. We do not know the precise nature of the chemical cues emitted in female urine that signal males that ovulation is likely. However, our study confirms that male giraffes detect signs of impending ovulation when they urine test females. Our pioneering research provides the first solid evidence that sexual selection has had an impact on male giraffe reproduction.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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