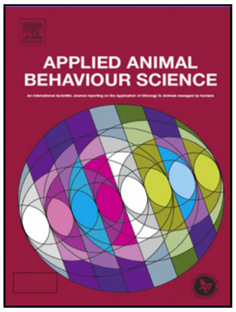

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PII: S0168-1591(18)30503-3
DOI: https://doi.org/10.1016/j.applanim.2019.03.005
Reference: APPLAN 4784

To appear in: *APPLAN*

Received date: 10 September 2018
Revised date: 19 February 2019
Accepted date: 4 March 2019

Please cite this article as: Jones MM, Nuñez CMV, Decreased female fidelity alters male behavior in a feral horse population managed with immunocontraception, *Applied Animal Behaviour Science* (2019), https://doi.org/10.1016/j.applanim.2019.03.005

Decreased female fidelity alters male behavior in a feral horse population managed with immunocontraception

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Highlights:

- Stallions experiencing increased female turnover engage in more frequent contests
- These stallions engage in more escalated contests and spend more time vigilant
- Habitat visibility but not female turnover influenced male-female aggression
- Home range overlap also influenced male-male interactions
- Immunocontraception management indirectly affects stallion behavior

Abstract

In social species like the feral horse (*Equus caballus*), changes in individual behavior are likely to affect associated animals. On Shackleford Banks, North Carolina, USA, mares treated with the contraceptive agent porcine zona pellucida (PZP) demonstrate decreased fidelity to their band stallions. Here, we assess the effects of such decreased mare fidelity on male behavior and address potential interactions with habitat visibility, a component of the environment shown to significantly affect feral horse behavior. We compared the frequency and escalation of male-male contests, rates of aggressive and reproductive behaviors directed toward females, and the percentage of time spent vigilant among males experiencing varying levels of mare group changing behavior. We found that regardless of habitat visibility, males experiencing more female group changes engaged in contests at a higher rate ($P = 0.003$) and escalation ($P = 0.029$) and spent more time vigilant ($P = 0.014$) than males experiencing fewer group changes. However, while visibility had a positive effect on aggression directed by stallions toward mares ($P = 0.013$), female group changing behavior did not influence male-female aggressive or reproductive behaviors ($P > 0.1$), showing that decreases in mare fidelity altered male-male but not male-female interactions. These results have important implications for feral horse management; PZP-contracepted mares demonstrating prolonged decreases in stallion fidelity may have a disproportionate effect on male behavior. Moreover, our results shed light on the relative influences of female behavior and environmental factors like habitat visibility on male behavior. Such findings can ultimately improve our understanding of how the social and physical environments interact to shape male-male and male-female interactions.

Key words: aggression; *Equus caballus*; immunocontraception; social stability; stallion;

vigilance

1. Introduction

Ungulate populations in the United States have expanded greatly in recent history (Eberhardt et al., 1982), demanding some form of management that balances efficient control of animal numbers and public opinion of the adopted strategy (National Research Council, 2013). While easily overlooked, maintenance of natural behaviors is an important consideration if management strategies are to sustain the health and wellbeing of wildlife populations. The feral horse (*Equus caballus*) provides an interesting case study for the impacts of management strategies on behavior and physiology at multiple levels. With few predators on the continent, feral horse populations have grown rapidly in the United States (Garrott et al., 1991; Turner et al., 1992; Turner and Morrison, 2001; DOI-OIG 2010). Subsequent overgrazing can be detrimental to native vegetation and wildlife communities and also conflicts with land use practices (Beever and Brussard, 2000; Levin et al., 2002). Viewed as both a culturally valuable species protected by federal law and a nuisance to humans and wildlife, feral horses present a unique challenge to wildlife managers (United States Congress, 1971). Recently, fertility control has become a popular option for managing feral horse populations. The immunocontraceptive agent porcine zona pellucida (PZP) has been used to manage several populations of feral horses and other social species including white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus*) (Kirkpatrick et al., 1997; McShea et al., 1997; Heilmann et al., 1998; Ransom et al., 2010).

In feral horses, both physiological and behavioral effects of PZP on treated mares have been widely documented, with varying results among populations (Kirkpatrick et al., 1996; Powell, 1999; Turner and Kirkpatrick, 2002; Nuñez et al., 2009; Nuñez et al., 2010; Ransom et al., 2010; Madosky, 2011; Nuñez et al., 2014). However, the potential effects of immunocontraception management on non-target individuals have received less attention (Ransom et al., 2010). Such effects are especially important to consider in social species like the feral horse, as the behavior of one individual is likely to influence the behavior of its associates. Feral horses live in social groups (called bands) consisting of one or sometimes multiple stallions, one or more associated females, and their offspring (Feist and McCullough, 1976; Rubenstein, 1981). Bands are typically stable groups with females staying with the same male (band stallion) for most of their adult lives (Klingel, 1975).

However, this stability may be compromised when the behavior of individual group members changes. The feral horse population on Shackleford Banks, North Carolina, USA provides such an example. Between 2000 and 2009, the population was managed by treating a subset of females with PZP. This resulted in several changes in the behavior and physiology of treated mares (Nuñez et al., 2010; Nuñez et al., 2014) including increased group changing behavior (Nuñez et al., 2009). While PZP treatment was largely suspended in 2010, increased group changing behavior is still exhibited by previously treated mares experiencing prolonged subfertility (Nuñez et al., 2017). The resulting decrease in group stability may have implications for other group members, particularly the band stallions (Madosky, 2011).

During periods of social instability, males exhibit increases in aggression and testosterone across diverse taxa including primates, birds, and fish (Wikelski et al., 1999; Cavigelli and Pereira, 2000; Hirschenhauser and Oliveira, 2006; Arlet et al., 2009). On Shackleford, the social instability primarily created by decreases in previously treated mares' fidelity to band stallions is likely to similarly increase the frequency and escalation of stallion contests. Furthermore, changes in female behavior and movement patterns are likely to influence how males interact with these females. For example, male harassment of females is common in ungulate mating systems, with males more likely to initiate mating than females (Bro-Jørgensen, 2011). However, harassment intensity can vary greatly with the strength of social bonds between males and females (Linklater et al., 1999). Females demonstrating weaker bonds with the dominant male and/or female associates often experience increased male harassment (Linklater et al., 1999). On Shackleford Banks (and in the western United States), treated mares demonstrate prolonged reproductive cycling (Nuñez et al., 2010; Ransom et al., 2010) and weakened social bonds (Nuñez et al., 2009; Madosky, 2011). Not surprisingly, treated mares are often subject to increased stallion harassment when compared to untreated mares (Madosky, 2011), demonstrating the profound impacts female behavior can have on male behavior.

Still, to fully understand how males respond to changes in female behavior, male behaviors should be considered in the context of the animals' surrounding environment. The interactions between animals and various facets of their physical environment such as visibility and resource distribution profoundly influence mating systems by dictating how males exploit female movement and dispersal patterns (Emlen and Oring, 1977;

Rubenstein, 1981). Historically, Shackleford has been defined by three distinct habitats: the East, which is flat and open with limited water sources (Marr, 1996); the Mideast, which is also flat and open with more evenly distributed water sources (Rubenstein, 1981); and the West, which contains two primary water sources and is dominated by high dunes and dense brush (Rubenstein, 1981). These regions vary in their resource availability and landscape features, both of which influence stallions' ability to defend territories (Rubenstein, 1981). For example, water and vegetation distribution determine the location and extent of horses' home ranges (Feist and McCullough, 1976; Miller, 1983; Manning and McLoughlin, 2017). Furthermore, the degree of visibility across a landscape can affect a stallion's ability to defend his band against intruders (Rubenstein, 1981). The history of territoriality in the eastern region of Shackleford demonstrates how high visibility and evenly distributed resources allow for the maintenance and defense of distinct boundaries (Rubenstein, 1981; Rubenstein, 1986). Therefore, shifts in resource availability or distribution, visibility, and the resulting degree of overlap between band home ranges are likely to impact the frequency of intergroup interactions and the degree of competition among stallions. Investigating the influences of both female behavior and ecological factors on male behavior can broaden our understanding of how these factors interact to shape male mating strategies and even the evolution of mating systems.

Here, we assessed the effects of decreased mare fidelity on male behavior in the feral horse population on Shackleford Banks, NC. We tested how the rate of female turnover experienced by males affected 1) the frequency and escalation of male-male contests and vigilance behavior and 2) the frequency of male aggression and reproductive behaviors directed toward mares. In addition, we tested the effects of ecological variables including visibility and home range extent on these behaviors. We hypothesized that males experiencing more group changes by females, defined as the number of times females enter or leave a stallion’s band, would engage in contests with other males more frequently and at a higher intensity than those experiencing fewer group changes. Furthermore, we predicted that stallions experiencing increased female turnover would exhibit increased rates of aggression and reproductive behavior toward females. Finally, we hypothesized that increased habitat visibility and overlap among home ranges would heighten aggression between males and increase male aggression and reproductive interest directed toward females. Because females previously treated with PZP exhibit prolonged decreases in stallion fidelity, this study can improve our understanding of the potential for indirect effects of contraception management on non-target animals. In addition, our evaluation of the relative effects of female behavior and ecological factors on male behavior may provide insights into their influence on the expression (and evolution) of male mate-guarding and reproductive behaviors, critical components of male mating strategies.

2. Methods

2.1. Study Site and Subjects

The focal population of feral horses lives on Shackleford Banks, a barrier island off the coast of North Carolina, USA. The island is approximately 15 km long and ranges from 0.2 to 1.2 km wide. As a part of Cape Lookout National Seashore, Shackleford Banks is visited by approximately 100,000 tourists annually (National Park Service, 2017). Therefore, the horses on the island are somewhat habituated to human activity (i.e., at distances of at least 15 m). The study was conducted during two breeding seasons, June through July, 2016 and May through August, 2017. In both years, we observed all band stallions and their females: 21 males and 70 females organized into 18 bands in 2016 and 20 males and 69 females organized into 19 bands in 2017. Most bands had one dominant stallion with the exception of two double-male bands in 2016 and one double-male band in 2017.

2.2. PZP Contraception

This feral horse population is federally protected under the Shackleford Banks Wild Horses Protection Act of 1997 and is managed jointly by the National Park Service and the Foundation for Shackleford Horses (United States Congress, 1997). To maintain the population at the predetermined size of approximately 120 animals, the National Park Service began utilizing immunocontraception in 2000. Mares received initial and booster doses of PZP, which were administered from February through April in designated years. Injections contained 100 µg of PZP plus an adjuvant, and initial doses contained Freund's Complete Adjuvant, Modified, *Mycobacterium butyricum* (Calbiochem #344 289). Booster doses contained Freund's Incomplete Adjuvant (Sigma #F5506). In 2010 and 2011, when the NPS and Foundation realized that some mares treated repeatedly experienced prolonged subfertility post-treatment, PZP treatment was suspended to allow the population to increase (Stuska, personal communication).

2.3. Behavioral Data

Behavioral data were collected primarily by one observer (M.M. Jones) and were supplemented with additional data from two other observers (M. Fatka and C.M.V. Nuñez). Both M.M. Jones and M. Fatka were trained by C.M.V. Nuñez. Study bands were located twice per week, and GPS location and group composition were noted. Individuals were identified with freeze brands, distinctive markings, coloration, and other identifying features. As witnessing group changing behavior by females was rare (n = 1), presence and absence of females in the band were noted to monitor this behavior. These data were used to calculate the number of group changes experienced by males (the number of times females entered or left a male's band).

Instances of rare behaviors were recorded *ad libitum* (Altmann, 1974). These included 1) male-male aggression (including biting, kicking, pushing, and kick threats), 2) male-female aggression (including both mate-guarding behaviors such as herding and chasing and the aggressive behaviors listed above), and 3) reproductive interest (including copulation, mounting, genital sniffing, and rump rubbing (Nuñez et al., 2009)). In addition, the amount of time that stallions spent vigilant was recorded during 15-minute focal samples (Altmann, 1974). The escalation of male contests was scored based on a scale of one to six adapted from Hynes (1998) with scores as follows:

1. Visual displays including parallel prance, pawing the ground, neck arch, and approach-retreat without physical contact.
2. Olfactory assessment including nose-to-nose and nose-to-genital contact and fecal pile sniff without physical contact.
3. Auditory assessment using aggressive male vocalizations called squeals.
4. Low intensity physical contact including pushing and kick threats.

5. Moderate intensity physical contact including hind and front kicks that may result in injury.
6. High intensity physical contact including biting and rearing while kicking with front legs that are likely to result in injury.

Frequencies of observed behaviors were significantly correlated with the total time individuals were observed (male-male contests: $\rho = 0.330$; $P = 0.035$; male-female aggression: $\rho = 0.486$; $P = 0.001$; male-female reproductive behavior: $\rho = 0.663$; $P < 0.001$). To control for this bias, rates of male-male aggression, male-female aggression, male-female reproductive interest, and female group changes were calculated as the number of instances an individual was observed engaging in those behaviors divided by the total time each horse was surveyed during the season (expressed as observation days, defined as 12 hour periods (the approximate length of daylight during our study period)). Additionally, the escalation scores of all male-male contests that a single stallion engaged in were averaged to calculate a mean escalation score for that stallion. Finally, percentage of time spent vigilant, which was characterized by ears erect and directed forward, head and neck held high, and eyes alert (Figure 1) (Feist and McCullough, 1976), was measured as the minutes spent vigilant over all focal samples conducted for each male divided by the total number of focal minutes for each male.

During observations, detailed descriptions of the behaviors exhibited, identity of the participating individuals, and the date and time were recorded. All observations were conducted by eye or with the aid of binoculars (Nikon Monarch M511 10x42) when necessary at least 15-20 meters away from the animals (National Park Service, 2004) to ensure that they were not disturbed by our presence.

2.4. Assessing Visibility Across Shackleford Banks

We assessed visibility across the island as a potentially important environmental factor that can influence male behavior and inter-band conflict (Rubenstein, 1981). Visibility was quantified through viewshed analysis in ArcMap 10.4.1 (Alonso et al., 2012; Aben et al., 2017). Digital surface models were created using USGS LiDAR point cloud data to account for both bare earth elevation and vegetation structure (U.S. Geological Survey, 2015). GPS locations collected at every band sighting using Garmin eTrex 10 units were used as observer points, and an observer offset of 1.2 m was used as the average height of the horses (Stuska, personal communication). The Visibility tool in ArcMap was then used to predict the cells visible from each point, and the number of visible cells was averaged across all points for each stallion to produce an average visibility score.

2.5. Estimating Home Range Size and Overlap

To quantify band home range size and degree of overlap, as these factors can also influence stallion behavior (Rubenstein, 1981), we used the R package *adehabitatHR* (Calenge, 2006). Home ranges for each band were estimated with GPS locations collected at all band sightings in 2016 and 2017 using the 95% adaptive kernel method as described by Worton (1989). Home range area and each band’s degree of overlap with other home ranges were then assessed in ArcMap. Home range overlap was calculated as the percentage of one stallion’s home range area that was overlapped by other home ranges. Home ranges for each year were estimated separately, and bands with fewer than ten GPS positions were excluded from the analysis (Börger et al., 2006).

2.6. Statistical Analysis

To assess whether the increased group changing behavior by females previously treated with PZP reported by Nuñez et al. (2017) persisted during our study period, we used a permutation test with a significance level of 0.05 in the *geomorph* package in R (version 3.4.1) (Adams et al., 2017; R Development Core Team, 2017). The rate of group changing behavior by females was the response variable, and mare age and a binary variable representing whether or not a mare had been previously treated with PZP were the independent variables. For this permutation test, F-statistics with total and residual degrees of freedom and *P*-values are presented.

We used generalized linear mixed-effects models to examine how changes in female behavior influenced male behavior. Separate models were run for each of the following dependent variables: male-male contest rate, average male-male contest escalation, male-female aggression rate, male-female reproductive interest rate, and percentage of time males were vigilant. Each model included the following independent variables as fixed effects: the number of group changes a male experienced divided by the total time he was surveyed (female turnover rate), average visibility score, male age, average female age (within the band), and the average number of adult females present. The latter three variables were included to control for their potential impacts on male behavior (Berger, 1977; Vervaecke et al., 2007; Nuñez et al., 2017). Finally, all models included stallion identity as a random effect to control for pseudo-replication.

For all models, dependent variables were log or square root transformed as necessary to account for non-normality of model residuals. Best fit models were then selected via backward elimination with a cutoff level of $P = 0.1$ to eliminate variables from the models. Additionally, Spearman correlation tests were used to assess correlations among independent factors and to avoid collinearity within models. Final models did not include any highly correlated variables. The significance level of 0.05 was used to evaluate the effects of predictor variables on male behavior.

All band stallions that were observed for more than two hours were included in the analysis (n = 23 (18 stallions were observed in both 2016 and 2017, 3 additional stallions were observed only in 2016, and 2 were observed only in 2017)).

While male rank is also often a predictor of male aggression (Vervaecke et al., 2007), it was not included in these models because dominance data were not available for approximately one third of the stallions. However, age is typically associated with rank, with older males having higher ranks, and thus the inclusion of male age in the models likely controlled for at least some of the variation due to dominance status (Vervaecke et al., 2007). Further, for those stallions whose dominance could be determined, rank was not significantly correlated with any dependent variables in Spearman correlation tests, and thus it is unlikely that including rank in the models would have altered our conclusions (Table 1).

Finally, because home ranges could only be estimated for 17 stallions (there was sufficient data to estimate home ranges for the same 8 stallions in both 2016 and 2017, 1 additional stallion in 2016 only, and 8 additional stallions in 2017 only), including home range size and overlap in our models reduced our sample size. Therefore, we ran two subsets of models: those that included home range size and overlap and those that did not. For these generalized linear mixed-effects models, parameter estimates, standard errors (SE), t-values, and *P*-values are presented in Tables 2 and 3.

3. Results

3.1. PZP Treatment and Female Group Changing Behavior

When accounting for mare age ($F_{1,136} = 0.62$, $P = 0.432$), females that were previously treated with PZP made on average 2.4 group changes per day more than those that had never been treated ($F_{1,136} = 4.72$, $P = 0.026$).

3.2. Male-male Interactions

The rate of female group changes experienced (Table 2, Figure 2a) and male age (Table 2) both had positive effects on the number of male-male contests that stallions engaged in per day. Similarly, males experiencing more group changes by females per day (Table 2, Figure 2b) and older males (Table 2) engaged in more highly escalated contests.

3.3. Male-female Interactions

Males living in higher visibility areas initiated more aggressive behaviors toward females per day (Table 2). However, the number of female group changes experienced per day did not affect male-female aggression and was dropped from the final model ($P > 0.1$).

Male age was the only variable retained in the final model investigating factors affecting the rate of reproductive behaviors per day but did not have a significant effect on this behavior (Table 2). The number of female group changes that males experienced was dropped from the final model, suggesting it did not explain variation in the rate of male reproductive behavior (*P* > 0.1).

3.4. Vigilance Behavior

The percentage of time males spent vigilant during focal samples increased with a greater number of female group changes experienced per day (Table 2, Figure 2c). Additionally, males with younger females in their bands also spent more time vigilant (Table 2).

3.5. Analyses Including Home Ranges

Both the percentage of home range overlap and the rate of female group changes had positive effects on the rate of male-male contests (home range overlap: estimate = 1.430, t = 2.93, *P* = 0.008; group changes: estimate = 0.048, t = 2.43, *P* = 0.024). Additionally, contests between males with a high degree of home range overlap were more highly escalated than those between males with a lower percentage of overlap (estimate = 3.505, t = 2.93, *P* = 0.008). Finally, stallions experiencing more female group changes per day tended to exhibit higher rates of reproductive behavior (estimate = 0.048, t = 1.90, *P* = 0.071). The behavioral and ecological variables of interest (female group changes, home range size, home range overlap, and visibility) did not affect the rate of aggressive behaviors directed toward females or the percentage of time males spent vigilant (Table 3).

4. Discussion

Effects of PZP treatment on female behavior, including increased group changing behavior and extended reproductive behavior and cycling, have been observed among feral horses on Shackleford Banks and in three western populations (Nuñez et al., 2009; Ransom et al., 2010). However, the potential consequences of such changes for their male associates have not yet been fully explored. Here, we demonstrate that increased group changing behavior by mares is correlated with an increase in aggression among stallions and heightened male vigilance. The implications of these results for management strategies deserve careful consideration and further investigation. Specifically, treating females with immunocontraceptives or other agents that decrease social fidelity can have broader behavioral impacts on males in the population. Moreover, our results provide new insights into the relative influences of female behavior and the surrounding environment, specifically habitat visibility and home range overlap, on male behavior and the expression of reproductive and mate-guarding behaviors involved in mating strategies.

4.1. Effects of Female Behavior on Male Behavior

Stallions that experience increased rates of group changes by females engage in contests with other males more often and at more escalated levels than those experiencing fewer group changes (Figure 2a, Figure 2b). The feral horse social system is typically characterized as harem defense polygyny, in which females form stable groups defended by males (Emlen and Oring, 1977; Rubenstein, 1986). Typically, mares stay with the same stallion for most of their adult lives, and attempts by intruding males to gain access to a resident male’s females are usually unsuccessful (Feist and McCullough, 1976; Rubenstein, 1981). In the Shackleford population, decreased female fidelity has compromised this group stability (Madosky, 2011); in response, it appears that males have responded by increasing their defense of females against rivals.

In addition, males that experience increased mare turnover spend a greater percentage of time vigilant than those experiencing fewer group changes (Figure 2c). Similar increases in vigilance behavior have been demonstrated across diverse taxa; during periods of high intrasexual competition, males spend more time monitoring conspecifics (Guillemain et al., 2003; Li et al., 2012). Such monitoring behavior allows males to guard female associates from rival males as well as detect and respond to wandering females (Guillemain et al., 2003; Li et al., 2012; Girard-Buttoz et al., 2014). In the Shackleford population, increased vigilance may allow stallions to monitor both rival males and group changing mares in the context of decreased social stability and high intrasexual competition.

Such changes in male behavior could have consequences for the overall health and wellbeing of these individuals. Male contests, particularly ones that are highly escalated, are costly, demanding significant energy expenditure and potentially leading to injury or even death in extreme cases (Feist and McCullough, 1976; Berger, 1986; Grogan and McDonnell, 2005). Devoting more time to vigilance behavior can detract from time available for other behaviors like foraging, limiting males' energy intake (Lima, 1998; Watson et al., 2007). This may be particularly consequential if this increase in vigilance behavior is also exhibited during the winter when horses spend more time grazing on lower quality forage (Stevens, 1990; Watson et al., 2007). Future work should explore the consequences of these changes in male-male competition and vigilance behavior on stallion health.

4.2. Male-female Interactions

Contrary to our expectations, the number of group changes experienced by males did not affect the rate of aggressive or reproductive behaviors initiated toward females. Although previous work with the same population indicates that PZP-treated females receive more harassment and reproductive interest from males (Nuñez et al., 2009; Madosky, 2011), it does not appear that males engage in varying rates of male-female interactions based on the rate of female turnover they experience. It may be that stallion harassment and reproductive interest directed toward treated females are influenced more by differences in estrous cycling among treated versus untreated mares (Nuñez et al., 2010; Ransom et al., 2013) rather than the differences in group changing behavior that we examined here. As they rarely become pregnant, treated mares exhibit increased reproductive cycling, which can persist even after immunocontraceptive treatment is suspended (Ransom et al., 2010; Nuñez et al., 2017).

However, it is important to note that this study was conducted solely during the breeding season, when reproductive behaviors and male-female interactions are at a peak (Stevens, 1990). In horses and other ungulate species, behavioral effects of PZP on mares are often more evident during the non-breeding season when reproductive behavior is more variable (McShea et al., 1997; Heilmann et al., 1998; Nuñez et al., 2009). Similarly, variation in stallions' interactions with females may be more evident during the non-breeding season when behavioral differences between treated and untreated mares are more pronounced (Nuñez et al., 2010). For example, on Assateague Island, Powell (1999) found no difference in the harassment received by untreated versus treated mares during the breeding season, though that study lacked true controls (mares that had never been treated with PZP) for comparison. On the other hand, Madosky (2011) found that treated mares on Shackleford did receive more harassment from stallions than untreated mares during the breeding season. Unfortunately, the definition of "harassment," specifically whether or not it included reproductive behaviors, is unclear, making it difficult to directly compare these studies.

Studies of other species examining male behavior in populations managed via fertility control reveal similar inconsistencies (Ji et al., 2000; Poiani et al., 2002; Druce et al., 2013; Duncan et al., 2017). Ji et al. (2000) found that male brushtail possums (*Trichosurus Vulpecula*) were attracted to sterilized females during and after the mating season; however, Druce et al. (2013) found no evidence that bull African elephants (*Loxodonta africana*) associated more with family groups containing more estrous females, suggesting that PZP treatment did not alter male-female associations. This level of variation among species supports the need for careful monitoring not only of target animals', but also non-target animals', behavior in response to contraception management.

4.3. Effects of Visibility

Habitat openness (or visibility) often plays an important role in male behavior because higher visibility allows males to defend home range or territory boundaries and track band member activity more easily (Rubenstein 1981). Our data show that males residing in higher visibility areas are more aggressive toward band females (herding, biting, and kicking their females more often) than males in lower visibility areas (Table

2). However, contrary to our predictions, we saw no effect of visibility on the frequency or intensity of male-male interactions (Table 2).

In high visibility areas, males can more easily see one another and each other's females, perhaps increasing the perceived threat of rival males. Males in these areas may herd their females more frequently to prevent them from wandering towards rivals. A higher frequency of herding behavior in high visibility areas may therefore allow for a lower frequency of male contests over roaming mares. In comparison, this pressure may be decreased in low visibility areas where stallions and their mares cannot readily see neighboring bands. In these areas, landscape features such as high dunes and dense brush likely make encounters between rival males relatively uncommon, limiting the frequency of male-male contests. Therefore, similar contest levels might be maintained in areas of low and high visibility albeit via different mechanisms, though future work should further explore this issue. Our use of viewshed analysis to assess the effects of visibility on feral horse behavior adds to a new but growing body of work that uses this GIS tool to explore questions in behavioral ecology (Alonso et al., 2012; Aben et al., 2017). Our results show that habitat visibility is an important environmental factor to consider in future studies. Visual cues are integral to the behavior of diverse taxa, and the reception and interpretation of these cues depend heavily upon the structure of the environment and the resulting visibility, as outlined by Aben et al. (2017).

4.4. Effects of Home Range Overlap

While both home range overlap and female group changes had positive effects on the frequency of male-male contests, only home range overlap influenced the escalation of those contests (Table 3). Because home range overlap affected both the frequency and escalation of these contests while female turnover only influenced contest frequency, home range overlap seems to be a stronger driver of whether and how males engage in fights with rivals. These results suggest that while female behavior is important, home range overlap and, potentially, habitat features like forage and water availability (which largely dictate the extent of home ranges), have a greater influence on male-male interactions (Feist and McCullough, 1976; Rubenstein, 1981; Miller, 1983; Manning and McLoughlin, 2017). In habitats necessitating a high degree of overlap between home ranges, inter-band interactions are likely to increase, with more heightened competition among band stallions over both females and resources (Michener and McLean, 1996; Haenel et al., 2003). However, the sample size from our analysis including home range variables was significantly reduced compared to our full analysis without those variables. Therefore, our results regarding the potential effects of home range size and overlap should be interpreted cautiously.

Nonetheless, these results provide preliminary but important insights into the factors driving the expression of behaviors involved in male mating strategies, specifically reproductive and mate-guarding behavior. In most wild populations, the effects of ecological patterns and female behavior on male behavior are nearly impossible to separate because ecological patterns typically dictate female behavior (Emlen and Oring, 1977; Kraus et al., 2003). However, on Shackleford, the increase in group changing behavior by previously treated females suggests that their behavior is now at least somewhat divorced from their physical environment; mares that would typically form stable groups are instead demonstrating behavior akin to a fission-fusion society (Rubenstein, 1986; Nuñez et al., 2009). Therefore, this system provides a rare opportunity to examine how female behavior and ecological factors may act separately or in interaction to drive male behavior. While visibility, home range size, and home range overlap do not encompass the full range of ecological variables that can influence animal behavior, they have been shown to be particularly important in determining stallion behavior and provide a platform for examining the effects of the surrounding environment (Rubenstein, 1981). Our data suggest that while both the social and physical environment influence male behavior, ecological factors may have a greater impact on the means by which males retain access to females (i.e. through herding of females and contests with rival males) than does female behavior itself. Future studies of similar populations (McShea et al., 1997; Ransom et al., 2010; Duncan et al., 2017), with larger sample sizes and long-term data have the potential to improve our understanding of how these factors influence important reproductive and mate-guarding behaviors that shape male mating strategies.

While we provide evidence that ecological factors like visibility and home range overlap play an important role in determining how males respond to changes in female behavior, much work remains to be done. Future studies should consider forage and water use and availability across seasons as these are dynamic factors that are likely to influence both male and female behavior (Feist and McCullough, 1976; Miller, 1983; Marr, 1996; Manning and McLoughlin, 2017). Furthermore, more intensive sampling of band locations to improve home range estimates, and use of more nuanced overlap calculations would allow for a clearer interpretation of how home range size and overlap influence males' intra- and inter-band interactions (Linklater et al., 2000; King, 2002; Fieberg and Kochanny, 2005; Börger et al., 2006). It is also important to note that while the extent of home ranges is strongly dictated by facets of the physical environment like food and water distribution (Feist and McCullough, 1976; Miller, 1983; Manning and McLoughlin, 2017), it can also be influenced by social factors such as dominance status (Höjesjö et al., 2007; Murray et al., 2007). How home range size and overlap represent an interaction between the social and physical environment should be considered when using these measures to assess the effects of the surrounding environment on behavior. Comparative studies in diverse environments with varying population densities may provide more generalizable conclusions about the influences of ecological factors on male behavior under different conditions.

4.5. Management Implications

While previous work has demonstrated the impacts of PZP on treated females in feral horses, white-tailed deer, and elk, this is one of only a few studies to explore the potential consequences of immunocontraception management for non-target individuals (Ji et al., 2000; Poiani et al., 2002; Gray and Cameron, 2010; Druce et al., 2013). If behavioral changes among treated females are likely to alter the behavior of untreated animals, we may need to reevaluate how we assess the behavioral impacts of immunocontraception. Our results show that increased mare group-changing behavior leads to increases in male-male competition and vigilance. Given the increased group changing among contracepted mares (Nuñez et al., 2009; Nuñez et al., 2017), it stands to reason that PZP-treated mares could have a disproportionate effect on these male behaviors. These results are not anomalous: indirect impacts of fertility control on male behavior have been demonstrated in three western horse populations in addition to brushtail possums (*Trichosurus vulpecula*) and eastern grey kangaroos (*Macropus giganteus*) (Ji et al., 2000; Poiani et al., 2002; Ransom et al., 2010). Compared to alternatives like gathers and removals, PZP is one of the best available options for controlling feral horse populations because it is generally highly effective, reversible, safe for pregnant animals, and relatively cost effective (Kirkpatrick et al., 1996; Kirkpatrick et al., 2009). However, when possible, care should be taken to develop management strategies that consider and attempt to minimize unintended consequences. Furthermore, as new methods of controlling these populations are developed (Ransom et al., 2014; Hall et al., 2016; Collins and Kasbohm, 2017), behavioral consequences for target animals and their associates should be monitored to ensure animal welfare and effective management.

Funding

This work was supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. 1744592 (to M.M.J.). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. This work was also supported by Iowa State University (the College of Agriculture and Life Sciences and the Department of Natural Resource Ecology and Management interdepartmental funds to C.M.V.N.) and by USDA National Institute of Food and Agriculture Hatch

Multistate Funding (project IOW05509 to C.M.V.N.).

Declarations of interest: none

Acknowledgements

We thank Dr. Sue Stuska for her help in the field and for her additional data. We would also like to thank Dr. James Adelman for his indispensable statistical advice. Finally, we extend our gratitude to Rachel Schwartzbeck for her help in the field and entering data and to Micah Fatka for her assistance in the field.

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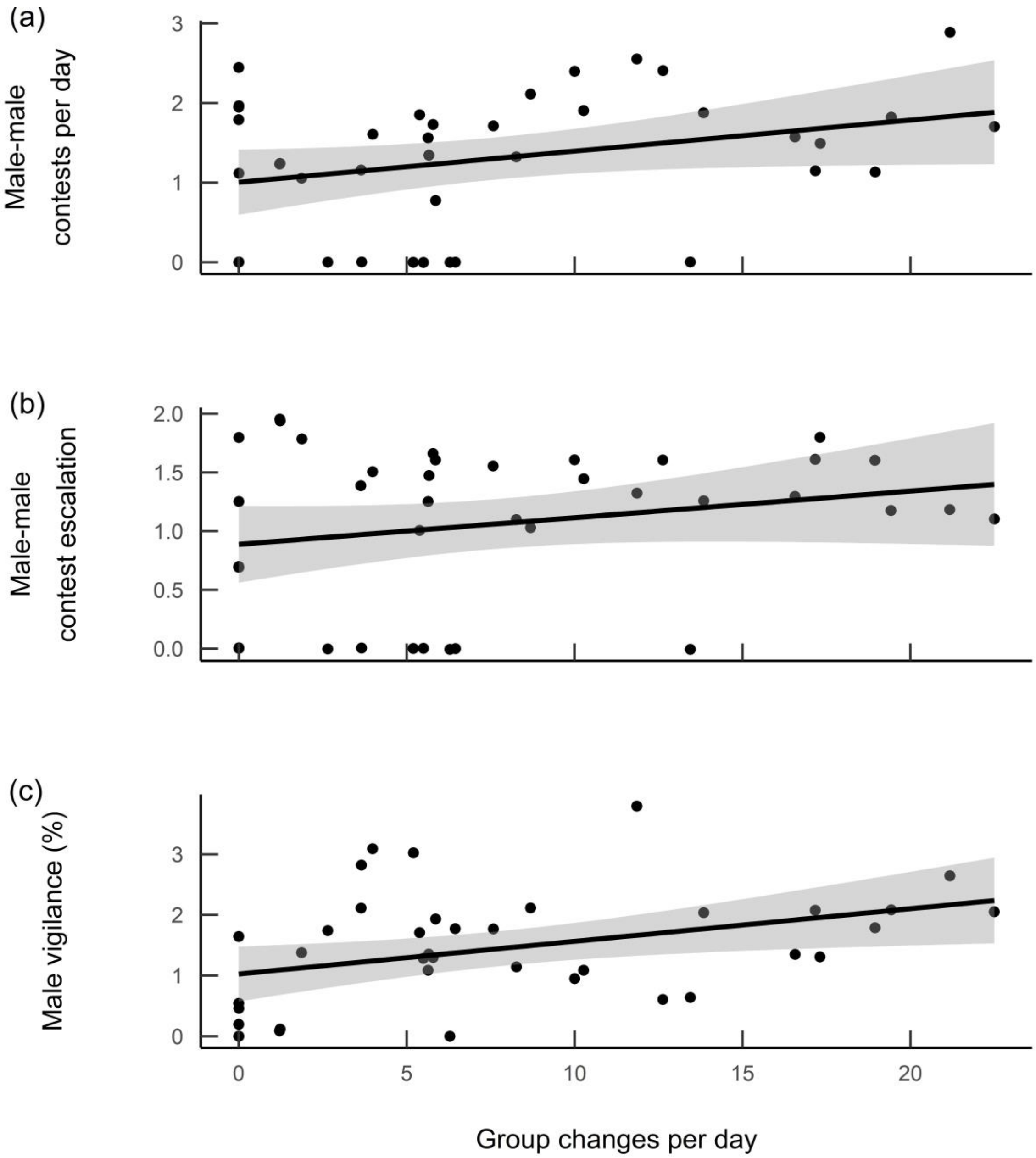
Figure captions

Fig. 1. Examples of (a) vigilant and (b) relaxed stances.


(a)
(b)

Fig. 2. The effect of the number of female group changes that males experienced per day on various male behaviors: (a) the number of male-male contests per day, (b) the average escalation of contests, and (c) the percentage of time spent vigilant (all response variables are log transformed). All response variables increased as the number of group changes increased. Shaded bands represent 95% confidence intervals. Points are jittered to allow clear visualization of all males observed.


(a)
Male-male contests per day
0
1
2
3
(b)
Male-male contest escalation
0.0
0.5
1.0
1.5
2.0
(c)
Male vigilance (%)
0
1
2
3
0
5
10
15
20
Group changes per day

Table

Table 1. Spearman rank correlations between response variables of interest and male rank.

Response variable	Correlation with male rank (ρ)	*P*
Male-male contests	-0.179	0.371
Average contest escalation	0.083	0.679
Male-female aggression	0.177	0.377
Reproductive behavior	0.180	0.370
Vigilance	0.184	0.367

Table 2. Summary of the final mixed effects models describing the effects of explanatory variables on each male behavior of interest (fixed effects only). Significant main effects are in bold.

Response variable	Explanatory variable	Estimate	SE	t	*P*
Male-male contests	Group changes per day	0.058	0.02	3.13	**0.003**
	Male age	0.076	0.02	3.33	**0.002**
Average contest escalation	Group changes per day	0.035	0.01	2.28	**0.029**
	Male age	0.050	0.02	2.65	**0.012**
Male-female aggression	Visibility	0.804	0.30	2.66	**0.013**
Reproductive behavior	Male age	-0.053	0.04	-1.47	0.156
Vigilance	Group changes per day	0.048	0.02	2.62	**0.014**
	Average female age	-0.048	0.02	-2.15	**0.039**

Table 3. Summary of the final mixed effects models describing the effects of explanatory variables on each male behavior of interest (fixed effects only) for analyses including only those males for which home ranges could be estimated (n = 17). Significant main effects are in bold.

Response variable	Explanatory variable	Estimate	SE	t	*P*
Male-male contests	Group changes per day	0.048	0.02	2.43	**0.024**
	Home range overlap	1.430	0.49	2.93	**0.008**
Average contest escalation	Home range overlap	3.505	1.20	2.93	**0.008**
Male-female aggression	Average female age	-0.545	0.22	-2.43	**0.023**
Reproductive behavior	Group changes	0.048	0.02	1.90	0.071
	# of adult females in band	0.184	0.09	1.94	0.065
Vigilance	Home range size	1.017	0.60	1.70	0.114