Rising up to the challenge of their rivals: Mare infidelity intensifies stallion response to playback of aggressive conspecific vocalizations

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Highlights

- Males were more vigilant after squeals (aggressive signals) than control playbacks
- Stallions were more likely to approach the speaker following squeals than controls
- Female turnover rate did not affect stallion responses to squeals
- Stallion responses were heightened during and after female group changes
- Altered social contexts in managed populations may affect male responses to rivals

Abstract

Management tools like immunocontraception can alter the behavior of target animals, but the extent to which they affect non-target individuals has received less attention. The feral horse (Equus caballus) population on Shackleford Banks, North Carolina is an ideal system with which
these questions may be explored, as management of the population with the immunocontraceptive agent porcine zona pellucida (PZP) has resulted in an increased propensity for females to change social groups and thus, decreased social stability. During the study, on average, females made 1.4 group changes per day (range = 0—18.5 group changes per day): females previously treated with PZP made 1.8 group changes per day, while females that had never been treated made 1.2 group changes per day. Between May and August, 2017, we used playbacks of aggressive male vocalizations (squeals) and human voices (reciting “hello horse”) to assess changes in stallion responses to male rivals versus socially irrelevant stimuli in the context of female turnover. Over the course of the study, males were observed for 9.4 hours on average (range = 2.4—20.5 hr). Males spent more time vigilant (estimate = 12.431, \( P = 0.016 \), \( \bar{x}_{\text{squeal}} = 30 \text{ s}, \bar{x}_{\text{control}} = 19 \text{ s} \)) and were more likely to approach the speaker following squeal playbacks than controls (estimate = 2.325, \( P = 0.039 \)). Males’ latency to return to normal behaviors varied depending on whether the playback was conducted in the weeks before, during, or after group changes occurred (\( P = 0.025 \), \( \bar{x}_{\text{before}} = 26 \text{ s}, \bar{x}_{\text{during}} = 39 \text{ s}, \bar{x}_{\text{after}} = 53 \text{ s} \)). Male responses were not affected by the total number of female group changes a male experienced during the observation period (\( P > 0.4 \)), suggesting the effects are more context-dependent and not long-lasting. These findings suggest mare turnover can impact stallion responsiveness to potential rivals. As previously contracepted mares change groups more often than untreated mares and stallions exhibit prolonged responses to aggressive vocalizations after experiencing a female group change, contraception-induced changes to mare behavior may lead to increased male aggression in response to intruding rivals, which could be associated with greater energy expenditure. Finally, our work demonstrates that playback experiments are a useful tool for studying feral horse behavior in the wild. As the need for population control of different species continues to expand, rigorous investigations of
immunocontraception’s effects on non-target animals are critical if agencies are to manage populations most effectively.

Keywords: *Equus caballus*, female turnover, immunocontraception, male aggression, playback, vocalization

1. Introduction

In the United States, feral horses (*Equus caballus*) are protected under federal law and are valued by much of the public for their historical and cultural associations (United States Congress, 1971). However, with few predators, feral horse populations have expanded rapidly (Garrott et al., 1991; Turner et al., 1992; Turner and Morrison, 2001; DOI-OIG, 2010), coming into conflict with human land use practices and certain native species (Beever and Brussard, 2000; Levin et al., 2002). As such, feral horses present a unique management concern. In lieu of traditional management practices like gathers and removals that tend to be costly, ineffective, and unpopular with the public, fertility control, and in particular the immunocontraceptive agent porcine zona pellucida (PZP), has become a popular option for managing feral horses and other ungulate populations (Kirkpatrick and Turner, 1991; Kirkpatrick et al., 1997; McShea et al., 1997; Heilmann et al., 1998; National Research Council, 2013).

In social species like the horse, maintenance of natural behaviors is an important consideration for managers trying to preserve the health and wellbeing of populations. Impacts of PZP on the behavior and physiology of treated mares have been explored in several 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), yet consequences of this management tool for non-target conspecifics have received considerably less attention (Ransom et al., 2010). Feral horses live in bands, social groups that remain together year-round and consist
of one or sometimes multiple stallions, one or more associated females, and their offspring (Feist and McCullough, 1976; Rubenstein, 1981). Given this complex social structure, changes in the behavior of one individual are likely to affect the behavior of its associates. The potential effects of changes in the behavior of PZP-treated mares on their associates are important to consider if managers aim to preserve more naturally functioning feral horse populations.

PZP was used to manage the feral horse population on Shackleford Banks, North Carolina between 2000 and 2009. In this population, mares treated with PZP change bands up to ten times more often than untreated mares (Nuñez et al., 2009). Regardless of contraception status, mares with a greater proportion of years pregnant are less likely to change groups (Nuñez et al., 2009), suggesting that decreases in pregnancy (and possibly lactation) may be the mechanism(s) by which PZP treatment increases mare propensity to change groups (Nuñez et al., 2009). Despite suspension of PZP administration in 2010, mares previously treated with PZP experience prolonged subfertility and still exhibit this increased group changing behavior, with previously treated mares making 1.8 group changes per day compared to untreated females making 1.2 group changes per day (Nuñez et al., 2017; Jones and Nuñez, 2019). Before PZP treatment began on Shackleford, only 10.8% of mares in the population changed groups (Rubenstein, 1981). While the immunocontraception program was active, this number increased to approximately 69.5%, and in 2017, after the suspension of treatment, group changing behavior remained more frequent with 34.8% of females changing groups throughout the breeding season (Madosky, 2011; Jones and Nuñez, unpublished data). These changes to female behavior have decreased the typically high degree of social stability that characterizes feral horse bands (Klingel, 1975; Madosky, 2011). Historically, contests between intruding and resident stallions were won by the resident; furthermore, in fights to regain mares separated from the band, the females were usually recovered.
(Feist and McCullough, 1976; Rubenstein, 1981). However, mare retention is now destabilized (Nunez et al., 2017), increasing male-male competition in this population (Jones and Nuñez, 2019). Such changes in male competition are not unique to feral horses: similar decreases in social stability have been associated with increases in male aggression in other species including ring-tailed lemurs (*Lemur catta*) and gray-cheeked mangabeys (*Lophocebus albigena*) (Cavigelli and Pereira, 2000; Arlet et al., 2009).

In feral horses, vocalizations are commonly used in a number of social contexts including mating, aggression, and the formation of social bonds, with different types of vocalizations containing varying social information (Feist and McCullough, 1976; Proops et al., 2009; Yeon, 2012). Specifically, squeals are often used during contests between rival males and indicate dominance status, with more dominant stallions emitting longer and more forceful squeals than subordinates (Rubenstein and Hack, 1992). Most male-male contests are characterized by a typical sequence of behaviors, beginning with approaches, olfactory assessment, and acoustic signaling (Feist and McCullough, 1976; Miller, 1981; Hynes, 1998). If contests become escalated, these assessments are followed by physical contact ranging from pushing to kicking and biting (Feist and McCullough, 1976; Rubenstein and Hack, 1992). As squeal vocalizations are a clear indicator of male-male conflict, they may be used to experimentally assess how males respond to signals from intruding males, including whether or not they choose to engage rivals and how escalated the response becomes.

Here, we use experimental playbacks of squeals to determine how stallions with varying levels of experience with female group changing behavior respond to these aggressive signals. To our knowledge, ours is the first experiment to assess the effects of female turnover on male competition in a natural setting. We predicted that all stallions would respond more aggressively
toward squeal playbacks than unrelated control stimuli (human voices), regardless of experience with female turnover. We also hypothesized that stallions experiencing more female group changes would exhibit heightened responses to squeal playbacks. We expected these males to approach the speaker more often and to exhibit decreased latency to respond, prolonged vigilance, and an increased latency to return to their normal behaviors when compared to stallions experiencing fewer group changes. This experiment provides a more complete understanding of the influence of female behavior on male competition. Furthermore, it provides insights into how playback experiments can be implemented as useful tools to assess feral horse behavior. Because mares previously treated with PZP demonstrate prolonged decreases in fidelity (Nuñez et al., 2017; Jones and Nuñez, 2019), investigating how increased female turnover affects male behavior will improve our understanding of the potential consequences of immunocontraception management for non-target animals.

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 the breeding season from May through August, 2017. We observed all band stallions and their females: 20 males and 69 females organized into 19 bands. Most bands had one dominant stallion with the exception of one double-male band. Individuals were identified by their sex, color, facial markings, and, in some cases, freeze brands. We conducted all observations from at least 15-20 meters away (National Park Service, 2004) to ensure the horses were not disturbed
by our presence. All observations were conducted by eye or with the aid of binoculars (Nikon Monarch M511 10x42) when necessary.

This study was approved by the Iowa State University Institutional Care and Use Committee (3-17-8456-E) and the National Park Service Institutional Care and Use Committee (1024-0265). The National Park Service granted permission to collect all observational data (CALO-2017-SCI-0006).

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 managing the population via immunocontraception in 2000. Designated mares received initial and booster doses of PZP, which were administered annually from February through April. Injections contained 100 micrograms 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 noted 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. Stimuli acquisition

Squeals made by stallions during male-male contests in a population of Misaki feral horses in Japan were recorded by L. Proops. Squeals tend to vary in duration with significant acoustic noise and medium to high amplitudes and tonality (Figure 1), differing in their acoustic properties from other vocalizations like whinnies and nickers (Yeon, 2012). Although stallions generally recognize familiar rivals using olfactory rather than auditory cues (Rubenstein and Hack, 1992),
we used recordings of males from another population to avoid any question of potential confounds related to individual recognition by Shackleford stallions. Stallions are capable of discriminating between dominant and submissive males on the basis of acoustic cues in squeals (Rubenstein and Hack, 1992). To help control for the potential effects of rank on stallion behavior, we used vocalizations from band-holding (relatively dominant) Misaki males exclusively. Recordings were collected using a Sennheisser ME66/K6 directional microphone attached to a Marantz PMD661 audio recorder at a sampling frequency of 44.1kHz and bit depth of 32 at distances between 5 and 30 m from the subjects. Seven high-quality recordings were chosen and used as auditory stimuli. Selected squeals ranged in duration from 0.6 s to 2.0 s with an average duration of 1.0 s.

Control recordings were made at Iowa State University. Seven adult males ranging in age from 22 to 68 years (mean = 41 years) were recorded reciting the phrase “hello horse” using an AKG D790 microphone and a Focusrite Scarlett 18i8 preamp connected to a Dell Latitude E7470 PC. Recordings were captured using Audacity 2.1.3 (Audacity Team 2017) at a sampling frequency of 44.1kHz and bit depth of 24. All squeal and control recordings were normalized to 75 dB in Praat (64-bit edition) (Boersma and Weenink, 2017) and were standardized to play at the peak sound pressure of 98 dB at 1 m from the source (Proops et al., 2009).

2.4. Playback procedure

All playback trials were conducted by a single observer (M. M. Jones) between June and August 2017. Vocalizations were played to all band stallions throughout the study, with each stallion receiving one squeal and one control playback, randomized in order with at least one day between trials. In the case of the double-male band, we presented the same squeal and control sounds to both males only once and recorded each male’s response separately.

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). The East, Mideast, and West were treated as blocks to account for the effect of region on stallion responses. Within each region, stallions were randomly assigned to different pairs of control and squeal exemplars.

Vocalizations were played on an Apple iPod Nano (2nd generation) connected to a small portable speaker (MIPRO MA-101C Personal PA System) hidden in vegetation approximately 20 m from the stallion. For each recorded exemplar, two minutes of silence were added before the vocalization to allow the observer time to move away from the speaker and out of sight of the stallion so as not to interfere with the subject’s response. After the two minutes of silence, the vocalization played twice with ten seconds of silence between iterations (Proops et al., 2009). To capture the stallion’s response, we conducted 15 minute focal samples (Altmann, 1974) starting immediately after the first vocalization played and recorded the following: whether stallions responded in any way to the stimulus, the latency to respond, whether stallions approached the speaker, length of vigilance behavior (characterized by ears erect and directed forward, head and neck held high, and eyes alert (Feist and McCullough, 1976)), and the latency to return to normal behaviors (measured as the time between the initiation of any response and the return to normal behaviors (e.g. grazing, standing)). A total of 40 trials were conducted among six horses in the East, eight in the Mideast, and six in the West receiving one squeal and one control vocalization. All playbacks were conducted when the stallion of interest was out of sight of other bands and when the weather was dry and winds were relatively calm.

2.5. Female group changing data

Study bands were located twice per week from late May to early August, and GPS location and group composition were noted. 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). The number and rate of group changes experienced by all males is detailed in Table 1. For most males that experienced group changes (100% in 2016; 92% in 2017), at least one of the females that left their band returned for some period of time.

2.6. Assessing visibility on Shackleford Banks

Habitat visibility can affect a stallion’s ability to protect his band from intruders because increased visibility allows males to defend territory boundaries and track band member activity more easily (Rubenstein 1981). Therefore, we included visibility as a potentially important environmental factor that could influence male responses to rival stallions. We quantitatively assessed visibility across the island by conducting a 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 take both bare earth elevation and vegetation structure into account (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 (the average height of Shackleford horses) was added to each observation point to account for the height at which a horse would be able to see across the landscape at eye level (Stuska, personal communication). The Visibility tool in ArcMap was then used to predict the number of 10 m x 10 m cells visible from each point, and the number of visible cells was averaged across all points for each stallion and converted to km² to produce an average visibility score.

2.7. Statistical analysis

We used generalized linear mixed-effects models with the lmerTest package (Kuznetsova et al., 2017) in R (version 3.4.1) to examine how female group changing behavior influenced male responses to playbacks (R Development Core Team, 2017). Playback type (squeal or control),
female turnover rate, and the timing of playback trials with regard to group changes were used as explanatory variables. Female turnover rate was calculated as the total number of group changes each male experienced divided by the total time that male was surveyed (expressed as observation days, which were defined as 12 hour periods (the approximate length of daylight during our study period)). Playback timing was determined by classifying each trial as occurring in the one to two weeks before the stallion experienced a female group change (weeks 1-2), during the week of and the week following a group change (weeks 3-4), or in the five weeks following a group change (weeks 5-10) (Figure 2). Interactions between playback type and female turnover rate as well as playback type and playback timing were included to assess whether experience with female group changes altered male responses to all stimuli or only relevant cues from potential rivals.

All response variables were analyzed independently due to small sample size. Binary response variables (whether stallions responded to the stimulus and whether they approached the speaker) were analyzed using models with binomial distributions, while models with normal distributions were used for continuous variables (response latency, length of vigilance behavior, and latency to normal).

The models also included male age, average female age (within bands), and the average number of adult females present as fixed effects as these factors are also likely to affect male behavior (Berger, 1977; Vervaecke et al., 2007; Nuñez et al., 2017). Visibility was included in the models to determine whether habitat openness affects how males respond to the stimuli. All models included stallion identity as a random effect to control for pseudo-replication. Best fit models were simplified using backwards elimination, removing terms with $P$-values $> 0.1$. In the final models, predictor variables were considered significant if $P \leq 0.05$. For these generalized linear mixed-effects models, parameter estimates, standard errors (SE), t- or z-values, and $P$-values are
3. Results

Males were more likely to approach the speaker (Figure 3; estimate = 2.325, SE = 1.13, \( z = 2.06, P = 0.039 \)) and spent more time vigilant (Figure 4; estimate = 12.431, SE = 4.56, \( t_{13} = 2.73, P = 0.016 \)) following squeal playbacks than control trials. The probability of showing any response and the response latency did not differ based on the type of playback trial (all \( P \)-values > 0.2).

Main effects of the number of group changes males experienced per day and interactions between this variable and playback type were dropped from all final models (all \( P \)-values > 0.4), indicating that the rate of female turnover did not affect male responses to either stimulus.

However, there was a significant interaction between playback type and playback timing with regard to female group changes (before, during, or after a male experienced a group change) in the final model for latency to return to normal behaviors (Figure 5; \( P = 0.025 \)). Tukey post-hoc pairwise tests revealed that males took significantly longer to return to normal following squeal trials after a group change (estimate = -53.380, SE = 16.77, \( t_{25} = -3.18, P = 0.040 \)), and there was a strong trend showing the same pattern for trials conducted during a group change (estimate = -23.232, SE = 7.68, \( t_{19} = -3.03, P = 0.065 \)). However, there was no difference in males’ latency to return to normal between squeal and control trials conducted before a group change (estimate = 0.738, SE = 9.44, \( t_{20} = 0.08, P > 0.9 \)).

With regard to the additional covariates, the average visibility score across all samples was 0.73 km\(^2\) (range = 0.06—3.97 km\(^2\)); however, visibility had no effect on male responses. The number of adult females was the only factor retained in the final model predicting the probability of any response. While the effect was not significant, there was a trend by which males with more females in their bands were more likely to respond to all playbacks (estimate = 1.118, SE = 0.62, \( Z = 1.80, P = 0.072 \)). Additionally, average female age and male age were retained in the final
model predicting response latency. Males with younger females in their bands took longer to respond to playbacks than males with older females (estimate = -0.185, SE = 0.09, \( t_{15} = -2.15 \), \( P = 0.048 \)). Finally, older males tended to take longer to respond than younger males (estimate = 0.035, SE = 0.01, \( t_{9} = 2.13 \), \( P = 0.062 \)).

4. Discussion

Here we show that males exhibit heightened responses to squeal playbacks compared to controls, and while the overall rate of female turnover does not affect those responses, responses to squeals are stronger during periods of group instability. These context-dependent responses suggest that female group changing events can influence how males respond to rivals. On Shackleford Banks, females previously treated with PZP change bands more often than untreated females, interfering with the population’s social stability (Nuñez et al., 2009; Madosky, 2011). This playback experiment provides a more controlled assessment of how this altered social context affects stallion response to rivals than can observation alone. While the impacts of fertility control on the behavior of treated animals have been studied in a variety of species and populations (Hayes et al., 1996; McShea et al., 1997; Heilmann et al., 1998; Powell, 1999; Ramsey, 2007; Nuñez et al., 2009; Ransom et al., 2010; Madosky, 2011), its consequences for non-target individuals have received considerably less attention (Ji et al., 2000; Poiani et al., 2002; Gray and Cameron, 2010; Ransom et al., 2010; Druce et al., 2013). Here we provide evidence that immunocontraception management can have indirect consequences for non-target males.

Among the response variables measured, males were more likely to approach the speaker and spent more time vigilant following squeal playbacks than control stimuli (Figure 3, Figure 4). Rubenstein and Hack (1992) found similar responses in stallion vigilance and likelihood of approach in their playback experiment investigating agonistic encounters among stallions. However, contrary to our expectations, the likelihood of any type of response and response latency
did not differ between squeal and control trials. The fact that playback type did not affect response likelihood may be attributed to a ceiling effect. Stallions responded in 80% of playback trials, suggesting that this measure may not be useful when examining response variation and that differences between playback types are more likely reflected in specific behaviors exhibited during responses. As horses are prey animals that respond to novel auditory stimuli as well as signals from conspecifics, it is important to consider which specific behaviors are relevant to the social context of interest when conducting playback experiments (Christensen et al., 2005). Vigilance and approaches are commonly demonstrated during male-male conflicts or when an intrusion by a rival is suspected, indicating that our playbacks served their intended purpose; males did engage, at least to some extent, in more typical contest behaviors following squeals than following control playbacks (Feist and McCullough, 1976; Miller, 1981; Hynes, 1998).

The rate of female group changes experienced by males did not explain variation in any of the measured response variables following squeal or control playbacks. Similarly, the likelihood of response, response latency, vigilance, and likelihood of approach did not differ depending on whether the trial was conducted before, during, or after a group change by a female. These findings contradict our prediction that males experiencing more group changes would exhibit more heightened responses to squeal playbacks than those experiencing fewer group changes. Low variation in male behavior may have been due to strong stabilizing selection on stallions’ responses to potential threats in the environment. More responsive males are likely better able to monopolize and protect their females and offspring from rivals, thus increasing their own reproductive success, than are less responsive males (Rubenstein 1981, Asa 1999). It may be then that in feral horses, males respond strongly to potential threats, regardless of the social circumstances.

Alternatively, limitations of the experiment may have contributed to the insignificant
findings. This study was conducted over the course of a single season with each male receiving only one squeal and one control playback. In addition, we could not control for the behavioral context at the time of testing; therefore, unknown external factors could have affected stallions’ responses (Tyack, 2009). Furthermore, we presented only auditory stimuli in this experiment, while a typical encounter between rival males would also include visual and olfactory stimuli that play a significant role in determining responses (Feist and McCullough, 1976; Miller, 1981; Rubenstein and Hack, 1992). The limitation of the auditory stimuli used here likely restrained stallions’ responses, potentially masking variation in the most aggressive behaviors that stallions would exhibit in response to visible intruders.

Moreover, our response measurements may not have been precise enough to capture subtle variations in stallions’ responses to squeals. Transcribing responses as they occurred restricted our ability to note nuances such as laterality, subtle changes in ear positions, and nostril lifts, all of which have been noted as relevant responses to conspecifics in domestic horses (Austin and Rogers, 2014; Wathan et al., 2015; Smith et al., 2016; Wathan et al., 2016). In future playback studies, video recordings that allow for more nuanced measurements of responses may be an important tool to reveal variation missed in the measurements reported here (McComb et al., 2000; Proops et al., 2009; Austin and Rogers, 2014).

It is of interest that the short-term context of female group changes did influence males’ latency to return to normal after playbacks. Males took longer to return to normal behaviors following squeals than controls but only when the playback was conducted during or after a group change by a female (Figure 5). This indicates that at least some responses are context-dependent, with males exhibiting prolonged responses to signals from perceived rivals compared to random stimuli in periods of social instability. Similar context-dependent changes in male behavior during
periods of social instability have been observed in other systems. For instance, male ring-tailed lemurs were more aggressive toward rival males throughout the relatively unstable mating period (during female estrus) than they were during the more stable premating period (Cavigelli and Pereira, 2000). As in our study, this short-term period of social instability altered male-male interactions during that specific time-frame only. Because previously contracepted females change groups more often than other females (Nuñez et al., 2017; Jones and Nuñez, 2019), males associated with such females are likely to exhibit this increased responsiveness more often than other males. Admittedly, the sample size for trials conducted following a group change is low in our study (n = 5); as such these results should be interpreted cautiously. Additional playback trials would help to more clearly assess such patterns in stallions’ latency to return to normal as well as other responses. Further, more nuanced exploration of these responses, perhaps in a more controlled setting, could reveal additional insights into the consequences of immunocontraception management for non-target animals.

More generally, our results support the use of auditory playbacks to experimentally investigate behavior in free-living populations (Reby et al., 2005; Fischer et al., 2013). Our data reinforce the importance of carefully considering the context of both stimuli and response measurements to ensure playback designs truly focus on behaviors relevant to the question of interest (Sayigh et al., 1999; McGregor, 2000). While playback experiments have been used extensively to assess domestic horse behavior (Lemasson et al., 2009; Proops et al., 2009; Lemasson et al., 2015; Smith et al., 2016; Wathan et al., 2016; Briefer et al., 2017), we posit that they are also a useful tool for feral horse behavior. They allow researchers to more precisely pinpoint causative relationships than do interpretations of correlative data and may be especially valuable when the behaviors of interest are rarely observed in the wild (Rubenstein and Hack,
1992; McComb et al., unpublished data).

Declarations of interest
None.

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Figure 1. Spectrogram of a squeal produced by a dominant Misaki stallion during an antagonistic encounter with another stallion.
**Figure 2.** Timeline for playback trials evaluating stallion responses to control and squeal sounds on Shackleford Banks, North Carolina from May-August 2017.

**Figure 3.** Stallion approaches toward the speaker for squeal and control playbacks on Shackleford Banks, North Carolina from May-August 2017. Black bars represent males that did approach the speaker; grey bars represent those that did not approach. Males were more likely to approach the speaker during squeal trials than controls.
Figure 4. The amount of time (seconds) stallions spent vigilant following control and squeal playbacks on Shackleford Banks, North Carolina from May-August 2017. Males spent significantly more time vigilant following squeal stimuli than control stimuli. Segmented lines indicate means for each group.
**Figure 5.** The length of time (seconds) between stallions’ initial response and their return to normal behaviors following squeal (black) and control (grey) playback trials conducted before, during, or after a mare entered or left their band on Shackleford Banks, North Carolina from May-August 2017. Males took longer to return to normal after squeals compared to control trials during and after experiencing a group change. Segmented lines indicate means for each group.
Table 1. The number and rate of female group changes experienced by all males observed in the playback experiment over the course of the 2017 breeding season on Shackleford Banks, North Carolina. Rates were calculated by dividing the number of group changes observed by the amount of time a given male was watched.

<table>
<thead>
<tr>
<th>Stallion</th>
<th>Number of female group changes experienced</th>
<th>Rate of group changes experienced (changes/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atari</td>
<td>5</td>
<td>17.3</td>
</tr>
<tr>
<td>Bilbo</td>
<td>16</td>
<td>17.2</td>
</tr>
<tr>
<td>Dale</td>
<td>1</td>
<td>1.2</td>
</tr>
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