

Examining the costs and benefits of male-male associations in a group-living equid

Pandora Pinto ^a, Renata S. Mendonça ^{a,b}, Satoshi Hirata ^a

^a Wildlife Research Center, Kyoto University, Kyoto, Japan

^b Centre for Functional Ecology – Science for People & the Planet, Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Keywords:

Male competition Stallion behaviour
Alternative mating strategies Equids
Cooperation

Abstract

In feral horse (*Equus caballus*) populations, females can associate in year-round social groups with one male (single-stallion (SS) groups) or multiple males (multi-stallion (MS) groups). Given the expectation of intense male competition for mating opportunities, male-male associations raise questions regarding their supporting mechanisms and adaptive consequences. The function of MS groups has been highly debated which led to a series of conflicting hypotheses attempting to describe the relationship between the males, and the costs and benefits of this association in relation to SS groups. Based on previous studies, our goal was to compare the group size and stability, males' defence behaviour against group outsiders and access to females, levels of male aggression to group members, and the activity budget and body condition of horses in MS and SS groups. Between the months of May to August (breeding season) from 2016 to 2021, we collected demographic and behavioural data from feral Garrano horses living in northern Portugal, where wolf (*Canis lupus signatus*) predation is an important factor. Contrary to mutualistic hypotheses, our results indicated that SS males had more breeding females than MS groups and group type did not influence female membership stability. However, dominant males in MS groups may be benefitting from the presence of another male via decreased effort in group defence and more resting time. We recorded less aggressive behaviours from dominant to subordinate males in MS groups when subordinates displayed more defensive behaviours against outsiders, but we found little evidence that subordinates were being rewarded with access to females, as proposed by reciprocal-altruism hypotheses. Females in MS groups did not suffer higher levels of male aggression when compared with SS females, and the number of males in the group had no significant influence on the time females spent grazing and resting, and ultimately on their body condition. Future research should explore how MS male associations shape male-female relationships, which could provide further insights to the existence and maintenance of subordinate males. This study is the first to report on predation impact by wolves in MS and SS groups, highlighting the need to further explore how different ecological pressures shape horse behaviour and social organization. Furthermore, our results provide information that can help improve the welfare of domestic horses and can make informed management decisions of feral and free-ranging horse populations, particularly in instances where males are targeted for removals. Considering the advantages, as well as costs, provided by extra males may be useful to managers and horse owners, who can then act accordingly and with the least disturbance to horse populations.

Introduction

Among equids, there are two types of social structure, each with its own type of bond stability between individuals; resulting from different ecological factors that influence resource availability and, consequently, females' distribution (Boyd et al., 2016). Species adapted to arid habitats exhibit resource defence polygyny and no permanent or long-lasting bonds between individuals, known as the type II system, and include Grevy's zebra (*Equus grevyi*), African wild ass (*E. africanus*), Asiatic wild ass (*E. hemionus*), and the kiang (*E. kiang*). Conversely, horses (*E. caballus*), as well as plains zebra (*E. quagga*), mountain zebra (*E. zebra*), and Przewalski's horses (*E. przewalskii*), are adapted to mesic habitats and display female defence polygyny with long-term, stable social groups – the type I system. However, one study hypothesizes that resource distribution may not be the only contributor to equids' social organization and that predation pressure may be an important factor as well (Feh et al., 1994).

Horses' main social unit, sometimes called a harem or band, is composed of at least one adult male (stallion), several unrelated females (mares) and their immature offspring (Boyd et al., 2016). Both sexes disperse from their natal group when they reach sexual maturity to avoid inbreeding (Linklater and Cameron, 2009; Monard and Duncan, 1996). Young females generally join another group soon after dispersal, whereas males frequently join all-male bachelor groups until they can acquire females (Boyd and Keiper, 2005). In different feral horse populations, multi-stallion (MS) groups, reported to range from two-to-four adult males in the same group (this study), to four (Linklater et al., 2000), or up to eight (Linnartz and Linnartz-Nieuwdorp, 2017), share overlapping home ranges with single-stallion (SS) groups. A strong hierarchy can be observed between the males in MS groups, where one male is dominant over the other(s) (Feh, 1999; Miller, 1981). These other males, known as subordinates, are integral adult members of the group, unrelated to the dominant, that may help with defence and acquire some paternity in the group, although less than the dominant (Boyd and Keiper, 2005; Feh, 1999). Nonetheless, considering that males compete for females, and a male's reproductive success is largely dependent on mating opportunities, we expect SS groups to be the predominant form of social organization.

Fierce competition amongst males for mating opportunities is at the centre of many theories for mating systems, sexual selection, and development of various phenotypic traits (Arak, 1983; Díaz-Munoz et al., 2014; McElligott et al., 2001). While working under this assumption, we may overlook the possibility that males could benefit from associating with other males, for example, as an anti-predator strategy (capuchin monkeys, *Cebus* sp.: van Schaik and van Noordwijk, 1989; cercopithecoid primates: Hill and Lee, 1998), during inter-group conflict (red-fronted brown lemurs, *Eulemur rufifrons*: Port et al., 2010), for resource access and defence (tufted capuchin monkeys, *Sapajus nigritus*: Scarry, 2013), or even to reproduce by working together to attract, gain, or maintain access to females (lions, *Panthera leo*: Bygott et al., 1979; bottlenose dolphins, *Tursiops* sp.: Connor et al., 1992; hamadryas baboons, *Papio hamadryas*: Chowdhury et al., 2015; chimpanzees, *Pan troglodytes*: Duffy et al., 2007). Provided that these advantages outweigh the potential loss of fitness for some members in association, selection may favour increased tolerance and cooperation among males.

In horses, the relationship between males of the same group has been hypothesized to be the result of alternative cooperative mating strategies, based on by-product mutualism (Feh, 1999; Miller, 1981; Stevens, 1990) – cooperation is a by-product of following the best strategy; or

reciprocal altruism (Feh, 1999) – the dominant male behaves altruistically, which implies a cost to him, and his reward, costly to the subordinate, is delayed in time. The larger average size and greater stability found in MS groups compared with SS ones have been attributed to the presence of subordinate males (Miller, 1981; Stevens, 1990). As a result, where group size positively correlates with group dominance, membership in an MS group was thought to ensure better access to limited resources and higher quality habitat (Miller, 1981). Potentially, it could also improve the reproductive success of its males: greater size and stability of the female group could mean greater access to breeding females during a dominant's lifetime compared with SS groups, while the subordinate male stood a greater chance of inheriting the group (Miller, 1981; Stevens, 1990). Feh (1999) showed that males in alliances sired fewer foals than males from SS groups, but subordinate males had higher relative reproductive success than bachelor males that adopted a “sneak” strategy. While having a subordinate incurred a fitness cost to the dominant male, in turn, he benefited from the subordinate's assistance during inter-group conflicts and increased time for grazing (Feh, 1999; Rubenstein, 1982). The relationship between the males is also attributed to reciprocal altruism, where the dominant male presumably “tolerates” the presence of the subordinate because of his help in group defence and rewards him with access to mares (Feh, 1999). It is also suggested that most MS groups are only temporary associations that serve as an alternative route for younger males to gain experience and to begin breeding early (but at the cost of lower reproductive success) before becoming the sole leaders of a group (Rubenstein, 1982; Rubenstein and Nunˆez, 2009).

Conversely, some studies show that the structure, composition, range-use, and behaviour of males in MS groups do not meet the predictions of cooperative hypotheses (Linklater and Cameron, 2000; Linklater et al., 2013). Furthermore, Linklater et al. (1999) showed that females in MS groups had lower body condition and fecundity due to greater harassment by the resident males. MS groups may simply be a by-product of strong selection pressure for stable, long-term male–female relationships, and thus the result of multiple males forming a “consort relationship” with the same female and male–male dominance behaviour during group formation (“the consort hypothesis”, Linklater et al., 1999). Additionally, more recent comparisons drawn between the social system of polygynous primates and feral horses did not explain the presence of multiple males (Ringhofer et al., 2017). Pandora and Hirata (2020) hypothesized that large body size was a favourable trait in stallion competition, and that MS groups were the result of smaller males banding together to improve their reproductive prospects, but found no differences in body length and height between males of different group types. Therefore, the proximate mechanism that leads to the formation of MS groups and their ultimate significance in horse societies remains largely unclear.

An understanding of males' natural behaviour, how they associate with conspecifics and the impact of these associations may contribute to the welfare of domestic horses. For example, it can help raise awareness to the importance of keeping horses in a species-appropriate manner and provide guidelines to identify and resolve causes of maladaptive behaviour (Briefer Freymond et al., 2013; Christensen et al., 2002; Hartmann et al., 2012). This may be more pressing in the case of males since, traditionally, they are often kept in isolated confinement which negatively affects their wellbeing (Christensen et al., 2002; Gehlen et al., 2021). Likewise, it can help make informed management decisions of feral and free-ranging horse populations, particularly in instances where males are targeted for removals, as is observed in some traditional husbandry systems (Lagos 2013). Information regarding the origin mechanisms of MS groups and advantages provided by extra males (such as defence against outsiders or predators), as well as costs, may be useful to managers and horse owners, who can then act accordingly and with the least disturbance to horse populations.

In the past, the function of multi-male groups in feral horses was highly debated which led to a series of conflicting hypotheses attempting to describe the relationship between dominant and subordinate adult males, and the costs and benefits of this association in relation to one-male groups. Using demographic and behavioural data, our goal was to test on the feral Garrano horse population living in Serra d'Arga, northern Portugal, what has been found and predicted in past studies. Thus, we intended to assess, based on by-product mutualism, (1) if MS groups have more females and are more stable than SS groups; and (2) if the subordinate male in MS groups contributes to group defence against outsiders, which benefits the dominant via reduced effort in female protection and increased time for grazing. Based on reciprocal altruism, we examined (3) if subordinate's help in group defence is rewarded with lower aggression from the dominant and access to females. Finally, in accordance with the consort hypothesis, we tested (4) if there are higher rates of male-to-female aggression in MS groups, which would compromise the activity budget and body condition of females. Additionally, due to the presence of natural predators in the study site, we examine if the number of males in a harem provides group advantages (i. e., loss of fewer members) in relation to predation pressure, which to our knowledge has not been addressed before.

Methods

Study site and population

Serra d'Arga in northern Portugal (41°48'22.0"N 8°42'49.0"W) is a mountainous region 825 m above sea level, comprising 4493 ha and characterized by a temperate Mediterranean climate with Atlantic influence. The high-altitude plateau features include Atlantic wet and dry heath, low-altitude hay meadows, granite outcrops, and sparse tree coverage, while the lower landscape consists mainly of agricultural fields, and maritime pine (*Pinus pinaster*) and eucalyptus forests (*Eucalyptus globulus*). During the spring and summer months, Garrano horses, an endemic breed of Portugal and Galicia, gather on the high-altitude plateau. At the end of summer, as the vegetation dries up, the horse groups spread out and come down the mountain slopes, closer to the forested areas. A small part of the feral horse population in Serra d'Arga is privately owned by livestock farmers, but the horses are free to form social groups, breed, and roam the whole mountain region year-round. Owners do not actively care for the horses, nor follow the traditional husbandry system observed in other populations in Galicia and Northern Portugal (Lagos, 2013). Removal of individuals from the population, particularly males, occurs arbitrarily and often at the end of breeding season (May–July). Furthermore, the Garranos are under intense predation pressure by the Iberian wolf (*Canis lupus signatus*) and since 2014 a wolf pack has been reproducing regularly in Serra d'Arga (Freitas, 2019; Nakamura et al., 2021).

Field research has been conducted in Serra d'Arga since 2016 focusing on the organization, and social and spatial behaviour of the horses (Inoue et al., 2019, 2020; Maeda et al., 2021a, 2021b; Mendonça et al., 2020, 2021; Pinto and Hirata, 2020; Ringhofer et al., 2017, 2020). From 2016–2021, more than 200 individuals in 35 groups have been identified based on their phenotypic characteristics, including SS, MS and bachelor groups. The population's sex ratio (> 1 yr. old) in 2016 was 2.38 females per male (114:48), in 25 harem groups (6 MS with 2–4 males, 19 SS) and one bachelor group (nine males), and an average foaling rate of 0.44 foals (n foals=47) per breeding female. We defined breeding females as adult females (≥ 4 yr. old), young females (2–4 yr. old) that have dispersed or joined the group, and young females that didn't disperse but have produced offspring while in the natal group. In 2017 the sex ratio was 2.11 females per male (118:56), in 30 harem groups (11 MS with 2 males each, 19 SS) and two bachelor groups plus solitary males (nine males in total), with an average foaling rate of 0.40 foals (n foals=41) per breeding female. In 2018 there were 2.36 females per male (104:44), in 25 harem groups (6 MS with 2 males, 19 SS) and two bachelor groups plus solitary males (12 males in total), with an average foaling rate of 0.32 foals (n foals=30) per breeding female. The horse population suffered a sharp decline between August 2018 and May 2019. The fate of individuals that disappeared from the population is likely connected to wolf predation or illegal human captures resulting in horses being removed from the study area. Consequently, in 2019 there were 2.85 females per male (37:13), in 11 harem groups (2 MS with 2 males, 9 SS) and no remaining bachelor males, with a foaling rate of 0.14 foals per breeding female (n foals=5). In 2020

the sex ratio was 2.90 females per males (29:10) in eight harems (2 MS with 2 males, 6 SS) and an average foaling rate of 0.28 foals (n foals=8) per breeding female. Prior to the start of the 2021 field season, a new SS group composed of individual adult horses previously removed from the mountain (5 adult females, 1 adult male, 2 juveniles (=1 yr. old) and 2 foals) was introduced in the population by a horse owner, while another SS group broke apart because the male disappeared. Thus, the sex ratio of the population > 1 yr. old in the 2021 field season was 3.27 females per male (36:11) in 7 harems (2 MS, 5 SS) and an average foaling rate of 0.55 foals (n foals=17) per breeding female. Foal mortality in the area is high and may be mostly attributed to predation (Supplementary material Fig. 1). Of the 47 foals born in 2016, 11 were still present in the following year. Between 2017 and 2019, only one foal born in 2018 lived past the first year and reached adulthood. Of the eight foals born in 2020, one disappeared within the first two weeks of life, and another disappeared between July 2020 and April 2021.

Data collection

Focal social groups and study period

We used the population's demography data over a six-year period (2016–2021) to analyse group size, stability and foaling rate of MS and SS groups. For behaviour analysis, we focused on a total of 14 SS groups and four MS groups (with two males), and the data collection period varied between the months of May and August (comprising the breeding and birthing season) from 2018 to 2020, amounting to a total of eight months of behavioural data (Table 1). Since the start of field studies in Arga, seven out of a total of nine MS groups changed into SS groups due to the disappearance, removal or leaving of one or more of the males in the harem. This was the case for three of the 14 SS groups we followed, as prior to the start of behavioural observations in 2018 they had more than one male (Table 1). Nijo, from the Hakata and Nijo group, was injured before we stopped seeing him on the mountain. In the other two groups, the males either left to form their own group or became bachelors. Because only one stallion remained in each group at the time of behavioural recording, we treated these three groups as SS.

From May to August 2018, we followed seven SS groups and four MS groups, with two males each. Horse numbers sharply decreased by the end of the field season in August 2018, because of predation pressure and/or illegal human interference, and only a third of the population was present on the high-altitude plateau by the spring of 2019. Of the groups we had previously followed, all seven males of the SS groups and three males of MS groups, plus most of their females, disappeared (Table 1). Consequently, in 2019 some new groups were formed and changes in membership were observed. From May to July 2019, we followed seven new SS groups and the two remaining MS groups. Another two SS groups disappeared from the mountain between August 2019 and May 2020, with at least one horse predated by wolves but mostly due to arbitrary human captures. Thus, in July 2020 our focal groups were the five remaining SS groups and the same two MS groups. While predation in this population was a considerable factor, it was mainly the significant human mismanagement that began in 2018 that affected group formation and composition. Consequently, since the start of behavioural studies, only three groups (2 MS, 1 SS) of the 18 we followed remained largely unaffected and were still present in 2020 (Table 1).

Population structure, group size and stability

We assessed the group size, structure and stability of SS and MS groups present in the population each field season (May-June 2016, May-July 2017, May-August 2018, May-July for 2019 and 2020, and June-July 2021). Harem groups were categorized as MS or SS, depending on the number of adult males in the group per field season. Groups were named after their stallion(s). The number of males, breeding females, young and foals in the groups were recorded during each field season. Female membership stability of each group was measured in three ways, based on Linklater et al. (1999): S1 – number of join events between consecutive field seasons (2016–2017, 2017–2018, 2018–2019, 2019–2020, 2020–2021); S2 – number of leave events between consecutive field seasons; and S3 – net change in female (♀) membership between consecutive field seasons, calculated as:

$$\frac{Q_{\text{joined}} - Q_{\text{left}}}{0.5 \times (\text{final } Q_{\text{group size}} + \text{initial } Q_{\text{group size}})}$$

Social behaviour and activity budget

We conducted daily 1-h focal group observations between 8:30 h and 19:00 h from 2018 to 2020, amounting to a total of 314 h of observation (ranging from 6 to 40 h per group in total). Groups were followed on foot at 5–10 m distance without disturbance (Pinto and Hirata, 2020) and only once per day, with effort made to alternate observations evenly per group and hours of the day. During 1-h observations, we recorded social behaviours of males and females as described by Feist and McCullough (1976) and McDonnell and Haviland (1995), using an all-occurrence sampling method with a video camera (Handycam HDR-CX500V; Sony, Tokyo, Japan). For each interaction, we recorded the type of behaviour, its duration and context, the actor, the receiver(s), and the latter's response. Simultaneously, every five minutes we scanned the activity of group's females and males (13 scans/h) to compare feeding and resting time of SS and MS groups. Both standing and recumbent postures were categorized as resting time.

Dominance rank in MS groups. We used the total number of unilateral agonistic events (i.e., kicks, bites, threats, and displacements) and the outcome of encounters between males in MS groups to determine dominance rank (Feh, 1999). An individual won an encounter when he was able to displace his opponent during an agonistic encounter. The highest-ranking male in a MS group was the one who won most encounters and directed the most aggression towards his counterpart whilst receiving the least (see Supplementary Material Fig. 2).

Males' group defence effort. Individuals may be proactive or reactive when faced with threatening situations (Budzyńska, 2014). The effort by males in defence of the females in the group was defined as the combined result of the following behaviours against other males and outsider individuals: (1) vigilance – when the horse assumes an alert posture by lifting its head and flicking the ears forward; (2) move to front of group – when the male clearly changes location to position himself between his group and an approaching group or outsider; (3) group herding – when the male brings group members together and/or moves the group from place to place by assuming a threatening posture, with ears laid back and neck and head stretched out; (4) inter-group aggression – when a male exhibits overt and threatening agonistic behaviours (displacements, bites, kicks, head threats with ears laid back, chases, and charges) towards non-group members; and (5) male-male encounters – when two males approach each other in a ritualized interactive sequence of dominance display. This type of display often involves synchronous defecation over dung piles, olfactory investigation, vocalizations, foreleg strikes and arched neck threats, prancing, and rearing, and can escalate into fights. The tone of encounters can be quite diverse: from serious aggression to playful fighting or even affiliative behaviour (Feh, 2005; Feist and McCullough, 1976; McDonnell and Haviland, 1995).

Males' access to females. We used two approaches to quantify male access to females in SS and MS groups: first, we used observations of behavioural interactions between males and females – mating (mounting with intromission and ejaculation based on visual assessment (McDonnell, 2005)) and grooming events, in the studied focal groups between 2018 and 2020 field seasons. A grooming event consisted in a reciprocal coat

care interaction between two partners in which the horses stand beside one another, usually head-to-shoulder or head-to-tail and gently nip, nuzzle, or rub the other's neck, mane, or rump (McDonnell and Haviland, 1995).

Second, we conducted paternity analysis of foals and young, and calculated the foaling rate of MS and SS groups between 2016 and 2021, considering the whole Garrano population of Serra d'Arga. This allows us to have a broader representation of reproductive success of the different types of males. Paternity analysis consisted in determining the genetic relatedness (i.e., the probability of two individuals sharing a gene) of foals and young horses to the adult male(s) in their natal group. Between May and August 2017–2021, we opportunistically collected a total of 290 faecal samples from visually recognized horses for genetic analysis. The protocol for DNA extraction, horse microsatellite markers and genotyping, is described in Mendonça et al. (2021). Afterwards, pairwise genetic relatedness (r) between 200 genotyped individuals was calculated using the Triadic likelihood estimator (TrioML) from the software COAN-CESTRY 1.0 (Wang, 2011). The methodology followed to select the best relatedness estimator can be found in Mendonça et al. (2021). The empirical TrioML estimates an r value, ranging from 0 to 1 with a 95% confidence interval (CI) for each dyad, where the probability of a dyad being: parent-offspring is 0.5 ($0.40 > CI < 0.66$), full-siblings is 0.45 ($0.24 > CI < 0.74$), half-siblings is 0.24 ($0.09 > CI < 0.54$), first cousins is 0.11 ($0.02 > CI < 0.42$), second cousins is 0.06 ($0.01 > CI < 0.35$) and unrelated is 0.05 ($0.00 > CI < 0.32$) (Nowak, 2006).

Male-to-female and dominant male-to-subordinate male aggression. Male aggression towards females was defined as the combined instances of individual females being herded and receiving active agonistic behaviours and threats by the group's male(s). We assumed targeted individual herding to be a form of aggression because it is often done in response to oestrus females, or as redirected aggression due to aggression or approach by another male (Miller, 1981; Rubenstein and Núñez, 2009). After determining male rank in MS groups, we defined dominant-to-subordinate aggression as all the directed events of active agonistic behaviours and threats towards a subordinate, through which the dominant asserted his superior ranking over the subordinate.

Body condition In 2019, two researchers (PP and RSM) assessed the summer body condition of 33 adult horses (nine males, 24 females) from five SS and two MS groups. Body condition scores were estimated by visual body fat distribution, 0 being "very thin" and 5 being "very fat", with 0.5 gradations (Carroll and Huntington, 1988; Rudman and Keiper, 1991). The condition of each horse was assessed from the closest distance possible without causing disturbance, once at the beginning of June and again at the beginning of July to calculate individual averages. We used weighted Cohen's kappa to determine inter-rater reliability, with moderate agreement between the two raters' scoring ($\kappa = 0.53$).

Impact of predation and human management

A study conducted in 2017–2018 examining wolf-horse interactions in Serra d'Arga showed that horses were being positively selected by wolves and comprised most of their diet in both years (Freitas, 2019). Overall, predation seemed to be biased towards males, in relation to their abundance in the area, and foals were the most affected age class (Freitas, 2019). In 2018, the predation impact of the estimated 12 wolves forming the local pack was 40% of the total horse population, with kill rates reaching 0.48 horses per wolf per month and 5.9 horses per wolf per year (Freitas, 2019), out of an estimated population of 178 horses, including foals and bachelor males. Based on this data, we determined the mortality status of 164 individuals, foals included, from MS and SS groups in 2018 (Supplementary material Fig. 1). Horses were considered "dead" if their carcasses were found or if they were detected in wolf scats, either due to predation or scavenging by wolves. By comparing horse genotypes found in wolves' scats with the genetic database of the Garrano population ($n = 200$), it was possible to identify some of the consumed individuals (Freitas, 2019; Mendonça et al., 2021). If the horses were detected in wolf scats and their carcasses found in wolves' killing sites, then they were considered to have been "pre-dated". Information on killing sites was obtained from opportunistic visual identification by researchers and through telemetry data from a collared wolf, between August and November 2018 (Freitas, 2019). Horses were considered to have been "removed" if we were able to confirm so with horse owners. However, it was difficult to reliably confirm if the disappearance of some horses was due to human intervention because of the irregular and often-illegal nature of these actions. If we were unable to verify the removal of a horse and no carcass was found during a one-year period (i.e., the interval between two field seasons), we assumed the reason for disappearance was "unknown". On the other hand, if the disappearance occurred within a field season, the horse was considered "dead" since removals were not taking place during this time and the more likely cause of absence may be attributed to natural death (i.e., sickness or predation).

Statistical analysis

Statistical analysis was performed via generalized linear mixed models (GLMM) using the R package *glmmTMB* (Brooks et al., 2017), except for body condition data, which was examined using one-way ANOVAs and t-tests. All analyses and graphical outputs were conducted with R version 3.5.2 (R Development Core Team, 2016).

To examine female group size, the number of females in each group per field season was modelled against group type, as a fixed effect, and group ID and year as random effects, with a gaussian distribution. The three female stability measures (S1, S2 and S3) were analysed in two separate GLMM with a Gaussian structure (Supplementary material Table 1). The number of females' join events (S1) and leave events (S2) for each group per field season interval (2016–2017; 2017–2018; 2018–2019; 2019–2020; 2020–2021) were evaluated in the same model, since both used female counts as the response variable. This model included stability measure (S1 or S2) and group type (MS or SS) as fixed factors with a nested structure, and consecutive field seasons period as another fixed factor. As random factor we included group ID. A second model was built to investigate the effect of group type on female membership (S3), having group type and consecutive field seasons period as the fixed factors, and group ID as the sole random factor.

We calculated foaling rate per group type as the number of foals born in a group divided by the number of breeding females in that group in that year. The GLMM for foaling rate had group type as a fixed effect, group ID and year as random effects and a gaussian distribution (Supplementary material Table 1).

Males' group defence effort against outsiders was assessed in two separate GLMMs with Poisson structure, one examining the effect of male status (MS dominant, MS subordinate, or SS male) and the other group type (MS or SS group; Supplementary material Table 1). Additionally, for both models, we added female group size as a fixed effect, but it only remained in the final models if it yielded a significantly better fit based on the lowest Akaike information criterion (AIC) value and chi-square test (χ^2). The model with male status as fixed effect included group ID, males' ID, observation day and year as random effects. The response variable was total counts of observed defence behaviour events per male per hour. The model with group type as fixed effect included group ID, observation day and year as random effects. In this model the response variable was counts of defensive behaviours per group (meaning that in MS groups we grouped the events of both dominant and subordinate, irrespective of the individual).

Similarly, male-to-female aggression was analysed in two separate GLMMs with *Poisson* structure: one investigating the effect of male status and another the effect of group type (Supplementary material Table 1). The effect of female group size was also analysed but was only included in the final models if it improved the fit of the model. We modelled counts of male to female aggression per male per hour against male status as the fixed effect; group ID, male ID, observation day and year were included as random effects. For the model with group type as fixed effect, we used counts of male to female aggression per group per hour as a response variable, with group ID, observation day and year as random effects.

To test if there was a negative relationship between subordinates' help in group defence and aggression received from their dominant counterparts, we also fitted a GLMM with *Poisson* structure (Supplementary material Table 1). The total number of defence behaviours across all observation hours by subordinates was regressed against the total number of agonistic behaviours by dominants, to subordinates, with male ID, observation day and year as random effects.

To analyse feeding and resting activity of females in MS and SS groups, and males of different status we used a total of four models with binomial structure (Supplementary material Table 1). For all models, the response variable was modelled as binary data where, every 5-min scan, females or males' activity was coded as 1 (if feeding or resting, depending on the model) or 0 (when displaying any other activity). The two models for females – feeding and resting, included group type as a fixed effect and group ID, female ID, observation day, daytime period and year as random effects. The feeding and resting activity of males was modelled against male status, with group ID, male ID, observation day, daytime period and year as random effects. In all models, the effect of female group size was also considered as a fixed effect, following the previous premise that it only remained in the final models if it improved the fit.

Results

Female group size and stability

When we compared the size of MS and SS groups in the Arga population from 2016 to 2021, we found that SS had considerably more females than MS groups (GLMM: $Z = 3.06$, $P < 0.01$) (Table 2). On the other hand, group type did not have a significant effect on any of the female stability measures: S1 - number of females' join events (GLMM: $Z = -0.47$, $P = 0.64$), S2 - number of leave events (GLMM: $Z = 1.14$, $P = 0.26$) and S3 - net change in female membership (GLMM: $Z = 0.20$, $P = 0.88$) (Table 2).

Males' group defence effort

In 314 h of focal group observations, vigilance was the behaviour most often observed ($n = 5665$), followed by "move to front of group" ($n = 374$), group herding ($n = 371$), inter-group aggression ($n = 194$), and male encounters ($n = 99$) (Supplementary material Fig. 3). Male status had a significant effect on group defence effort against outsiders (Table 2, Fig. 1). Dominant males in MS groups spent significantly less effort in defending the group from outsiders compared to SS males (GLMM: $Z = 3.89$, $P < 0.001$). In MS groups, subordinate males displayed more defensive behaviours than their dominant counterparts, however this trend only had a marginal effect (GLMM: $Z = 1.91$, $P = 0.06$). Subordinate males in MS and males in SS groups were not statistically different in their defence effort (LSmeans: t -ratio = -0.72 , $P = 0.75$) (Supplementary Material Table 2). The combined defence effort of dominant and subordinate males in MS groups was significantly higher than that of SS males (GLMM: $Z = -3.07$, $P < 0.01$) (Table 2, Fig. 1). Models that included female group size as a second fixed effect did not have a better fit (Supplementary material Table 4) and therefore female group size was not included in the final model.

Males' access to females

Using mating and grooming events between males and females as a proxy for males' access to females, we counted five mating (plus seven matings observed outside of focal group observations) and 82 grooming interactions during 314 h of focal group observations from 2018 to 2020. Of the total 12 mating events, only two were performed by the dominant male in MS groups and the rest were by SS males. The only clear instance we observed of a subordinate male attempting to mate was quickly thwarted by the dominant in the group. With respect to grooming, we observed an average of 0.19 events/ hour/ year ($n = 52$) by SS males. We recorded an average 0.20 events/ hour/ year for MS dominant males ($n = 17$) and 0.15 events/ hour/ year for subordinate males ($n = 13$), but the latter occurred between the same subordinate male-female pair. Furthermore, this was a natal female that had been born in the group in 2016 and never dispersed.

When we compared the foaling rate of all MS and SS groups in the Arga population between 2016 and 2021, we did not find significant difference between the two (GLMM: $Z = 1.18$, $P = 0.24$). Due to the short life of foals because of wolf predation, it was not possible to collect faecal samples of all the observed 143 foals born between 2016 and 2021. Nonetheless, we determined the genetic relatedness between 60 immature individuals (17 MS, 43 SS), of which 33 were foals and 27 were young horses, and their natal group male(s) (Supplementary material Table 3). In some foal/young-natal male pairs, the genetic relatedness estimator was lower than that attributed to offspring-parent and we were unable to identify a relationship with other males from the population. This may be because the parent was not genotyped, either because we were unable to collect samples or because the individual was no longer present in the population. In other cases where the estimator was relatively low for a clear offspring-parent relationship, but still within the CI, we assumed the natal male(s) sired the offspring if we found a sibling/half-sibling relationship with other horses that we knew were sired by the male. Of the 43 horses born in SS groups, 67% ($n = 29$) were sired by the natal male, while 19% ($n = 8$) resulted from extra-pair copulations. For the last 14% ($n = 6$) paternity could not be determined. By contrast, in MS groups, 59% (10) of the 17 genotyped horses were sired by the dominant male, 12% ($n = 2$) were from extra-pair copulations and 18% ($n = 3$) were undetermined. The remaining 12% ($n = 2$) correspond to one MS group with two males, the Hakata and Nijo group, where both males sired a foal each. However, we did not have behavioural data to identify the males' rank. If, based on general sightings of the group, we assume Nijo was the dominant male in the association (who we believed to be a senior individual that disappeared after injury), that would raise the percentage of foals sired by the dominant male in MS groups to 65% ($n = 11$), while subordinate males only sired 6% ($n = 1$) of the foals.

Male-to-female and dominant-to-subordinate aggression

In 314 h, we observed 192 intra-group instances of individual herding and 188 agonistic interactions from MS and SS males to their group females during the 2018–2020 field seasons. Subordinate males in MS groups were significantly less likely to behave aggressively towards females compared with their dominant counterparts (GLMM: $Z = -4.69$, $P < 0.001$) (Table 2, Fig. 2), but only showed a marginal difference from SS males (LSmeans: t -ratio = 2.36, $P = 0.05$; Supplementary material Table 2). Dominant males and males from SS groups were not statistically different to each other in their rates of female harassment (GLMM: $Z = 0.40$, $P = 0.69$; Fig. 2). Male-to-female aggression was lowest in SS groups, but this disparity did not reach statistical significance (GLMM: $Z = 1.03$, $P = 0.31$) (Table 2, Fig. 2). We did not observe significant influence of females' group size on males' levels of female harassment (Supplementary material Table 4) and therefore did not include it as a predictor in the final models.

There was a significant negative relationship between subordinates' effort in group defence and the number of agonistic behaviours received from the dominant males (GLMM: $Z = -2.74$, $P < 0.01$) (Table 2, Fig. 3).

Activity budget and body condition

Females in SS groups spent slightly more time grazing but less time resting than females in MS groups, however these differences were not statistically significant (feeding: GLMM: $Z = 1.04$, $P = 0.30$; resting: GLMM: $Z = -0.88$, $P = 0.38$) (Table 3, Fig. 4). We found that the number of females in the group had a significant negative effect on female feeding activity (GLMM: $Z = -2.31$, $P < 0.05$) (Table 2), but did not affect resting time or confer a better fit to that model (Supplementary material Table 4), therefore the final model for resting activity only included group type as a predictor. Body condition of 24 females from SS groups and MS groups was not statistically different (t -test for normalized data: $T(6.11) = 0.93$, $P = 0.39$), scoring 2.8 and 3, respectively, which is considered to be "good" condition.

Dominant males in MS groups did not show differences in grazing time (GLMM: $Z = 0.91$, $P = 0.36$) but spent significantly more time resting than males in SS groups (GLMM: $Z = -1.97$, $P < 0.05$) (Table 2, Fig. 5). Compared with their subordinate counterparts, feeding activity was similar but dominant males had more resting time (feeding: GLMM: $Z = 0.66$, $P = 0.51$; resting: GLMM: $Z = -2.54$, $P < 0.05$) (Table 2, Fig. 5). Subordinate and SS males were not statistically different in their activity budget (feeding: LSmeans: t -ratio = -0.74 , $P = 0.74$; resting: LSmeans: t -ratio = 1.29 , $P = 0.40$) (Supplementary material Table 2). Female group size had a significant influence on males' activity budget: the higher the number of females, the lower the amount of time grazing, but the higher the amount of time resting (feeding: GLMM: $Z = -2.64$, $P < 0.01$; resting: GLMM: $Z = 2.19$, $P < 0.05$) (Table 2). Finally, the body condition of nine males was not affected by their status (one-way ANOVA test for normalized data: $F(2,6) = 0.41$, $P = 0.68$), with an average score of 3.0 for males of SS groups, and 3.1 for dominant and 3.4 for subordinate males in MS groups.

Impact of predation and management on MS and SS groups

Based on existing wolf predation data and confirmation of horse removals with owners, we determined the status of 164 individuals from MS and SS groups in 2018 (Supplementary material Fig. 1). In general, approximately 9% ($n = 2$ MS, $n = 12$ SS) of the horses in that year were confirmed to have been killed by wolves and a further 13% ($n = 7$ MS, $n = 15$ SS) were found dead, the exact reason was unknown. Horse owners admitted to having captured 23% ($n = 7$ MS, $n = 31$ SS) of the MS and SS horses, but we were unable to determine the cause of disappearance of 27% ($n = 10$ MS, $n = 34$ SS) of the animals. Only 1 out of 22 foals from SS groups survived past the first year of age. Of the 22

foals that did not survive or remain on the range, 59.1% ($n = 13$) died due to undetermined reasons, 27.2% ($n = 6$) were confirmed to have been killed in wolf attacks, 4.5% ($n = 1$) disappeared and another 4.5% ($n = 1$) were captured by owners. By contrast, of the eight foals born in MS groups, 62.5% ($n = 5$) died, 25% ($n = 2$) disappeared and 12.5% ($n = 1$) were captured. Of the 20 SS males, 20% ($n = 4$) were killed by wolves, 30% ($n = 6$) were removed by owners and 25% ($n = 5$) disappeared. As for the 12 MS males, 16.7% ($n = 2$) were killed and a further 16.7% ($n = 2$) died. 8.3% ($n = 1$) were captured and another 8.3% ($n = 1$) disappeared. Of the 78 SS females, 2.6% ($n = 2$) were predated and 2.6% ($n = 2$) died. 30.8% ($n = 24$) were captured and 37.2% ($n = 29$) disappeared. Of the 24 females in MS groups, 20.8% ($n = 5$) were removed and 29.2% ($n = 7$) disappeared. No MS females were detected to have died, due to predation or otherwise (Supplementary material Fig. 1).

Discussion

Group size and stability of MS and SS groups

Based on by-product mutualism, it was proposed that MS groups have more females and are more stable than SS groups because of the number of males in the group (Miller, 1981; Stevens, 1990). Our results indicated that SS males from the Garrano population in Arga had more breeding females than MS groups, however group type did not influence female membership stability. The difference in group size may have resulted from a few non-exclusive explanations. A priori, the female biased sex-ratio may have influenced harem formation and size. Since populations with fewer males tend to form larger groups because of less competition for females (Boyd and Keiper, 2005), we would expect SS groups in Serra d'Arga to be the predominant form of social organization and well-established groups to be larger. By contrast, newly formed groups where the male did not take-over an existing harem but bonded with a dispersing or lone female would be smaller. The latter was the case for a few unstable MS groups consisting of two to four bachelor males and one female that formed between 2016 and 2017 and broke apart by 2018, which may have influenced the results. In some of these MS groups only one male remained, the other(s) left to form their own group or they became bachelors, thus transitioning into SS groups. Furthermore, the considerable human interference and wolf attacks, which intensified in 2018, may have exacerbated the female-biased sex ratio and group formation issues since males were being targeted. Thus, it is possible that the larger size of SS groups may be a by-product of the field site characteristics rather than an inherent trait or a perceived ability to acquire and maintain females of SS males.

While Pacheco and Herrera (1997) reported a positive correlation between number of males and number of females in Venezuelan feral horse groups, other studies have found that the number of males does not seem to directly affect group size nor stability (Linklater and Cameron, 2000; Linklater et al., 2013). Rather, group cohesion may be better explained by stable long-term bonds between group members (Stanley et al., 2018; Mendonça et al., 2021). The fact that we observed MS groups that lost males with no effect on the maintenance of the group, further reiterates the superfluosity of subordinate males in this respect (Linklater et al., 1999). Studying the spatial organization of horse societies, our team found that larger harem groups were more likely to occupy the centre of the herd – an indication of dominance, regardless of whether they were

SS or MS groups, as opposed to smaller harems and bachelor groups, which were relegated to the periphery of the herd (Maeda et al., 2021a). This organization may explain why our results show that the combined group defence effort of dominant and subordinate males together is higher than that of males leading SS groups. MS groups in our population, because of their number of members, may be occupying a position in the herd that is exposing them to more confrontations with other groups. Similarly, Feh (1999) reported that the combined total fighting frequency for both males in MS groups was significantly higher than that of SS strategists, but suggested it was because MS males were lower ranking and so had to defend their females more often. For horses in the Red Desert, group dominance determined access to limited water sources and was correlated with group size, not number of males (Miller and Dennisto, 1979). Therefore, the literature and our results seem to give little support for mutualism hypotheses centred on net benefits to group size, stability, dominance, and resource access as a result of number of males in the group.

Relationship between the males in MS groups

Similar to what has been observed in other populations (Feh, 1999; Linklater and Cameron, 2000; Linnartz and Linnartz-Nieuwdorp, 2017; Miller, 1981; Stevens, 1990), in MS Garrano horse groups, the dominant male maintained proximity to the females, while the subordinate was positioned more often at the edge of the group (unpublished results) and played an active role in its defence against outsiders. Conflict between the males in MS groups was rarely observed and affiliative behaviours even less so, although to varying degrees among the different MS groups. In practice, it seems clear that there are variations in individual temperament and how the males in MS groups associate with each other, which could hint at different pathways male horses take to acquire females (Rubenstein and Nunˆez, 2009). For example, male associations where the males are familiar with each other, by bonding during bachelorhood or infancy in a shared natal group, could be more cooperative than associations where the subordinate joined a fully formed harem. In lions, it is suggested that males' ability to form successful large coalitions, which grant higher reproductive success, may be dependent on long-term bonds formed early in life, since lions tend to be intolerant of strangers (Bygott et al., 1979). In horses, Feh (1999) described alliance males in Camargue as low-ranking, unrelated individuals of similar age that developed strong bonds during bachelorhood. Conversely, while Mountain gorillas (*Gorilla gorilla beringei*) exhibit a similar social system to horses, the multi-male group structure may be better explained by demographic factors, male-male competition and male-female relationships than any benefits subordinate and dominant gorillas may derive from their association (Robbins, 1996).

Male's group defence effort and activity budget

Some studies have suggested that MS males have a mutualistic relationship wherein the subordinate contributes to group defence, which benefits the dominant via reduced effort in female protection and increased time for grazing (Feh, 1999; Rubenstein, 1982). Our study shows that dominant MS males clearly benefitted from the presence of another male, by spending less energy watching and fending off threats to the group (and avoiding potential injuries in the process) when compared with other males. However, in relation to their subordinates, this trend only had marginal significance, so more data may be needed to conclude if there is a difference between the number of defence behaviours shown by dominant and subordinate MS males. It can be argued that this pattern may arise as a result of different probabilities of the MS males facing outsiders, because of the spacing and competitive nature of their association (Linklater et al., 1999; Linklater and Cameron, 2000). However, dominant males in our population consistently displayed fewer of the targeted defence behaviours than other males, such as vigilance, and did not dissuade the subordinate from herding the group away (Supplementary Material, Fig. 3). In addition to less effort employed in group defence, dominant males in MS groups also benefitted from the presence of subordinate males via increased resting time when compared with the other males. Conversely, feeding time and body condition were not significantly different depending on male status. This could mean that subordinate males and males from SS groups may be sacrificing their resting time, but not the time spent grazing, to perform other activities, such as group defence. Therefore, our results show that dominant males in MS groups may receive a net-benefit gain by having another adult male in the group.

Subordinate's help vs. dominant's aggression and access to females in MS groups

It is hypothesized that males in MS groups have a relationship based on reciprocal altruism whereby subordinate's help in group defence is rewarded with lower aggression from the dominant and access to females (Feh, 1999; Rubenstein, 1982; Rubenstein and Nunˆez, 2009). In Kaimanawa horses, Linklater and Cameron (2000) observed a positive relationship between dominant's aggression and subordinate's effort in group defence and proximity to females. By contrast, our results showed that increasing help in defence against outsiders meant less dominant's aggression to the subordinate. On the other hand, we found little evidence that subordinates were being rewarded with access to females, both through behavioural interactions and reproductive concessions. Our observations of matings and paternity analysis seem to conform to reports from previous studies, where subordinate strategists sire very few foals, if any (Asa, 1999; Feh, 1999; Linklater and Cameron, 2000; Linnartz and Linnartz-Nieuwdorp, 2017; Rubenstein and Nunˆez, 2009). Hence, among Garrano horses we did not find evidence of altruism, as proposed by Feh (1999), in terms of reproductive concessions, even though dominants were not aggressive towards subordinates. Subordinates' actual access to the group females appears restricted and may be dependent on factors such as female rank, age, and her affiliation with the dominant. In our population, dominant males in MS groups had similar siring rates to SS males and there was no difference in the overall foaling rate between MS and SS groups. However, paternity analysis revealed fewer offspring from extra-pair copulations in MS groups. While this could be an indication that having extra adult males in the group may provide an advantage in deterring group females and outsiders from interacting, it may have come partially as a result of human disturbance and wolf attacks, since some SS females were forced to change groups post-breeding season, after their males disappeared. It is also important to remark that we could not determine the paternity of approximately 2/3 of the foals born between 2016 and 2021, because they were predated before we could collect samples, and in a few cases where foal samples were analysed we could not identify the parent because they were not genotyped.

Male to female aggression and female activity budget

Sexual coercion or harassment by males can have deleterious effects on females and lead them to alter their behaviour as an adaptive counterstrategy (Darden and Watts, 2012; Sundaresan et al., 2007). Previous studies have suggested that male harassment, rather than cooperation, better explains horses' mating system, and male-to-female aggression is higher in MS groups, with negative effects on females' body condition and reproductive success (Linklater et al., 1999; Cameron et al., 2009). In our population, we found no statistical support that females in MS groups suffered higher levels of male aggression when compared with females in SS groups. Furthermore, the number of males in the group had

no significant influence on the time females spent grazing and resting, nor on their body condition. Subordinate males were significantly less aggressive to females than dominant males but only showed a marginal difference from SS males, although SS males had similar levels of aggression to MS dominants. This difference might be related to having more statistical control in comparisons of sub-ordinates vs dominants, than in those of subordinates vs SS males. If in every MS group, the dominant is more “reliably” aggressive, even if the dominant and subordinate values have a wide range, this should still yield a significant difference between the two. In contrast, there might be too much variation within the SS group, considering the sample size (four MS subordinates vs 14 SS males), to accurately measure the “expected” difference between subordinates and SS males.

Predation impact in MS and SS groups

Horses and wolves have long coexisted in a prey-predator relationship and, although predation on horses is generally low, in some parts of southern Europe and central Asia horses are an important prey species (especially in areas with low density of wild prey or under free-ranging husbandry) and are positively selected by wolves, to varying degrees (Freitas et al., 2021). The actual predation impact in our population may be higher than what the available wolf data of 2018 indicates since horses termed “dead” were probably killed in wolf attacks, as all animals observed were in good body condition and foals are especially vulnerable in the first months of life (Freitas, 2019; Freitas et al., 2021). There’s also a high percentage of individuals that went missing, probably as a combination of wolf killings and furtive human removals, which was also a big source of disturbance in 2018 and makes it difficult to discern and measure the effects of predation on horse groups and its determinants. Nonetheless, some general trends seem present and are worth speculating. Some researchers noticed an increase in the number of males in harems after wolf attacks (Linnartz and Linnartz-Nieuwdorp, 2017), which may suggest that male horses play an important role in protecting the group from predators, for example by cohesively leading the group away or by fighting. Foals were the most affected class in both MS and SS, with the only surviving individual belonging to a SS group isolated from the rest of the population. Since almost all foals born in 2018 disappeared, it is difficult to say if the number of adult males in the harem provides any actual advantages for foal survivability in relation to predation. While we do not have accurate wolf data for other years, in 2016 human interference was negligible and 40% of the foals in MS groups survived compared to only 19% of the foals in SS groups. As the wolf pack grew and human disturbance intensified, no foals reached one year of age until 2020, when both pressures were declining (personal observation). In respect to males, they were the most affected class after foals, which may be expected given that males are active protectors of the group against outsiders and predators, thus being more prone to injuries and at a higher risk of being preyed (Garrott and Taylor, 1990). In 2018 there seemed to be a higher predation tendency for males in MS groups, and both dominant and subordinate males were affected. We often observed the disappearance of the subordinate first, followed by the dominant but, again, it is difficult to say how meaningful this trend is considering males were being heavily targeted in captures. On the other hand, females were less affected by these pressures in MS groups than in SS groups, even if we consider the individuals that disappeared for an unknown reason. While it is possible that the trends observed may be clearer under more balanced circumstances, considering our field site characteristics - the female biased sex-ratio, small number of MS groups, the confounding effect of human disturbance and the predator data available, more studies are needed to draw proper conclusions in relation to defensive advantages resulting from the number of males in the group against predators.

Conclusion

Encompassing the breeding and birthing season periods, we studied a population of feral horses in northern Portugal to examine the costs and benefits predicted for one-male and multi-male groups. Although our study site characteristics may have posed some limitations, for the most part, demographic and behavioural data showed that reciprocal altruism and by-product mutualism hypotheses may not entirely explain the relationship between males in the same group. Dominant males in MS groups may profit from the presence of subordinates, but the latter seems to benefit little in relation to their efforts. Conversely, we did not find discernibly clear negative effects of male-to-female harassment in MS groups as proposed in some studies. Future research should explore how male associations in harems shape male-female relationships, which could provide further insights into the existence and maintenance of subordinate males in horse groups. One distinctive aspect of the Garrano population is the presence of natural predators, Iberian wolves, which are absent from many studies investigating MS groups, even though additional males could prove to be advantageous against predators. Only by studying horse populations with different compositions, living in diverse environments with different ecological pressures, can we expect to gain a better understanding of the strategies, cognitive capabilities, and adaptive evolution of this species.

Declaration of Competing Interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The field observations conducted in this study complied with the Wildlife Research Center of Kyoto University’s guidelines for animal studies in the wild. We are grateful to Shinya Yamamoto, Monamie Ringhofer, Sota Inoue, Tamao Maeda and Tetsuro Matsuzawa for their support throughout this study. Thank you to James Brooks for his help during the statistical analysis. We also thank the municipality of Viana do Castelo, residents of Montaria and field volunteers for their assistance to this project. This study was financially supported by the Japanese Government’s MEXT Monbukagakusho scholarship; Japan Society for the Promotion of Science (JSPS) LJP-U04; JSPS’ Grants-in-Aid for Scientific Research KAKENHI Nos.: 18H05524, 19H00629; and Kyoto University SPIRITS. We thank Clio Reid, PhD, from Edanz (<https://jp.edanz.com/ac>) for editing a draft of this manuscript.

References

- Arak, A., 1983. Sexual selection by male–male competition in natterjack toad choruses. *Nature* 306 (5940), 261–262.
- Asa, C.S., 1999. Male reproductive success in free-ranging feral horses. *Behav. Ecol. Socio* 47 (1), 89–93.
- Boyd, L., Keiper, R., 2005. Behavioural ecology of feral horses; in D. S. Mills, McDonnell S.M. (Eds.) *The domestic horse: the origins, development, and management of its behaviour*. Cambridge: Cambridge University Press, pp. 55–82.
- Boyd, L., Scorolli, A., Nowzari, H., Bouskila, A., 2016. Social organization of wild equids; in: Ransom, J.I., Kaczensky, P. (Eds.) *Wild equids: Ecology, management, and conservation*. Johns Hopkins University Press, Baltimore, Maryland, pp.7–22.

Briefer Freymond, S., Briefer, E.F., Niederhauser, R.V., Bachmann, I., 2013. Pattern of social interactions after group integration: A possibility to keep stallions in group. *PLoS One* 8 (1), e54688.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Maechler, M., Bolker, B.M., 2017. glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R. J.* 9 (2), 378–400.

Budzyńska, M., 2014. Stress reactivity and coping in horse adaptation to environment. *J. Equine Vet. Sci.* 34 (8), 935–941.

Bygott, J.D., Bertram, B.C., Hanby, J.P., 1979. Male lions in large coalitions gain reproductive advantages. *Nature* 282 (5741), 839–841.

Cameron, E.Z., Setsaas, T.H., Linklater, W.L., 2009. Social bonds between unrelated females increase reproductive success in feral horses. *Proceedings of the National Academy of Sciences* 106 (33), 13850–13853.

Carroll, C.L., Huntington, P.J., 1988. Body condition scoring and weight estimation of horses. *Equine Vet. J.* 20 (1), 41–45.

Chowdhury, S., Pines, M., Saunders, J., Swedell, L., 2015. The adaptive value of secondary males in the polygynous multi-level society of hamadryas baboons. *Am. J. Phys. Anthropol.* 158 (3), 501–513.

Christensen, J.W., Ladewig, J., Søndergaard, E., Malmkvist, J., 2002. Effects of individual versus group stabling on social behaviour in domestic stallions. *Appl. Anim. Behav. Sci.* 75 (3), 233–248.

Connor, R.C., Smolker, R.A., Richards, A.F., 1992. Two levels of alliance formation among male bottlenose dolphins (*Tursiops sp.*). *Proc. Natl. Acad. Sci.* 89 (3), 987–990.

Darden, S.K., Watts, L., 2012. Male sexual harassment alters female social behaviour towards other females. *Biol. Lett.* 8 (2), 186–188.

Díaz-Mun˜oz, S.L., DuVal, E.H., Krakauer, A.H., Lacey, E.A., 2014. Cooperating to compete: altruism, sexual selection and causes of male reproductive cooperation. *Anim. Behav.* 88, 67–78.

Duffy, K.G., Wrangham, R.W., Silk, J.B., 2007. Male chimpanzees exchange political support for mating opportunities. *Curr. Biol.* 17 (15), R586–R587.

Feh, C., 1999. Alliances and reproductive success in Camargue stallions. *Anim. Behav.* 57 (3), 705–713.

Feh, C., 2005. Relationships and communication in socially natural horse herds, in: Mills, D.S., McDonnell, S.M. (Eds.) *The domestic horse: the origins, development, and management of its behaviour*. Cambridge University Press, Cambridge, pp.83–93.

Feh, C., Boltsukh, T., Tourenq, C., 1994. Are family groups in equids a response to cooperative hunting by predators? The case of Mongolian kulans (*Equus hemionus luteus* Matschie). *Rev. D'Écol.*

Feist, J.D., McCullough, D.R., 1976. Behavior and communication patterns in feral horses. *Z. Tierpsychol.* 41, 337–371.

Freitas, J., 2019. Patterns and Behavioral Determinants Related to Wolf Predation on Free-Ranging Horses. PhD Thesis. University of Porto.

Freitas, J., Lagos, L., Álvares, F., 2021. Horses as prey of wolves: worldwide patterns and management implications. *Carniv. Damage Prev. N.* 23, 1–11.

Garrott, R.A., Taylor, L., 1990. Dynamics of a feral horse population in Montana. *J. Wildl. Manag.* 603–612.

Gehlen, H., Krumbach, K., Thöne-Reineke, C., 2021. Keeping stallions in groups—species-appropriate or relevant to animal welfare? *Animals* 11 (5), 1317.

Hartmann, E., Søndergaard, E., Keeling, L.J., 2012. Keeping horses in groups: a review. *Appl. Anim. Behav. Sci.* 136 (2–4), 77–87.

Hill, R.A., Lee, P.C., 1998. Predation risk as an influence on group size in cercopithecoid primates: implications for social structure. *J. Zool.* 245 (4), 447–456.

Inoue, S., Yamamoto, S., Ringhofer, M., Mendonça, R.S., Pereira, C., Hirata, S., 2019. Spatiotemporal position of an individual in a group of feral horses: a case study using drone technology. *Mamm. Res.* 64 (2), 249–259.

Inoue, S., Yamamoto, S., Ringhofer, M., Mendonça, R.S., Hirata, S., 2020. Lateral position preference in grazing feral horses. *Ethology* 126 (1), 111–119.

Linklater, W.L., Cameron, E.Z., 2000. Tests for cooperative behaviour between stallions. *Anim. Behav.* 60 (6), 731–743.

Linklater, W.L., Cameron, E.Z., 2009. Social dispersal but with philopatry reveals incest avoidance in a polygynous ungulate. *Anim. Behav.* 77 (5), 1085–1093.

Linklater, W.L., Cameron, E.Z., Minot, E.O., Stafford, K.J., 1999. Stallion harassment and the mating system of horses. *Anim. Behav.* 58 (2), 295–306.

Linklater, W.L., Cameron, E.Z., Stafford, K.J., Veltman, C.J., 2000. Social and spatial structure and range use by Kaimanawa wild horses (*Equus caballus*: *Equidae*). *N. Z. J. Ecol.* 139–152.

Linklater, W.L., Cameron, E.Z., Stafford, K.J., Minot, E.O., 2013. Removal experiments indicate that subordinate stallions are not helpers. *Behav. Process.* 1–4, 94.

Linnartz, L., Linnartz-Nieuwoudorp, E., 2017. The social organisation of natural herds of koniks (*Equus caballus*): subordinate stallions, rule or exception. *Lutra* 60 (1), 27–42.

Maeda, T., Sueur, C., Hirata, S., Yamamoto, S., 2021b. Behavioural synchronisation in a multilevel society of feral horses. *bioRxiv*.

Maeda, T., Ochi, S., Ringhofer, M., Sosa, S., Sueur, C., Hirata, S., Yamamoto, S., 2021a. Aerial drone observations identified a multilevel society in feral horses. *Sci. Rep.* 11 (1), 1–12.

McDonnell, S.M., 2005. Sexual behaviour. In: Mills, D.S., McDonnell, S.M., McDonnell, S. (Eds.), *The domestic horse: the origins, development and management of its behaviour*. Cambridge University Press, pp. 110–125.

McDonnell, S.M., Haviland, J.C.S., 1995. Agonistic ethogram of the equid bachelor group. *Appl. Anim. Behav. Sci.* 43 (3), 147–188.

McElligott, A.G., Gammell, M.P., Harty, H.C., Paini, D.R., Murphy, D.T., Walsh, J.T., Hayden, T.J., 2001. Sexual size dimorphism in fallow deer (*Dama dama*): do larger, heavier males gain greater mating success? *Behav. Ecology Sociobiol.* 49 (4), 266–272.

Mendonça, R.S., Ringhofer, M., Pinto, P., Inoue, S., Hirata, S., 2020. Feral horses' (*Equus ferus caballus*) behavior toward dying and dead conspecifics. *Primates* 61 (1), 49–54.

Mendonça, R.S., Pinto, P., Inoue, S., Ringhofer, M., Godinho, R., Hirata, S., 2021. Social determinants of affiliation and cohesion in a population of feral horses. *Appl. Anim. Behav. Sci.* 105496.

Miller, 1981. Male aggression, dominance and breeding behavior in Red Desert feral horses. *Z. Tierpsychol.* 57, 3–4.

Miller, R., Dennisto, R.H.N., 1979. Interband dominance in feral horses. *Zeitschrift für Tierpsychologie* 51 (1), 41–47.

Monard, A.M., Duncan, P., 1996. Consequences of natal dispersal in female horses. *Anim. Behav.* 52 (3), 565–579.

Nakamura, M., Rio-Maior, H., Godinho, R., Petrucci-Fonseca, F., Álvares, F., 2021. Source-sink dynamics promote wolf persistence in human-modified landscapes: Insights from long-term monitoring. *Biol. Conserv.* 256, 109075.

Nowak, M.A., 2006. Five rules for the evolution of cooperation. *Science* 314, 1560–1565.

Pacheco, M.A., Herrera, E.A., 1997. Social structure of feral horses in the llanos of Venezuela. *J. Mammal.* 78 (1), 15–22.

Pinto, P., Hirata, S., 2020. Does size matter? Examining the possible mechanisms of multi-stallion groups in horse societies. *Behav. Process.* 181, 104277.

Port, M., Johnstone, R.A., Kappeler, P.M., 2010. Costs and benefits of multi-male associations in redfronted lemurs (*Eulemur fulvus rufus*). *Biol. Lett.* 6 (5), 620–622.

R Development Core Team, 2016. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org>.

Ringhofer, M., Inoue, S., Mendonça, R.S., Pereira, C., Matsuzawa, T., Hirata, S., Yamamoto, S., 2017. Comparison of the social systems of primates and feral horses: data from a newly established horse research site on Serra D'Arga, northern Portugal. *Primates* 58 (4), 479–484.

Ringhofer, M., Go, C.K., Inoue, S., Mendonça, R.S., Hirata, S., Kubo, T., Ikeda, K., Yamamoto, S., 2020. Herding mechanisms to maintain the cohesion of a harem group: two interaction phases during herding. *J. Ethol.* 38 (1), 71–77.

Robbins, M.M., 1996. Male-male interactions in heterosexual and all-male wild mountain gorilla groups. *Ethology* 102 (7), 942–965.

Rubenstein, D.I., 1982. Reproductive value and behavioral strategies: coming of age in monkeys and horses. In: Bateson, P.P.G., Klopfer, P.H. (Eds.), *Ontogeny*. Springer, Boston, MA, pp. 469–487.

Rubenstein, D.I., Nunes, C.M., 2009. Sociality and reproductive skew in horses and zebras, in: Hager, R., Jones, C.B. (Eds.) *Reproductive skew in vertebrates: Proximate and ultimate causes*. Cambridge University Press, pp.196–226.

Rudman, R., Keiper, R.R., 1991. The body condition of feral ponies on Assateague Island. *Equine Vet. J.* 23 (6), 453–456.

Scarry, C.J., 2013. Between-group contest competition among tufted capuchin monkeys, *Sapajus nigritus*, and the role of male resource defence. *Anim. Behav.* 85 (5), 931–939.

Stanley, C.R., Mettke-Hofmann, C., Hager, R., Shultz, S., 2018. Social stability in semiferal ponies: networks show interannual stability alongside seasonal flexibility. *Anim. Behav.* 136, 175–184.

Stevens, E.F., 1990. Instability of harems of feral horses in relation to season and presence of subordinate stallions. *Behaviour* 112 (3–4), 149–161.

Sundaresan, S.R., Fischhoff, I.R., Rubenstein, D.I., 2007. Male harassment influences female movements and associations in Grevy's zebra (*Equus grevyi*). *Behav. Ecol.* 18 (5), 860–865.

van Schaik, C.P., van Noordwijk, M.A., 1989. The special role of male Cebus monkeys in predation avoidance and its effect on group composition. Behav. Ecol. Sociobiol. 24 (5), 265–276.

Wang, J. 2011. COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. Mol. Eco. Res. 11, 141–145.

Lagos, L. (2013). *Ecología del lobo (Canis lupus), del poni salvaje (Equus ferus atlanticus) y del ganado vacuno semiextensivo (Bos taurus) en Galicia: interacciones depredador-presa* (Doctoral dissertation, Ph. D Thesis. Universidad de Santiago de Compostela, Santiago de Compostela).

Figures

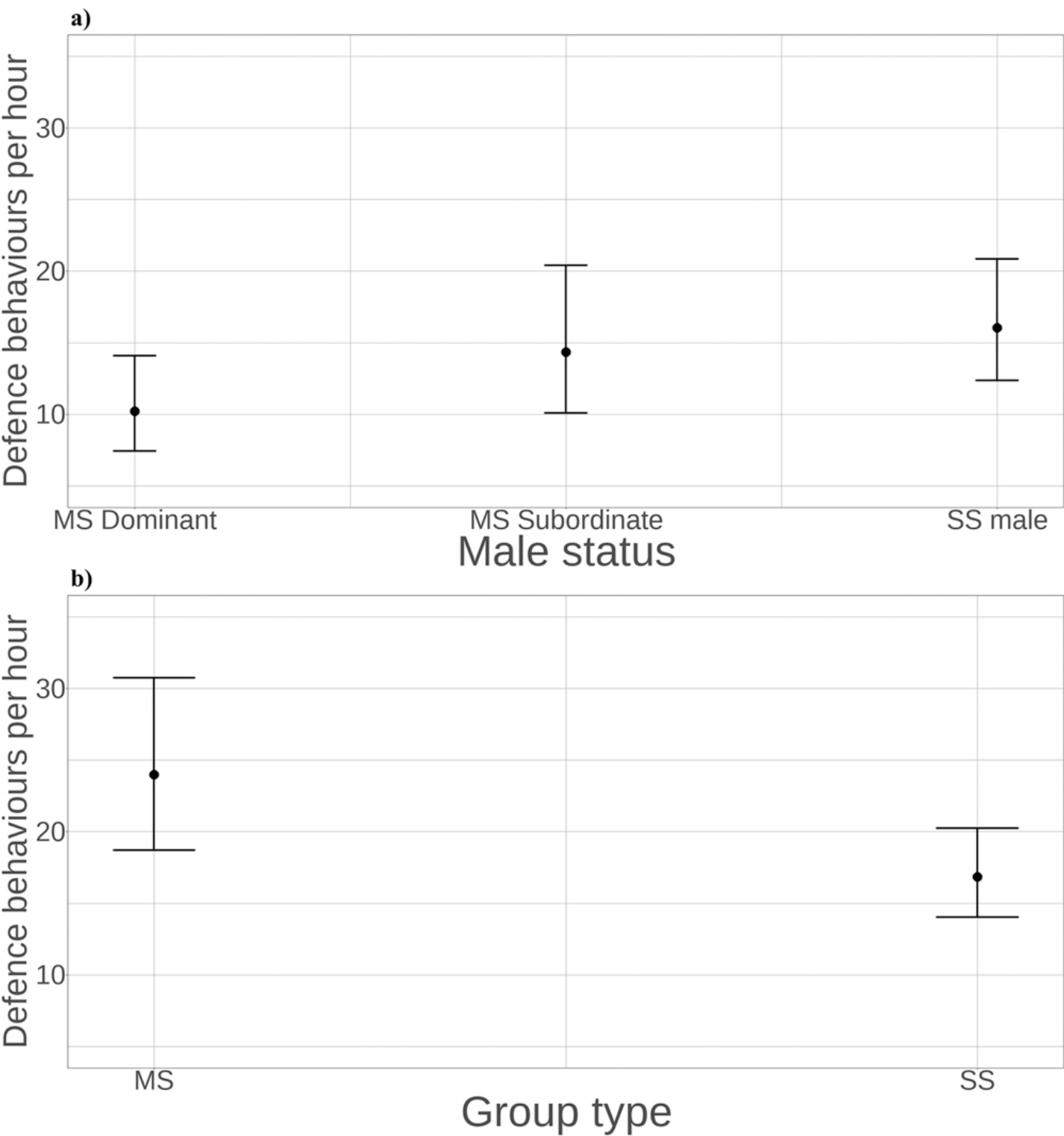


Fig. 1. Relationship between the number of defence behaviours per hour displayed by stallions and **(a)** male status – MS dominant, MS subordinate or SS male; and **(b)** group type – multi-male (MS), or one-male (SS). The values plotted correspond to predicted values (marginal effects) held constant at their proportions, and the bars represent standard error (SE).

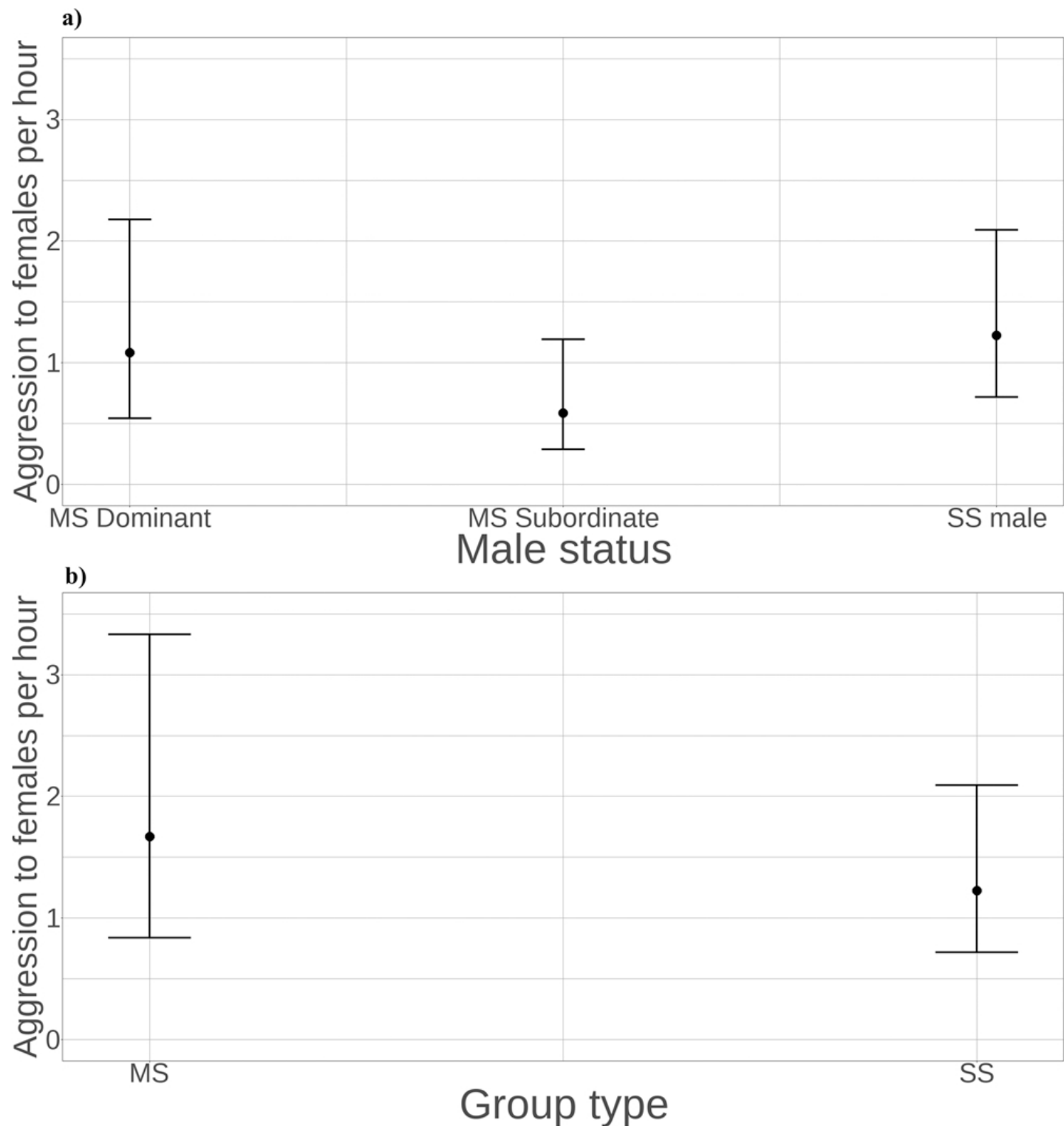


Fig. 2. Relationship between the number of male-to-female aggression events per hour and **(a)** male status – MS dominant, MS subordinate or SS male; and **(b)** group type – multi-male MS, or one-male SS. The values plotted correspond to predicted values (marginal effects) held constant at their proportions, and the bars represent standard error (SE).

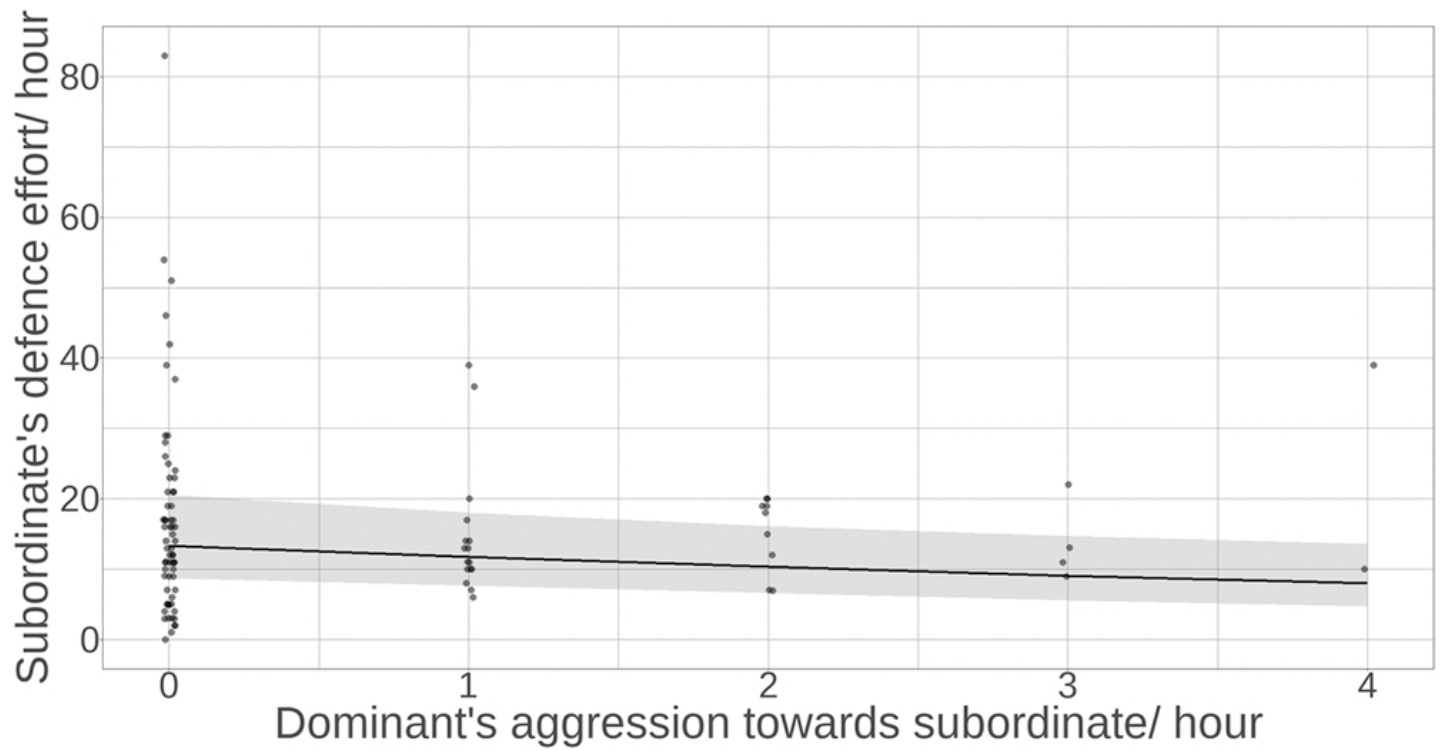


Fig. 3. The relationship between subordinates' observed defence effort and the rate of aggression of the dominant males in MS groups. The values plotted correspond to predicted values (marginal effects) held constant at their proportions. The shaded area represents the pointwise 95% confidence interval for the fitted value. Jitter is included in the observed data points.

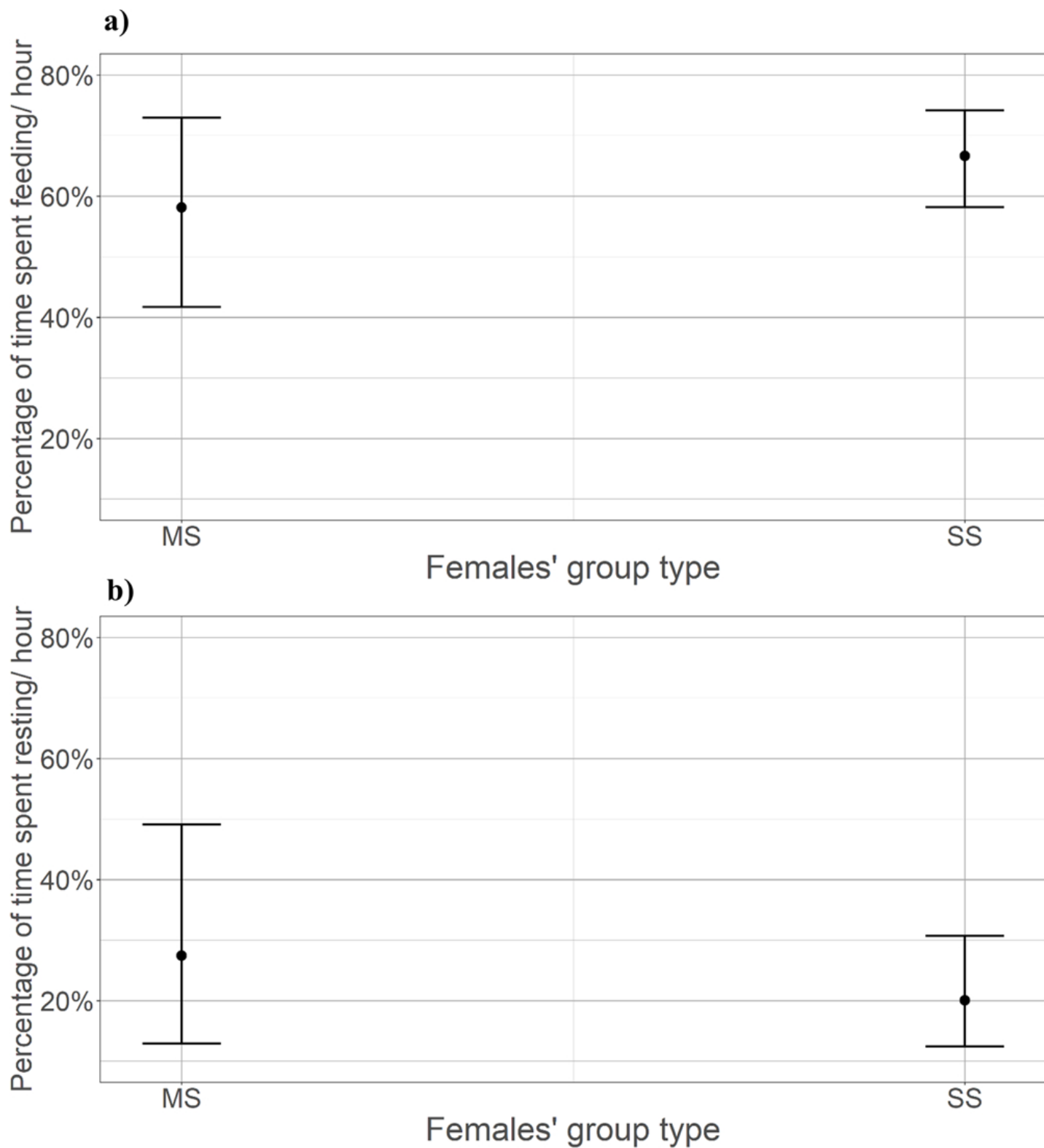


Fig. 4. Relationship between the **(a)** feeding and **(b)** resting activity budget per hour of females and their group type – multi-male MS, or one-male SS. The values plotted correspond to predicted values (marginal effects) held constant at their proportions, and the bars represent standard error (SE).

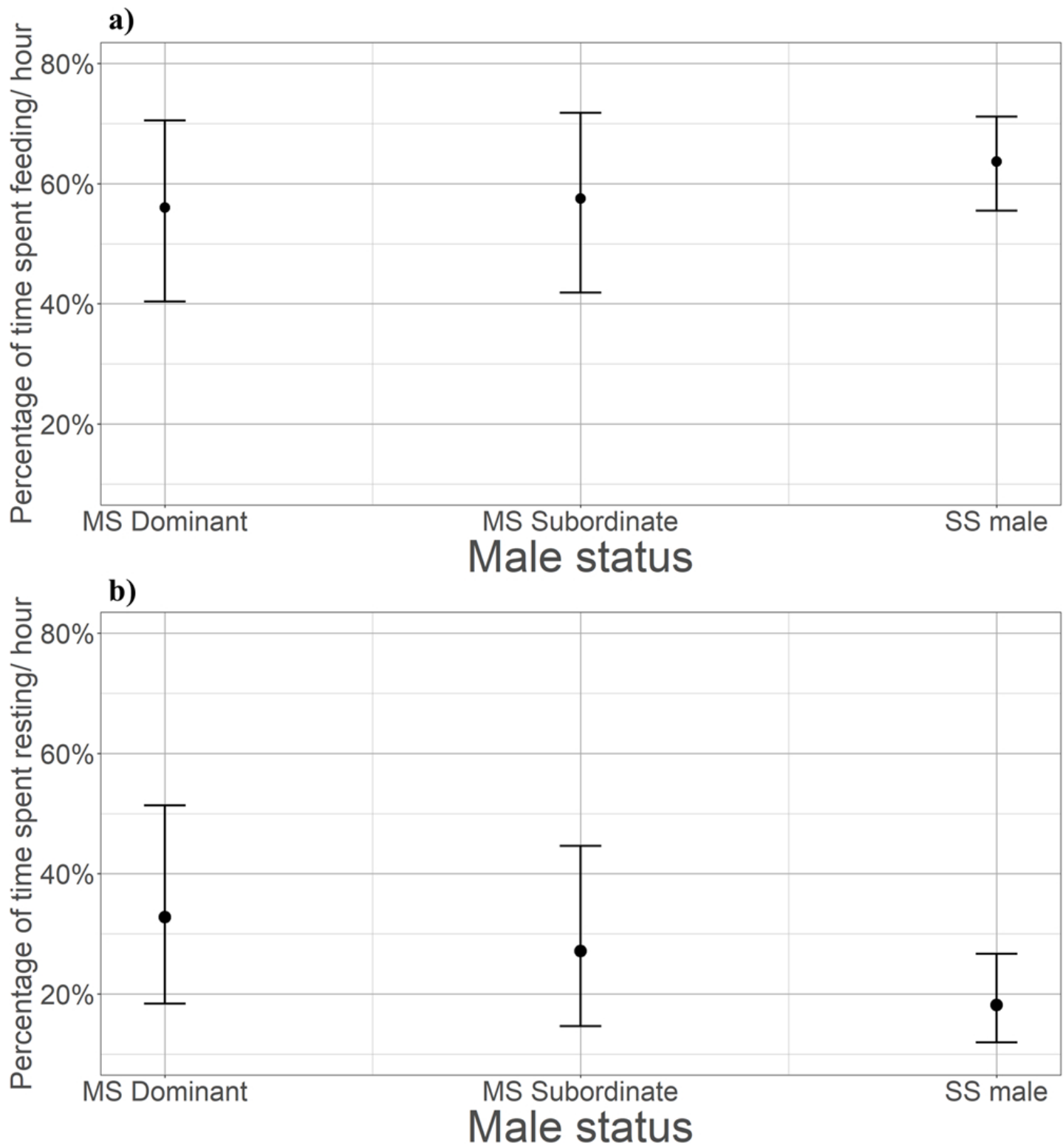


Fig. 5. Relationship between the **(a)** feeding and **(b)** resting activity budget per hour of males and male status. The values plotted correspond to predicted values (marginal effects) held constant at their proportions, and the bars represent standard error (SE).

Group type	Group ID	Total group size	Breeding females			Young		Foals		Group history		
			n	range	core	n	range	n	range	First seen	Last seen	Notes
MS	Gozen & Nagaoka	5.2	2.8	2–3	2	0.0	0	0.4	0–2	2016	Alive in '20	
MS	Nanba & Tennoji	7.3	4.3	2–6	2	0.0	0	1.0	0–2	2016	2018	Group captured (except Nanba)
MS	Kamakura & Zama	9.0	3.3	4–5	4	0.7	0–1	2.0	1–3	2016	2018	Zama disappeared (died). Kamakura predated afterwards
MS	Takaoka & Uozu ^a	6.0	2.4	2–3	2	0.4	0–1	1.2	1–2	2016	Alive in '20	
SS	Toki  ¹	5.2	2.0	1–3	1	0.6	0–1	0.8	0–2	2016	Alive in '20	Seki formed own group. Gujyo became bachelor
SS	Hakata	7.7	3.0	2–4	2	0.7	0–1	2.0	0–4	2016	2018	Nijo disappeared (died). Hakata predated afterwards. Unnan took over the females
SS	Kitakami	7.0	4.0	1–7	1	0.0	0	1.5	0–3	2016	2018	Natori and Kurihara formed own group in 2017. Kujiin became bachelor in 2018. Kitakami group captured
SS	Aso	4.7	3.3	2–5	0	0.0	0	0.3	0–1	2019	Alive in '20	Lost all the females of 2018. New group in 2019
SS	Daisen	5.0	3.5	2–5	1	0.0	0	0.5	0–1	2017	Alive in '20	
SS	Hirosaki	5.5	4.0	4	4	0.0	0	0.5	0–1	2019	Alive in '20	Hirosaki formed a MS group in 2017. Became bachelor in 2018 and followed Takaoka & Uozu group, before forming own group in 2019
SS	Harajuku	14.3	8.7	6–13	5	0.3	0–1	4.3	3–5	2016	2018	Group captured
SS	Hitachi	10.7	5.0	3–7	3	2.0	2	2.7	2–4	2016	2018	Group disappeared
SS	Kobe	9.3	6.0	5–7	4	0.7	0–1	1.7	1–2	2016	2018	Male predated
SS	Nagoya	8.3	5.0	4–6	1	1.3	0–2	1.0	0–1	2016	2018	Group captured
SS	Nanba	8.0	5.0	5	0	0.0	0	2.0	2	2019	2019	Group captured
SS	Takamatsu	7.3	4.0	4	4	0.3	0–1	2.0	0–3	2016	2018	Male predated
SS	Seki	3.0	2.0	1–3	1	0.0	0	0.0	0	2018	2019	Group captured
SS	Unnan	5.0	3.0	0–5	0	0.0	0	1.0	0–2	2019	Alive in '20	Formed a SS group in 2017 but became bachelor in 2018. New group in 2019 (Hakata females)

^a ¹These groups had each a female born in 2016 that hasn't dispersed and had a foal in 2020.

Table 1

The composition and history of the 18 (4 MS, 14 SS) focal groups followed for behaviour analysis between May–August 2018, May–July 2019, and July 2020. Groups were named after their stallion(s). Group type was determined based on the number of males still present in the group at the start of behavioural observations in 2018. Breeding females include adult females (≥ 4 yr. old), young females (2–4 yr. old) that have dispersed or joined the group, and young females that didn't disperse but have produced offspring while in the natal group. Young horses are natal fillies (immature females) and colts (immature males) ≥ 1 and < 4 yr. old. Average (n) total group size, number of breeding females, young and foals in the groups were calculated from the number of horses observed during each field season. Female core represents the number of females continuously associating with the same stallion, from the time of the group's first observation to its last.

Response	Fixed Effects	Estimate	SE	Z	P
Female group size	(Intercept)	2.78	0.46	5.99	< 0.001
	Group type (SS)	1.42	0.46	3.06	< 0.01
Number of females' joint events (S1) and leave events (S2)	(Intercept)	0.75	0.33	2.29	< 0.05
	Stability measure (S2)	-0.41	0.35	-1.17	0.24
	Year 2017–2018	0.17	0.25	0.68	0.50
	Year 2018–2019	0.35	0.41	0.85	0.40
	Year 2019–2020	-0.26	0.36	-0.72	0.47
	Year 2020–2021	0.76	0.40	1.89	0.06
	Stability measure (S1):				
	Group type (SS)				
	Stability measure (S2):				
	Group type (SS)	0.41	0.36	1.14	0.26
	(Intercept)	-0.15	0.13	-1.15	0.25
Net change in female membership (S3)	Group type (SS)	0.03	0.13	0.20	0.84
	Year 2017–2018	0.32	0.12	2.61	< 0.01
	Year 2018–2019	0.34	0.20	1.68	0.09
	Year 2019–2020	0.31	0.17	1.79	0.07
	Year 2020–2021	0.02	0.13	0.15	0.88
Foaling rate	(Intercept)	0.39	0.07	5.67	< 0.001
	Group type (SS)	-0.10	0.09	-1.18	0.24
Males' defence effort	(Intercept)	2.33	0.16	14.22	< 0.001
	Male status (MS subordinate)	0.34	0.18	1.91	0.06
	Male status (SS male)	0.45	0.12	3.89	< 0.001
	(Intercept)	3.18	0.13	25.11	< 0.001
Male-to-female aggression	Group type (SS)	-0.35	0.16	-3.07	< 0.01
	(Intercept)	0.08	0.36	0.23	0.82
	Male status (MS subordinate)	-0.62	0.13	-4.69	< 0.001
	Male status (SS male)	0.12	0.31	0.40	0.69
	(Intercept)	0.51	0.35	1.45	0.15
MS subordinate help vs. dominant's aggression	Group type (SS)	-0.31	0.30	-1.03	0.31
	(Intercept)	2.59	0.22	11.77	< 0.001
	Dominant's aggression	-0.13	0.05	-2.74	< 0.01
Females' feeding activity	(Intercept)	0.81	0.36	2.23	< 0.05
	Group type (SS)	0.36	0.35	1.04	0.30
	Nr. females	-0.11	0.05	-2.31	< 0.05
Females' resting activity	(Intercept)	-0.97	0.48	-2.04	< 0.05
	Group type (SS)	-0.41	0.47	-0.88	0.38
Males' feeding activity	(Intercept)	0.88	0.38	2.34	< 0.05
	Male status (MS subordinate)	0.06	0.09	0.66	0.51
	Male status (SS male)	0.32	0.35	0.91	0.36
Males' resting activity	Nr. females	-0.17	0.06	-2.64	< 0.01
	(Intercept)	-1.29	0.45	-2.87	< 0.01
	Male status (MS subordinate)	-0.27	0.11	-2.54	< 0.05
	Male status (SS male)	-0.79	0.40	-1.97	< 0.05
	Nr. females	0.15	0.07	2.19	< 0.05

Table 2

Summary of statistics for 13 separate generalized linear mixed models (GLMMs), used to compare the female group size and stability, foaling rate, males' defence effort, male-to-female aggression, dominant-to-subordinate aggression, and activity budget in multi-male (MS) and one-male (SS) groups. Significance is set at $P < 0.05$.