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Horns honestly advertise parasite infection in male and female African buffalo

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The evolution and maintenance of elaborate secondary sexual characters in males have been the subject of intense interest since Darwin's time. Parasite-mediated sexual selection (PMSS) suggests that elaborate ornaments serve as honest indicators of male health and parasite resistance. Studies testing this key prediction of PMSS have largely focused on ornaments, with the role parasites might play in the maintenance of elaborate weapons being relatively understudied. Here, we tested whether weapon (horn) size was an indicator of health status in male and female African buffalo, *Syncerus caffer.* We examined whether individuals with larger horns were less likely to be infected with parasites, and had lower parasite loads and stronger immune systems. In males, horn size was significantly negatively correlated with the number of different parasites infecting an individual (parasite richness), the likelihood of infection with strongyle nematodes and coccidia, and strongyle intensity. In females, horn size was significantly negatively correlated with parasite richness, occurrence of coccidia infection, coccidia intensity and white blood cell count. These findings were robust when the effects of body condition, age and season were controlled for, consistent with the idea that horns function as honest indicators of health in both sexes. Our study provides new insight into the evolution and maintenance of elaborate weapons in mammals, suggesting a role for PMSS in both males and females.

Keywords: African buffalo; Hamilton–Zuk hypothesis; handicap hypothesis; parasite-mediated sexual selection; *Syncerus caffer*; weapon

Over the past few decades, a considerable body of theoretical and empirical work has been aimed at understanding the evolution and maintenance of elaborate secondary sexual traits in males (Andersson 1994). Far less attention has focused on secondary sexual characters in females, despite the fairly widespread occurrence of these traits across multiple taxonomic groups (Amundsen 2000). Handicap and reliable signalling theories propose that elaborate sexual traits honestly advertise individual phenotypic and/or genotypic quality (Zahavi 1975; Zahavi & Zahavi 1997; Grafen 1990). In particular, parasite-mediated sexual selection (PMSS) suggests that secondary sexual traits function as indicators of health and resistance to parasites. Only males in good health are able to produce the most elaborate sexual ornaments, and females who prefer showy males benefit by passing down heritable disease resistance genes to their offspring and/or by mating with parasite-free males and avoiding sexually transmitted or contact-transmitted diseases (Hamilton & Zuk 1982; Borgia & Collis 1989).

Most studies testing predictions of PMSS have focused on female choice for exaggerated male ornaments (Møller et al. 1999). Although less well studied, the role of PMSS in intrasexual selection (i.e. male–male competition) is equally compelling, since the development of weapons (e.g. horns, antlers) may be constrained by parasites, and parasites can in turn influence male competitive ability (Howard & Minchella 1990; Mulvey & Aho 1993). Whether female weapons might also function as indicators of individual health and parasite resistance status has yet to be considered. However, given evidence that parasites can affect weapon development in females of

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some species (e.g. reindeer; Folstad et al. 1996), similar mechanisms for indicating individual health might also operate in females, with weapons potentially advertising female quality to males or competitive ability to other females.

In polygynous ungulates, the use of horns and antlers in male contests is well established (Clutton-Brock 1982). Because fights can be costly, direct contests normally occur only between well-matched individuals, and mechanisms for assessing potential opponent's fighting ability have evolved in many species (Clutton-Brock & Albon 1979; Barrette & Vandal 1990). Horns and antlers often feature prominently in assessment rituals (Geist 1971), and therefore could serve as honest signals of male quality and competitive ability. Indeed, recent studies of weapons in deer suggest that antler size in males may be a reliable signal of traits, ranging from parasite load and genetic variability to sperm production and body mass (Ditchkoff et al. 2001; Malo et al. 2005; Vanpé et al. 2007). Although evidence is accruing that weapons serve as honest signals of quality in male ungulates, their contribution as honest signals of quality in females remains largely unknown despite the fairly widespread occurrence of female hornedness. Key hypotheses on the function of weapons in female ungulates include predator defence, male mimicry and resource competition (reviewed in Roberts 1996). However, if weapons are associated with individual quality and reproductive success in females, these structures may also fulfil a signalling function in females.

We investigated sex-specific patterns of association between weapon size and health status in African buffalo, *Syncerus caffer*, a species in which both sexes have horns. Male horns are larger and thicker than female horns. In males, horns are important in intrasexual conflict, featuring prominently in agonistic encounters and displays that determine dominance and access to oestrous females (Sinclair 1977; Mloszewski 1983; Prins 1996). In females, the function of horns remains unclear, although threat displays and agonistic interactions involving horns have been documented in this sex (Sinclair 1974). To examine the potential role of weapons as honest signals of health in male and female buffalo, we tested the following specific predictions: (1) males with larger horns are less likely to be infected with parasites, and when infected, will have fewer parasites; (2) males with larger horns have stronger immune systems, reflecting their higher overall ability to fight parasites; and (3) horns also function as signals in females, leading to similar associations between horn size, parasites and immunity as predicted for males.

METHODS

Study System

We collected data on African buffalo captured as part of the Bovine Tuberculosis Control Program at Hluhluwe-iMfolozi Park (HIP), South Africa (28°10′–28°14′S; 31°54′–32°03′N). HIP comprises almost 900 km^2, with a buffalo population of approximately 3000 individuals. Animals were captured in the Masinda section of the park over a 2-week period in both October 2005 and May 2006. Captures were carried out by KwaZulu-Natal Wildlife, the park management organization, using a helicopter and funnel system to drive buffalo herds into a capture corral. Once corralled, buffalo were anaesthetized for bovine tuberculosis (TB) testing, during which we collected data on animal age, horn size, condition and reproductive status, as well as faecal and blood samples for parasite and immunological analysis. All captured animals were marked with brands to allow for identification of recaptured animals during subsequent capture periods. To avoid pseudoreplication, we eliminated all recaptured individuals from the May 2006 data set. The final data set of 393 TB-negative individuals included 226 females and 167 males.

Age, Horn Size, Condition and Reproductive Status

Buffalo under the age of 2.5 years were aged using body size and degree of horn development (Sinclair 1977). Animals between 2.5 and 5.5 years were aged by tooth eruption patterns (Sinclair 1977; Jolles et al. 2005). For adults (>5.5 years), tooth wear of the first incisor was used as an indicator of age (Jolles et al. 2005). We estimated horn size for individuals 1 year old and older by measuring the width (in centimetres) of the horns at the point of maximum curvature (Fig. 1, inset). Since horn width increased with age in both males and females (horn width versus age: male, $F_{1,166} = 334$, $r = 0.82$, $P < 0.0001$; female, $F_{1,225} = 346$, $r = 0.78$, $P < 0.0001$; Fig. 1), we corrected for age effects by using the residuals from the regression of horn width versus age as a measure of relative horn size. For both sexes this procedure effectively removed the correlation between age and horn size (residual horn width versus age: male, $F_{1,166} = 0.004$, $r = 0.005$, $P = 0.9477$; female, $F_{1,225} = 1.114$, $r = 0.005$, $P = 0.2923$).

Body condition for all study animals was assessed using a manual index after Prins (1996), which included visual inspection and manual palpation of four areas of the

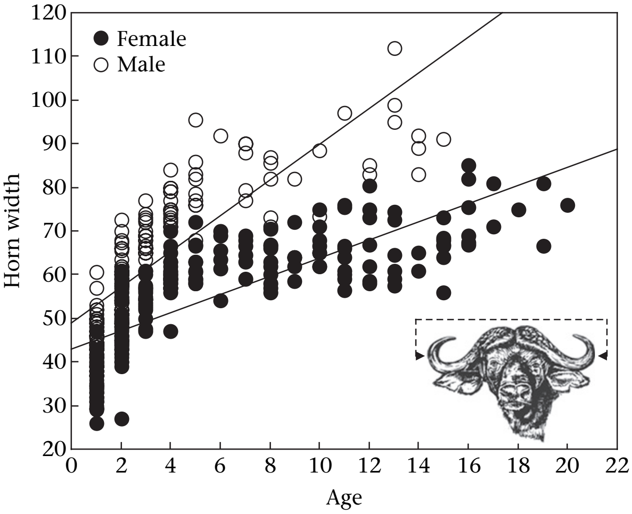

Figure 1. Associations between age and horn width in male and female African buffalo.

body where buffalo store fat: ribs, spine, hips and base of the tail. Each body part was given a score between 1 and 5, with a higher score reflecting greater fatness. The sum of all four scores (maximum = 20) was used as a composite measure of overall body condition, and this measure of condition is significantly correlated with the kidney fat index, a widely used estimate of body condition in ungulates (Ezenwa et al., in press). We also used haematocrit (HCT) as a second measure of buffalo condition. HCT provides information on nutritional condition and reflects nutritional deprivation in both domestic and free-ranging ungulates (DelGiudice et al. 1992). To determine HCT, blood samples were collected from the jugular vein into 10 ml EDTA vacutainer tubes. Samples were immediately placed on ice and shipped to the laboratory of Dr Bouwer & Partners Inc. (Durban, South Africa) for complete blood cell (CBC) counts and haematological estimates. CBC and haematological values were determined using an ADVIA 120 automated analyser (Bayer Diagnostics, Tarrytown, NY, U.S.A.). Reproductive status was assessed for all females 3 years old and older. Pregnancy status was determined using rectal palpation, and lactation was evaluated by manual milking.

Parasites and Immune Status

We assessed gastrointestinal (GI) parasite loads from faecal samples collected during capture. Samples were collected directly from the rectum of anaesthetized animals and stored at approximately 4°C until processing. Parasite output in host faeces was quantified using a modification of the McMaster faecal egg counting technique (Ezenwa 2003). Five distinct GI parasite types were distinguished, including three nematodes (strongyles (Nematoda: Strongylida), *Trichuris* spp., *Strongyloides* spp.), a cestode (*Moniezia* spp.), and a protozoan (coccidia (Apicomplexa: Eimeriidae)). We used the number of different parasite types infecting each host as a measure of parasite richness. For the two most prevalent parasites, strongyle nematodes and coccidia, we used the number of eggs and oocysts per gram of faeces as estimates of parasite intensity, with intensity defined as the number of parasites per infected host. Although the exact relationship between faecal egg counts and the number of adult parasites in the gut can vary, egg counts provide a valuable noninvasive means of assessing relative infection levels across hosts (Bryan & Kerr 1989; Stear et al. 1995). Immune status was measured as the total white blood cell (WBC) count. White blood cells are the first line of defence against parasite attack, playing a prominent role in recognizing antigens, killing parasites and pathogens, and destroying infected host cells. To measure WBCs, blood samples were collected and processed as described above for HCT.

Statistical Analyses

To ascertain whether our measure of horn size accurately reflected the sexual dimorphism in horn morphology between male and female buffalo, we tested for differences in mean uncorrected horn width using an analysis of variance (ANOVA) with age, sex and the interaction between age and sex as factors. We also tested for differences in the degree of variation in horn width among males and females by performing an equality of variances *F* test on log-transformed horn width data (Sokal & Rohlf 1995).

Because we expected condition to be correlated with horn size, we tested for associations between horn size and both measures of buffalo condition, body condition and HCT (nutritional condition), using general linear models (GLM). In these models, condition was used as the dependent variable, and age-corrected horn size, age and season (May versus October) were included as predictor variables. The model for HCT also included body condition as an additional predictor. To test associations between horn size, parasite load and immune status, we used the following analyses. First, we tested whether horn size was correlated with the number of different parasite types infecting an individual (parasite richness) using GLM. The number of parasite types was included as the dependent variable with age-corrected horn size as the main predictor variable. Body condition, age and season were included as covariates in the model to control for effects of these factors on parasite infection, and account for associations between body condition and horn size. Second, we examined whether horn size was associated with infection status for the two most prevalent parasites (strongyle nematodes and coccidia) using logistic regression. Infection status was the dependent variable in all regression models and age-corrected horn size, body condition, age and season were predictor variables. Last, we tested whether horn size was a significant predictor of parasite intensity and WBC count using GLM similar to those described for parasite richness. For these analyses, parasite intensity data were log-transformed to normalize data distributions.

In addition to the condition, parasite and immune function analyses, we also examined the links between horn size and female quality by testing for associations between horn size and female reproductive status. To do this, we used logistic regression models that tested for effects of horn size, body condition, age and season on female pregnancy and lactation status. For all analyses, statistical tests were two tailed and the significance level (α) was set at $P \leq 0.05$.

RESULTS

Horn Size: Males versus Females

Mean (±SD) uncorrected horn width was 62.6 ± 15.8 cm ($N = 167$) for males and 54.3 ± 13.1 cm for females ($N = 226$). Sex, age and the interaction between sex and age were all significant predictors of horn size (ANOVA: sex, $F_{1,389} = 22.6$, $P < 0.0001$; age, $F_{1,389} = 653$, $P < 0.0001$: sex*age, $F_{1,389} = 68.0$, $P < 0.0001$). Males had significantly larger horns than females, suggesting that our index of horn size captured the sexual dimorphism in horn morphology present in African buffalo. However, despite obvious dimorphism in horn size between the sexes, there was no significant difference in levels of within-sex variation ($F_{225,166} = 1.076$, $P = 0.6138$).

Horn Size and Condition

In males, age-corrected horn size was a significant and positive predictor of body condition, and this relationship persisted even after controlling for the effects of season and age (GLM, horn size: $F_{1,163} = 7.452$, $P < 0.007$; season: $F_{1,163} = 137.645$, $P < 0.0001$; age: $F_{1,163} = 0.653$, $P = 0.4203$). Unlike with body condition, horn size did not predict nutritional condition (HCT) in males (horn size: $F_{1,160} = 1.169$, $P < 0.2812$; season: $F_{1,160} = 13.831$, $P < 0.0003$; age: $F_{1,160} = 20.768$, $P < 0.0001$; body condition: $F_{1,160} = 0.856$, $P < 0.3563$). In females, age-corrected horn size was significantly and positively correlated with body condition, and once again this relationship was robust after controlling for the effects of age and season (horn size: $F_{1,222} = 4.581$, $P = 0.0334$; season: $F_{1,222} = 188.279$, $P < 0.0001$; age: $F_{1,222} = 23.786$, $P < 0.0001$). Horn size was also a significant positive predictor of nutritional condition in this sex (horn size: $F_{1,220} = 5.494$, $P = 0.02$; season: $F_{1,220} = 6.301$, $P = 0.0128$; age: $F_{1,220} = 0.395$, $P = 0.5305$; body condition: $F_{1,220} = 11.232$, $P < 0.0009$).

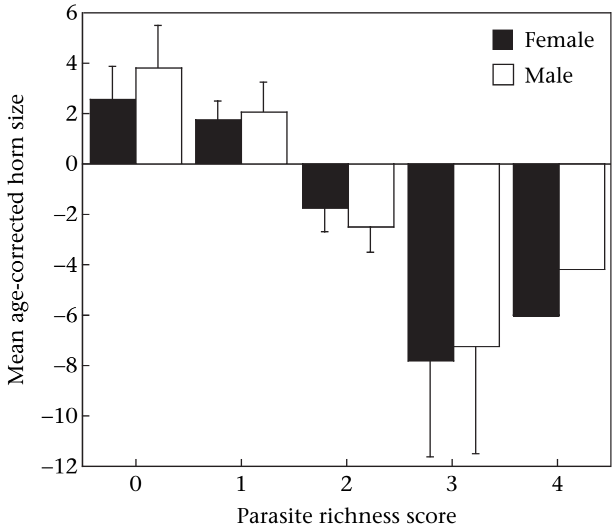

Figure 2. Mean age-corrected horn size as a function of parasite richness in male and female African buffalo.

Horn Size and Parasite Infection Status

Eighty-nine per cent (149 of 167) of males and 85% (194 of 226) of females were infected with at least one parasite. Out of the five parasite types identified, strongyle nematodes were the most prevalent, infecting 68% (114 of 167) of males and 72% (164 of 226) of females, followed by coccidia with 68% (115 of 167) of males infected and 54% (123 of 226) females infected. Infection prevalence for the other three parasites ranged between 4% and less than 1% in males, and between 3% and less than 1% in females.

There was a significant association between age-corrected horn size and parasite richness for both males and females. In both sexes, individuals with smaller horns had higher parasite richness scores (Fig. 2), and these results were robust after controlling for body condition, age and season (male, horn size: $F_{1,162} = 18.823$, $P < 0.0001$; condition: $F_{1,162} = 1.929$, $P = 0.1668$; age: $F_{1,162} = 10.507$, $P = 0.0014$; season: $F_{1,162} = 0.131$, $P = 0.7174$; female, horn size: $F_{1,221} = 12.951$, $P = 0.0004$; condition: $F_{1,221} = 0.105$, $P = 0.7463$; age: $F_{1,221} = 20.783$, $P < 0.0001$; season: $F_{1,221} = 0.357$, $P = 0.5507$).

Focusing specifically on the two most prevalent parasites, we found that horn size was a significant predictor of coccidia and strongyle infection status in males. Males with larger horns were less likely to be infected with both parasites (likelihood ratio test: coccidia, $N = 167$, horn size: $\chi^2_1 = 11.57$, $P = 0.0007$; condition: $\chi^2_1 = 3.531$, $P = 0.0602$; age: $\chi^2_1 = 6.66$, $P = 0.0099$; season: $\chi^2_1 = 1.339$, $P = 0.2472$; strongyles, $N = 167$, horn size: $\chi^2_1 = 5.821$, $P = 0.0158$; condition: $\chi^2_1 = 0.009$, $P = 0.9251$; age: $\chi^2_1 = 1.846$, $P = 0.1743$; season: $\chi^2_1 = 3.184$, $P = 0.0744$). In females, horn size was also a significant predictor of coccidia infection status, but it was only marginally correlated with strongyle infection status. Females with larger horns were less likely to be infected with coccidia ($N = 226$, horn size: $\chi^2_1 = 7.482$, $P = 0.0062$; condition: $\chi^2_1 = 2.253$, $P = 0.1333$; age: $\chi^2_1 = 19.708$, $P < 0.0001$; season: $\chi^2_1 = 5.104$, $P = 0.0239$), but horn size had only a weak negative effect on strongyle infection status ($N = 226$, horn size: $\chi^2_1 = 3.299$, $P = 0.0693$; condition: $\chi^2_1 = 1.042$, $P = 0.3074$; age; $\chi^2_1 = 3.573$, $P = 0.0587$; season: $\chi^2_1 = 3.765$, $P = 0.0523$).

Horn Size, Parasite Intensity and Immune Function

With regard to parasite intensity, strongyle intensity was significantly correlated with horn size in males, and males with larger horns had fewer parasites (Table 1, Fig. 3a). Age was also a significant predictor of strongyle intensity in males. Younger animals had more intense infections, but neither age, body condition nor season influenced the relationship between horn size and strongyle burden (Table 1). There was no significant correlation between horn size and coccidia intensity in males (Table 1, Fig. 3b), but both age and season were significant predictors of coccidia burden (Table 1). Horn size did not predict WBC count in males, but age was significantly and negatively correlated with WBC count (Table 1). In females, horn size was a significant negative predictor of coccidia intensity (Table 1, Fig. 3c). Age and body condition were also significantly and negatively correlated with coccidia intensity in females, and intensity was higher in the wet season (Table 1). There was no association between horn size, or any covariate, and strongyle intensity in females (Table 1, Fig. 3d). Unlike in males, however, horn size was significantly and negatively correlated with WBC count in females, even after controlling for body condition and age, both of which had significant effects on this trait (Table 1).

Horn Size and Reproductive Status in Females

Given the observed associations between horn size, parasites and condition in females, we also examined

Table 1. General linear models testing for effects of age-corrected horn size on parasite intensity and immune status in male and female African buffalo

		Males			Females		
Dependent variable	Independent variable	*df*	Coefficient	*P*	*df*	Coefficient	*P*
Coccidia intensity	Horn size	1	−0.008	0.2337	1	−0.013	0.0379*
	Condition	1	−0.007	0.8510	1	−0.041	0.0309*
	Age	1	−0.090	<0.0001*	1	−0.046	0.0001*
	Season: May	1	0.343	<0.0001*	1	0.137	0.0337*
	Residual	110			118		
Strongyle intensity	Horn size	1	−0.019	<0.0001*	1	−0.004	0.3341
	Condition	1	0.019	0.4390	1	−0.024	0.1172
	Age	1	−0.029	0.0328*	1	−0.006	0.5028
	Season: May	1	−0.052	0.3762	1	−0.003	0.9502
	Residual	109			159		
WBC	Horn size	1	−0.023	0.4658	1	−0.067	0.0172*
	Condition	1	0.247	0.1493	1	0.315	0.0004*
	Age	1	−0.320	0.0010*	1	−0.280	<0.0001*
	Season: May	1	0.553	0.1689	1	−0.400	0.1935
	Residual	160			220		

Body condition, age and season were included as covariates in all models (* indicates significance at $P \leq 0.05$).

whether horn size was an important predictor of reproductive status in females. Controlling for age, body condition and season, all of which had significant effects on reproductive status, we found that horn size had no effect on the likelihood of a female being pregnant (likelihood ratio test: $N = 132$, horn size: $\chi^2_1 = 0.178$, $P = 0.6733$; body condition: $\chi^2_1 = 13.763$, $P = 0.0002$; age: $\chi^2_1 = 5.26$, $P = 0.0218$; season: $\chi^2_1 = 17.663$, $P < 0.0001$), but females with larger horns were significantly more likely to be lactating ($N = 131$, horn size: $\chi^2_1 = 5.820$, $P = 0.0158$; body condition: $\chi^2_1 = 28.616$, $P < 0.0001$; age: $\chi^2_1 = 4.546$, $P = 0.0330$; season: $\chi^2_1 = 14.833$, $P < 0.0001$).

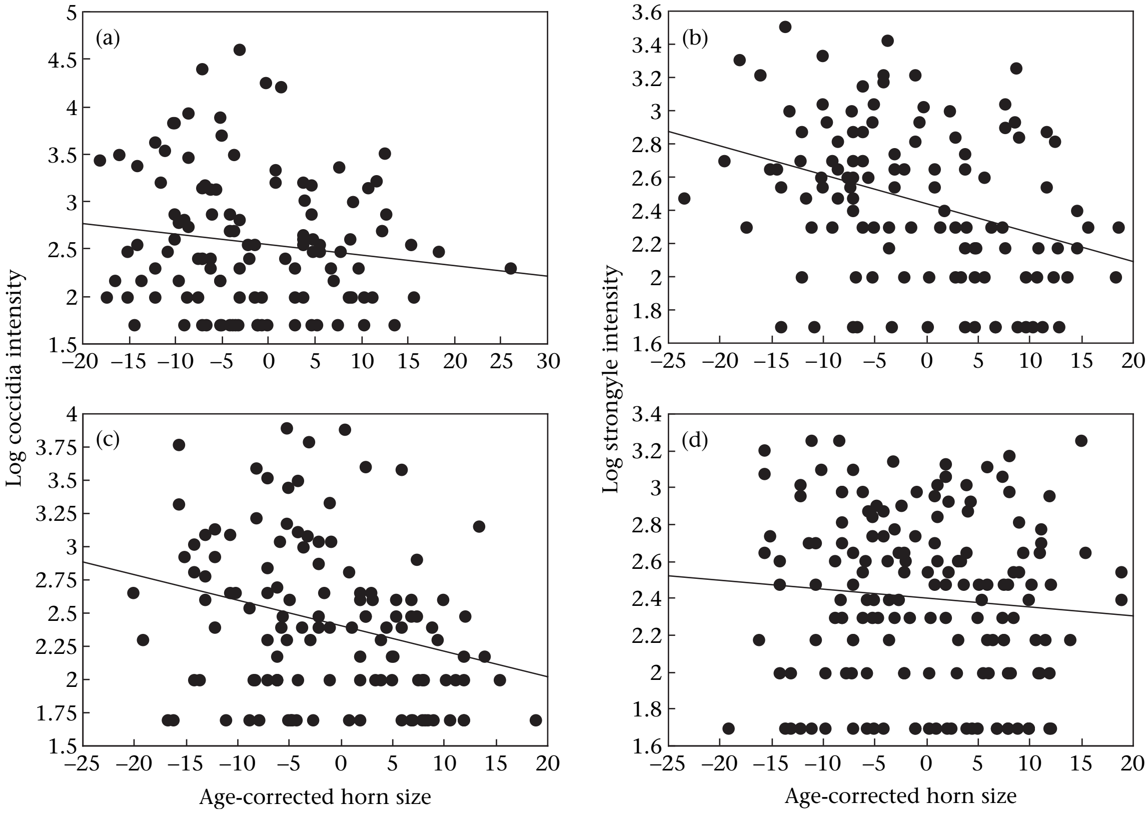

Figure 3. Associations between horn size and parasite intensity in (a, b) male and (c, d) female African buffalo.

DISCUSSION

The idea that exaggerated secondary sexual traits reveal information about the ability of individuals to resist parasites has been examined across a growing number of species (Møller et al. 1999). This work has focused almost exclusively on males, but in species where both males and females express secondary sexual characters, PMSS might also function in females. In this study, we tested whether horn size in African buffalo might convey information about parasite infection and immune function in both sexes. We found that males infected with multiple parasite types had smaller horns; smaller-horned males were also more likely to be infected with coccidia and strongyles, and had more intense strongyle infections. For females, infection with multiple parasites was also correlated with smaller horn size; furthermore, individuals with smaller horns were more likely to be infected with coccidia, and had higher coccidia burdens and more WBCs. In addition to correlations between horn size and health indicators, horn size was also correlated with condition in both sexes, confirming that horns are a condition-dependent trait in this species. In females, horn size was also associated with reproductive status. Pregnancy status did not differ with horn size, but females with larger horns were significantly more likely to be lactating, suggesting that horn size is correlated with calf survival. Overall, these results are consistent with the idea that weapons function as honest indicators of individual health and quality.

In males, associations between weapon size and parasite richness and intensity could signal genetic quality to females, competitive ability to other males or both. Both strongyle and coccidia resistance show moderate heritability in domestic and free-ranging ungulates (Smith et al. 1999; Gauly et al. 2001; Beraldi et al. 2007). Since female choice based on male phenotype has been documented in several polygynous ungulates where males do not provide parental care, as is the case in buffalo (Clutton-Brock et al. 1989; Balmford et al. 1992; Charlton et al. 2007), it is possible that female buffalo choosing males with large horns benefit by passing down resistance genes to their offspring. However, given that male–male competition is probably the primary determinant of male reproductive success in buffalo (Sinclair 1977), signalling of infection status is likely to be directed, in large part, towards other males. There is considerable evidence suggesting that parasites can have important effects on the outcome of intrasexual competition, and negative effects of parasites on host agonistic behaviour and competitive ability have been documented for a variety of species (Freeland 1981; Maksimowich & Mathis 2000; Gourbal et al. 2002). For example, male red grouse, *Lagopus lagopus scoticus*, with experimentally reduced nematode infections responded more aggressively to territorial intrusions and won more territorial disputes than controls (Fox & Hudson 2001). Such effects could be mediated, in part, by changes in host metabolism and energetics that make parasitized individuals less able to engage in physically demanding activities (Schall & Sarni 1987; Marden & Cobb 2004).

In African buffalo, overt dominance interactions between males are rarely observed (Sinclair 1977; Mloszewski 1983; Prins 1996). In over 200 000 h of observation, Prins (1989a) reported only 22 cases of male–male agonistic interactions, all of which were settled without further escalation. Escalation of threat displays into outright fighting is extremely rare, and in three cases described by Prins (1989a), both contestants died as a consequence of the interaction, highlighting the extreme cost of fighting in this species. Because fighting is so costly, agonistic interactions generally occur only between opponents of similar size or physical condition (Prins 1989a, 1996). Across the entire bovine tribe (buffalo, bison and cattle), visual displays are commonly used as a means of assessment (Estes 1991a); and it has been suggested that buffalo use body condition as a major indicator of an opponent's strength prior to engaging in costly fights (Prins 1996). Horn size is probably easier to assess visually than is condition, and our results show that horns convey reliable information on body condition as well as disease status, both of which can influence fighting ability. The fact that threat displays in buffalo prominently showcase the horns (Sinclair 1977) further supports the idea that horns probably serve as important visual signals of fighting ability. Thus, we suggest that in addition to their direct role as weapons in fights, horns in male buffalo may also serve the function of visually deterring inferior competitors by providing honest information about the bearer's infection status, condition and overall competitive ability.

Although an increasing number of studies have begun to examine the extent to which parasites might influence the evolution and maintenance of secondary sexual traits in females, most of this work has focused on ornaments (e.g. Potti & Merino 1996; Roulin et al. 2001; Setchell et al. 2006; Weiss 2006). We tested predictions of PMSS focusing on female weapons, and found that female horn size was negatively correlated with parasite richness, presence of coccidia infection and coccidia intensity. In African buffalo, male horns are clearly larger and more elaborate than female horns. Reduced expression of male secondary sex traits in females has often been considered to be the result of genetically correlated responses to selection in males (Lande 1980; Amundsen 2000). However, several mechanisms of direct (natural) selection have also been proposed to explain the evolution and maintenance of horns in female ruminants, including predator defence (Packer 1983), male mimicry (Estes 1991b) and female competition (Roberts 1996). Our findings that female horns grow throughout life and show within-sex variability in size equivalent to that of male horns are most compatible with the idea of female competition. If female ruminants use their horns to compete directly over resources, or to improve their rank or position within the herd, leading to higher reproductive success, then there should be a selective advantage for larger horns in females (Alados & Escos 1992; Roberts 1996). Our results show that females with larger horns are not only in better physical and nutritional condition, but are also more likely to be lactating than females with smaller horns. These results suggest a link between horn size, resource acquisition and reproductive success in female buffalo. Although aggressive interactions between adult female buffalo are rarer than between males, displacements and attacks do occur in females (Sinclair 1974; Prins 1989b). Female aggression generally takes the form of ‘a sweep of the horns’ at target individuals, and is primarily directed towards other females (Sinclair 1974). In particular, Prins (1989b) documented that adult females with calves tended to be the most aggressive, executing a majority of observed displacements over food. If females actively compete over resources, then much like males they should also have mechanisms by which to assess each other’s competitive ability. The observed correlation between horn size, parasite infection, condition and reproduction in females suggests that horns may function as a means of assessment in females, with females with larger horns able to monopolize more resources by signalling their health status and competitive ability to would-be competitors, resulting in higher reproductive success.

In addition to female competition as a possible mechanism driving the evolution and/or maintenance of horns as signals in female buffalo, evidence that dominant males preferentially aggregate in the front of herds where high-quality females have been found to occur disproportionately (Prins 1996) is also suggestive of some degree of male choice in this system. Although females are generally considered to be the choosy sex in polygynous mammals, certain conditions can select for male choice, including high variance in female quality and situations in which mating with one female requires a male to forgo mating with others (Andersson 1994). Our results directly link horn size with female reproductive success, suggesting that horn size is correlated with variance in female quality. Furthermore, because buffalo bulls form tending bonds with oestrous females that can last for several days (Sinclair 1977), and associating with one female might result in the loss of mating opportunities with other females, dominant males may actively choose to consort with particular females. Indeed, work on a closely related species, American bison, *Bison bison*, showed that older bison bulls choose females based on their reproductive potential (Berger 1989). In combination, these results suggest that the occurrence of male choice in African buffalo is highly plausible; if so, male buffalo may use horn size to assess relative female quality. Although detailed behavioural studies will be needed to ascertain whether there is indeed active male choice in African buffalo, and whether choice is correlated with horn size, our results suggest that sexual selection via ‘cryptic’ male choice could be important in maintaining horns in females.

Since most individuals are infected with multiple parasites, many of which may not exert strong fitness costs on the host, measures of immune status may potentially provide more accurate tests of PMSS than analyses of individual parasites (Møller et al. 1999; Mougeot et al. 2004). For this reason, we also examined associations between horn size and WBC counts in buffalo, predicting that individuals with larger horns would have more WBCs. Contrary to our expectations, we found no association between horn size and WBCs in males, and a significant negative association between the two variables in females. Because WBC counts can reflect levels of exposure to disease, current infection status, or the ability to fight new infections, a possible explanation for the negative pattern in females could be that healthier individuals have lower WBC counts than more infected individuals, reflecting their lower exposure to parasites (Møller et al. 1999; Mougeot & Redpath 2004). Past studies, mostly in birds, have reported negative, positive or nonexistent associations between WBCs and secondary sexual traits (Dufva & Allander 1995; Zuk 1996; Saino et al. 1997). Given these highly variable results, future work using direct estimates of immunocompetence (the ability of an individual to respond to challenge by a novel pathogen) may be more effective at clarifying relationships between weapon size and immune status in buffalo.

In conclusion, we tested whether horn size was a reliable indicator of health status in African buffalo. Both male and female horn sizes were negatively correlated with parasite infection, suggesting that weapons may function as honest signals of parasite resistance in both sexes. In males and females, it is likely that a combination of mechanisms drives the maintenance of horns as signals. In males, horns may reliably signal competitive ability to other males as well as genetic quality to females. In females, horns may be used as signals in competitive interactions for resources and also as indicators of reproductive potential to males. Future work will be needed to assess the degree to which horn size influences competitive ability and mate acquisition in both sexes. Nevertheless, since very few studies of mammals have tested whether weapons serve as honest indicators, and none to our knowledge have examined whether weapons function as signals of health status simultaneously in both males and females, our results provide important new insights on the evolution of weapons in mammals. In particular, they suggest a possible role for sexual selection in the maintenance of horns in female ungulates.

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