

## ORIGINAL RESEARCH

# Condition-dependent maternal sex allocation in horses can be demonstrated using a biologically relevant, multivariable condition measurement

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## Keywords

sex allocation; sex ratio; horse; evolutionary biology; reproduction; Trivers-Willard Hypothesis; multivariate model.

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## Introduction

Condition-dependent sex allocation is predicted when offspring of one sex has more variable survival and reproductive success, which are affected by parental inputs. Therefore, females would benefit from producing the sex with more variable survival and reproductive success when in better condition and vice versa when in poor condition (Trivers-Willard Hypothesis, TWH; Trivers & Willard, 1973). The rationale is that in polygynous species, for example, only high-quality males gain the opportunity to breed (Clutton-Brock & Huchard, 2013; Trivers & Willard, 1973). However, while investment in a male offspring provides the greatest return on maternal investment when successful, this is a high-risk strategy, as only the most robust male offspring will have an opportunity to reproduce. The conservative option is a female offspring that will perpetuate the genotype, but at a much lower rate, but more than an unsuccessful male, who is unlikely to breed. Therefore,

## Abstract

Maternal condition is influenced by multiple variables that individuals experience at seasonal and local levels, and thus condition-dependent sex allocation is likely also multifactorial. Here, we test the Trivers-Willard Hypothesis (TWH) using a multivariable approach on a dataset of thoroughbred mare breeding records. There is no sex ratio variation when examined at univariable level, mirroring the usual approach to test condition-dependent sex allocation. Conversely, the multivariate model shows multiple variables interact to influence the likelihood of producing a male. Mare and management variables that represent better body condition is associated with an increase in likelihood of a male offspring. The magnitude and direction of sex ratio skew correspond closely with predicted mare energy balance, consistent with TWH predictions. Our findings therefore support the TWH and show that, while sex allocation is multifactorial, maternal energy status emerges as the dominant driver. The additive and agonistic interactions of different variables demonstrate that a multifactorial approach should be a key consideration in sex allocation studies.

mothers with more to invest ('good condition') would be advantaged by producing a male offspring, as extra resources invested could yield more grand offspring, if he was one of the successful breeding males.

When Trivers and Willard proposed their hypothesis, maternal condition was used loosely to refer to a mother's ability to invest. Many different measures of maternal conditions have been used (e.g. dominance, range quality, previous reproduction, age, weight, density, group, litter size, condition, time of breeding season, time of insemination, food), and have produced inconsistent results (Cameron, 2004). Earlier studies also focused on investigating a single, or small number of maternal condition measures at a time, expecting that these would represent 'condition'. Few considered several maternal condition measures simultaneously (see studies reviewed in Cameron, 2004). Since maternal condition can be influenced by different environmental variables simultaneously, the outcome will likely be influenced by the combination of variables that each

individual female was exposed to at seasonal, local, and individual levels. Therefore, a multivariate approach considering multiple condition variables would be more suitable for indexing maternal ability to invest.

The condition-dependent sex allocation model has been applied across different species and produced mixed results because the biology (i.e. size and growth rate of offspring, survival, litter size), mating system (degree of polygyny), and social characteristics (transmission of maternal rank, philopatry, matriline structure) did not always fit the assumptions of the TWH (Armitage, 1987; Cameron, 2004; Hewison & Gaillard, 1999; Kruger *et al.*, 2005; Lloyd & Rasa, 1989; Nygrén & Kojola, 1997; Schwartz & Hundertmark, 1993; Silk *et al.*, 1981; Simpson & Simpson, 1982). It is vital that the TWH is tested in species that fit all the TWH assumptions (that 1) parental condition influences offspring condition, (2) offspring condition advantages persist into adulthood, and (3) condition advantages differentially assists the reproductive success of the reproductively variable sex (Hewison & Gaillard, 1999) as other aspects of biology change the predictions of the model resulting in a number of revisions to the condition-dependent model (e.g. Local Resource Competition/Enhancement, density dependence) (Allainé, 2004; Komdeur, 1994, 1996; Kruuk *et al.*, 1999; van Schaik & Hrdy, 1991; West, 2009). Furthermore, many tests of the TW Model have relied on small datasets, which may produce aberrant results (Festa-Bianchet, 1996). Therefore, a comprehensive test of the TWH would consider these factors (multivariate measurement of condition, meet the model's assumptions and a multi-year test on a large number of individuals), and ideally test lifetime reproductive success rather than a shorter window of reproduction.

The horse is a suitable model to test the TWH as it is a polygynous species (Linklater, 2000; Linklater & Cameron, 2009), with minimal sexual dimorphism, and neither sex is more costly to produce just because of its higher growth rates (Feh, 1990). The confounding effect of litter size on sex ratio adjustment is limited in the horse as they only produce a single offspring at a time. Nonetheless, the variance in reproductive success is higher in males than females (Hewison & Gaillard, 1999; Hoogland & Foltz, 1982). The survival of male offspring is more severely affected by poor maternal nutritional conditions (Monard *et al.*, 1997). These characteristics enable clear assumptions to be formed in terms of sex differences in the direction of sex bias.

Due to the regulations associated with the Thoroughbred racing industry in New Zealand, the lifetime production data of Thoroughbred horses are well documented and extensive (multi-year data of a large number of individuals). Multiple mare environment and management variables influencing the nutritional condition and maternal investment potential of the mare can be derived from the production data, providing multivariate measurement of conditions and enabling multi-year tests on large numbers of individuals. The structure of the thoroughbred breeding industry mean that variables, such as mare age and parity, can be used to account for the documented endometrium and placenta degenerative changes associated with age and parity which can affect foeto-maternal exchange of nutrients (Derisoud *et al.*, 2021; Wilsher & Allen, 2003, 2012).

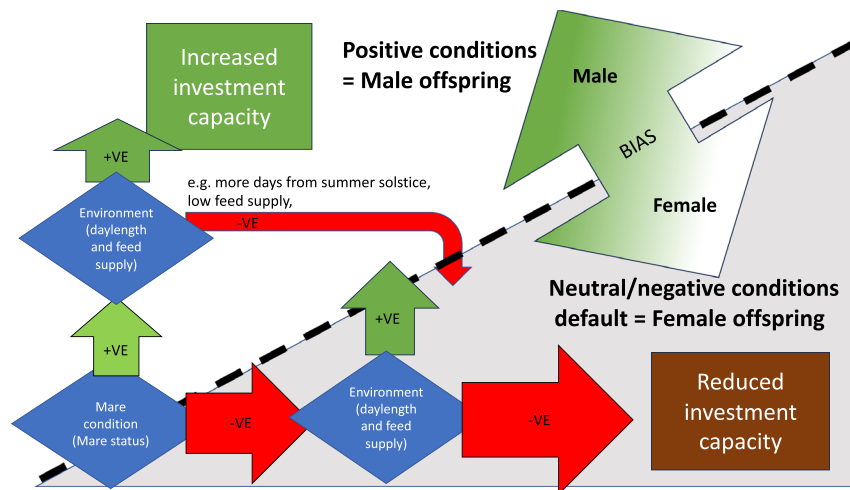
Thoroughbred breeders in New Zealand aim to have foals born as close to the official birth date of August 01. To achieve this, breeders will attempt to mate mares as early in the breeding season as possible, which differs from the natural, or evolutionary programmed, reproductive pattern of the horse to have reproduction coincide with summer solstice (increased day length/photoperiod) when pasture/feed supply would be at its greatest (Chin *et al.*, 2020; Williams *et al.*, 2012). Therefore, the date of last service (conception date) provides a measure of the deviation of conception from the biological ideal conception date near summer solstice. Across the breeding farms in New Zealand, the management strategies for the different mare class (non-pregnant, pregnant, and lactating) are relatively homogeneous between farms, with breeders prioritizing feed supply in relation to reproductive status of the mare (Bengtsson *et al.*, 2018; Gee *et al.*, 2017; Rogers *et al.*, 2007). Thus, reproductive status provides a descriptor of the long term (previous year) and short term (within months of mating) feed supply for that mare. Within New Zealand's pasture-based management system non-pregnant/lactating mares are kept at a lower body condition score than lactating mares and are under greater short term and sustained nutritional challenge due to lower pasture mass on offer and greater stocking density compared to lactating mares. At the time of conception, despite adequate pasture supply, lactating mares experience a negative energy balance that peaks around day 15–35 of lactation, at the time period when many lactating mares are mated. A conceptual model of the interaction of these production variables on mare condition and sex ratio adjustment is presented in Fig. 1.

The aim of this study was to test the TWH and the hypothesis that sex allocation is multifactorial using (1) the horse as a suitable model, (2) a large multi-year dataset, and (3) a multivariate approach in examining the maternal, environment, and management variables within Thoroughbred stud book records that reflects maternal nutritional status and investment capacity. In conclusion, our study shows that no single variable revealed sex ratio variation, but that biologically relevant multifactorial analysis on both horse-specific and management variables showed significant effects in support of the TWH. Incorporating multiple variables that consider the multiple influences on female condition are essential for testing for sex ratio effects that may have been missed if only a single variable had been used.

## Materials and methods

### Study population

In New Zealand, the Thoroughbred industry operates on a pasture-based system where pasture quality and growth are seasonal. The commercial breeding season is short (September–November) and is displaced from the natural breeding season due to the commercial pressure to produce foals close to 1 Aug (universal birthdate of Thoroughbred racehorses in Southern Hemisphere) (Chin *et al.*, 2021a, 2021b; Gee *et al.*, 2017). Under pastoral management, the feed availability and environment conditions can be different for mares mated earlier versus later in the season (Chin, 2018).



**Figure 1** A conceptual model of the interactions between the Thoroughbred horse production variables reflecting mare condition, mare's investment capacity and sex ratio adjustment.

## Data collection

The breeding records of all Thoroughbred stallions covering more than 10 mares during the 2005/06 to 2015/16 breeding seasons and the lifetime breeding records of all mares covered by these stallions were extracted from the New Zealand Thoroughbred Racing website (<https://loveracing.nz/stud-book/search.aspx>). The records for stallions that mated less than 10 mares are not included due to largely incomplete breeding records and no foal sex records available. The stallion details available from the downloaded records were name, year of birth, and for each mating, the mare's name and year of birth, the last mating date, mating outcome (became pregnant, pregnancy loss or carried to term, survival of foal), and foal sex. The stud farm and stud farm location (region) were obtained from the Register of Thoroughbred stallions of New Zealand (NZ Thoroughbred Breeders Association (Inc)) for each of the years examined. The lifetime breeding records of mares mated by the stallions were extracted to derive parity information (parity 0, nulliparous/multiparous) and cross validate data. The mare information available were name and year of birth, sire mated to, parturition date, mating outcome, and foal sex. These two datasets were combined to provide the full database used in the analysis. Mare name, date of birth, and sire mated to were used as primary keys to merge datasets and cross validate data.

## Mare reproductive information

Each year, mares were categorized according to mating outcome from the previous season. Lactating mares were mares that had foaled in the current season, nulliparous mares were mares first presented for breeding (parity 0, nulliparous) and a non-lactating mare was a mare that had not produce a foal due to pregnancy loss or was not mated in the previous season. Mare age was determined based on year of birth and the year

when mating occurred. Foaling and conception interval (FCI) was calculated for lactating mares by calculating the number of days between the last mating date of the current pregnancy and the parturition date of the pregnancy from last year's mating.

## Statistical analysis

Data visualizations and descriptive analysis were used to examine the data, check for errors, and examine the distribution of the data. Outliers (values outside of three standard deviations) and observations with unrealistic values (i.e. negative FCI) were removed. Foal sex was coded as a dichotomous variable (1 (male)/0 (female)) within the database. All variables were assessed for collinearity using spearman rank correlation. Strong collinearity was observed between mare age and parity ( $R^2 = 0.81$ ,  $P < 0.001$ ). Therefore, only age was used in the model.

The structure of data was examined, there were multiple observations per individual mare and stallion. Different mares and stallions were mated across each year and thus each conception occurred under a different combination of variables. Seasonal distribution of observations for the different groups of mare reproductive status (lactating, non-lactating, and nulliparous) were examined using histograms. When examined at a daily or weekly interval across the different mare reproductive status, the number of observations within each mare reproductive status group were low, or there were consecutive days where no conceptions were recorded.

The binomial outcome of offspring sex was investigated using logistic regression using the generalized linear model in 'lme4' package within Rstudio. Separate models were constructed for all mares and lactating mares as some variables are exclusive to lactating mares. The variables considered are presented in Table 1. All variables were initially examined using univariate analysis. Significance of each variable was

**Table 1** Variables considered during the model building procedure of the generalized linear models for estimating the binomial outcome of foal sex in New Zealand Thoroughbred mares mated between 2005 and 2015

Variables	Levels and unit
<b>All mares</b>	
Region	North Island, South Island (Factor)
Season	Year (Factor), 2005–2015
Mare age (years)	≤12, ≥13 (Factor)
Month of conception	Month, September–December (Factor)
Month of parturition	Month, August–December (Factor)
Reproductive status	Non-lactating (mare that failed to conceive the previous season) Lactating (mares with foals) Nulliparous (mare being mated for the first time)
<b>Lactating mares</b>	
Region	North Island, South Island (Factor)
Season	Year (Factor), 2005–2015
Month of conception	Month, September–December (Factor)
Month of parturition	Month, August–December (Factor)
Month of parturition in the previous season	Month, August–December (Factor)
Mare age	≤12, ≥13 (Factor)
Foaling to conception interval	1 (<15 days postpartum) 2 (16–35 days postpartum) 3 (>35 days postpartum)

obtained using Chi-square test (Wald's). Variables identified in the univariate analysis with  $P < 0.20$  were then added as fixed effects into the multivariate model in a forward, stepwise approach. For each new variable being retained in the model, model fit was compared between the new model and the previous model.

To describe variations associated with stallions and farm environment at which the stallions were based, stallions were included in the model as random effects. Time of conception and foaling were first modelled as continuous variables as days followed by using week. Using either day or week as a continuous variable led to convergence issues due to low sample sizes and lack of observations for some predictors which prevented coefficient estimates from being calculated, and resulted in large standard error around the estimate, and complete- or quasi-separation of observations.

The model output using week at univariable level and within the multivariable model without sire as random effects revealed clustering of estimates that reflected the month grouping. This indicated an underlying temporal feature of climate, feed supply, and management that was associated with month. Thus, month was subsequently used as the unit for time of conception and foaling as it reflected the underlying temporal and biological pattern and was not associated with the convergence issues faced when using either day or week.

To investigate the population sex ratio, the proportion of male foals was calculated for each year by reproductive status, month of conception, month of parturition and age group. To report the within month (conception) variation in gestation

length and parturition date and the respective variation in sex ratio was investigated by grouping mares by both parturition and conception month and the gestation length and proportion of male foals were calculated. To investigate the dynamic relationship between sex ratio and maternal energy status a parallel analysis of sex ratio and energy balance dynamics of lactating mares was conducted. Lactating mares were grouped by their months of parturition for the previous pregnancy and foaling to conception interval and the proportion of males were calculated. Foaling to conception interval indicates the stage of lactation when conception occurred. The energy balance data of Thoroughbred mares were adapted from Chin *et al.* (2020) and overlaid with the sex ratio of lactating mares in this study.

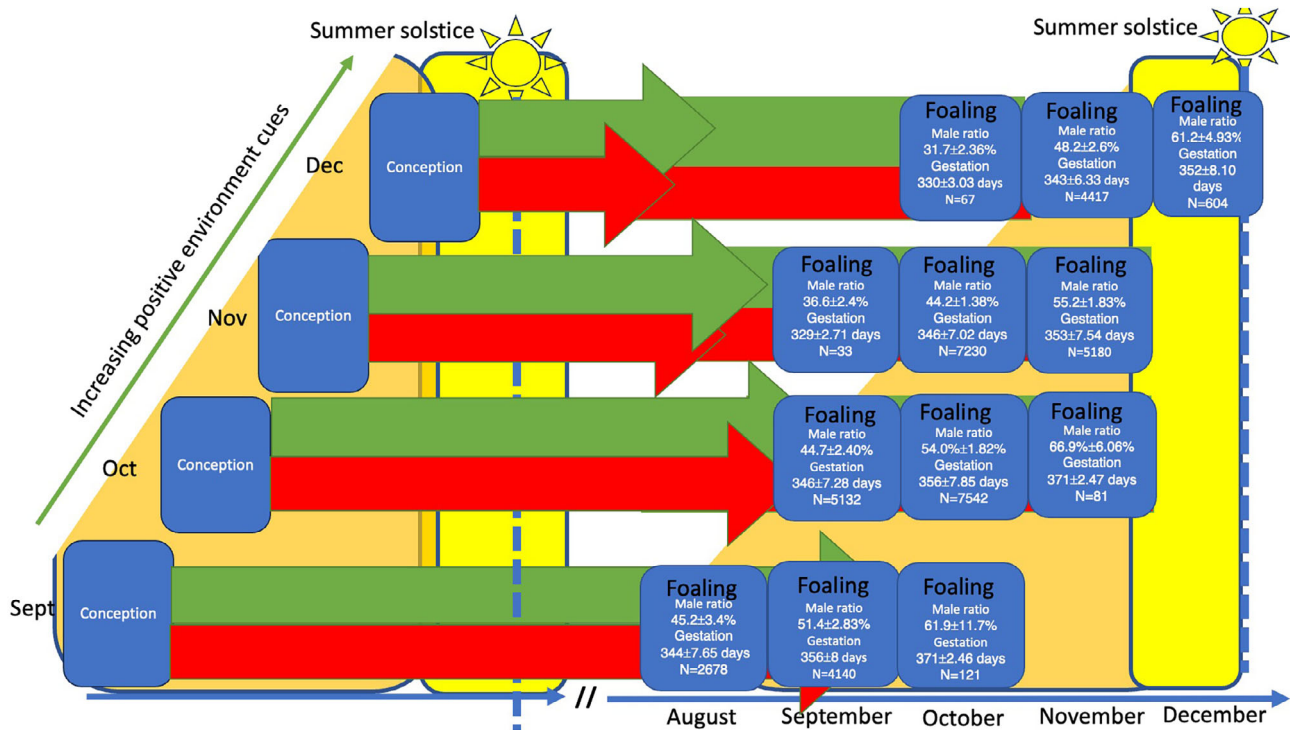
All data manipulation and analysis were conducted in RStudio (version 1.2.5033 ©2009–2019 Rstudio, Inc., Boston, MA, US).

## Results

After removing outliers and observations with incomplete records, there were 37 369 foal sex observations contributed by 12 991 mares and 221 stallions over a 10-year period. Mares were represented by 2 (1–4) foals and produced 2 (1–2) foals in consecutive breeding seasons. The sires were represented in  $4.3 \pm 2.8$  seasons. Within a season, a stallion would be represented by  $21.6 \pm 18.4$ ,  $12.7 \pm 8.3$ , and  $6.3 \pm 4.9$  progeny from lactating, non-lactating, and nulliparous mares, respectively. Across all seasons investigated, the percentage of male foals was  $49.7 \pm 0.05\%$ . At the univariable level, there was no difference in the population sex ratio by reproductive status, month of conception, month of parturition and age (Table S1). There was a trend for the percentage of males to increase as the foaling season progressed ( $R^2 = 0.84$ ,  $P < 0.001$ ). The interaction between conception month, foaling month, gestation length, and sex ratio are presented in Fig. 2. Foaling month delayed with conception month. Within a given month of conception, the gestation length and therefore the foaling month varied and there was a greater proportion of mares which had longer gestation length and therefore gave birth later which produced a male foal (Fig. 2).

The results for the multivariate model (all mares) for odds ratio of conceiving a male are presented in Table 2. Increase in mare age and a later conception month lowered the odds of conceiving a male. Having a foal at foot (i.e. lactating at the time of conception) increased the odds of a male offspring (Table 2). In an expanded model describing production variables describing maternal investment associated with a colt foal, month of birth was included to describe a longer gestation length associated with a colt foal, reflecting the increased maternal investment (Table 3). In this model a colt foal was associated with the mare giving birth later in the season. In lactating mares, longer FCI lowered the odds of a male offspring. The odds of a male were relatively lower in later months of previous parturition and this reduction in odds was only significant in November (Table 4).

In the parallel analysis of sex ratio and energy balance dynamics of lactating mares, the pattern of sex ratio fluctuation followed a similar pattern to the energy balance fluctuation in



**Figure 2** The variation in gestation length of mares in a given conception month, across conception months and the respective foaling month, and proportion of males born.

mares that gave birth in October and November (Fig. 3a,b). A decrease in the proportion of males were observed with a decline in energy balance and vice versa when the energy balance increased. A more constant sex ratio pattern was observed in mares that gave birth in August and September, with a very marginal (2%) increase for mares that gave birth in September across increasing FCI (Fig. 3a). In mares that foaled in August mares, the proportion of males decreased for conceptions more than 35 days after parturition (Fig. 3a). For conceptions between 15 and 35 days post-partum, the degree of female skew corresponded with the magnitude of energy deficit depending on the mares last parturition date, which increased from August to November (Fig. 3a,b). This pattern, however, was not observed in the other foaling to conception interval groups.

## Discussion

The overall population sex ratio was 1:1 across the 10 years examined, as expected, since condition dependent models predict variation at the individual level, not for populations. When examined at the univariable level there were virtually no sex ratio variations between mares of different reproductive status, month of conception and age except, an increase in proportion of males were observed as the months of parturition progressed. However, when all mare variables were considered using a multivariate approach, the variables together were significantly associated with the conception being a male. This

supports the concept that the determination of offspring sex is multifactorial and is a function of multiple variables reflecting cues from the internal and external environment that there is capability to meet the maternal investment required. The temporal signalling of which may be both immediate at the time of mating and in some cases cumulative over several months prior to mating. Stud farms attempt to keep mares in good body condition to maintain reproductive performance therefore the variation in body condition score can be less than in feral horses (Gee *et al.*, 2017). However, due to seasonal pasture availability and lactation energy demands, the energy status and thus, the body reserve can still vary considerably and may not be reflected in subcutaneous fat mobilization as there are evidence that horses preferentially mobilize visceral fat reserves and lean body mass when in good condition (Dugdale *et al.*, 2011; Lawrence *et al.*, 1992; Manso Filho *et al.*, 2008; Pagan *et al.*, 2006). Therefore, offspring sex bias can be less likely to be predicted with just body condition score and requires a multivariate approach.

The multivariate model shows non-lactating mares were less likely to produce males than lactating mares. In lactating mares, mating at 15–35 days and >35 days post-partum reduced the odds of male offspring. These findings reflect that prior to mating non-lactating mares are expected to be under greater short term, and sustained nutritional challenge (lower pasture mass on offer and greater stocking density) compared to lactating mares, due to commercial management conditions (Bengtsson *et al.*, 2018; Gee *et al.*, 2017). Lactating mares

**Table 2** Mare level variables retained in the generalized linear model associated with the odds of conceiving a male in New Zealand Thoroughbred mares mated in 2005–2015

Variables	Coefficient (SE)	OR (95% CI)	P-value	Wald's test
Month of conception				<0.001
September		Ref		
October	−0.269 (0.042)	0.76 (0.70–0.94)	<0.001	
November	−0.680 (0.053)	0.51 (0.46–0.56)	<0.001	
December	−0.975 (0.067)	0.38 (0.33–0.43)	<0.001	
Age (years)				0.028
<5		Ref		
6–14	−0.098 (0.037)	0.91 (0.84–0.97)	0.008	
>14	−0.098 (0.044)	0.91 (0.83–0.99)	0.027	
Status at conception				0.603
Non-lactating		Ref		
Foaled	0.260 (0.087)	1.30 (1.09–1.54)	0.003	
Nulliparous	−0.063 (0.108)	0.94 (0.76–1.16)	0.558	
Parturition month: status				<0.001
Non-lactating: September	0.364 (0.074)	1.44 (1.25–1.66)	<0.001	
Foaled: September	0.076 (0.074)	1.08 (0.93–1.25)	<0.001	
Nulliparous: September	0.420 (0.099)	1.52 (1.25–1.85)	<0.001	
Non-lactating: October	0.698 (0.083)	2.01 (1.71–2.36)	<0.001	
Foaled: October	0.511 (0.078)	1.67 (1.43–1.94)	<0.001	
Nulliparous: October	0.723 (0.104)	2.06 (1.68–2.52)	<0.001	
Non-lactating: November	1.186 (0.095)	3.27 (2.72–3.94)	<0.001	
Foaled: November	0.942 (0.085)	2.57 (2.17–3.03)	<0.001	
Nulliparous: November	1.221 (0.120)	3.39 (2.68–4.29)	<0.001	
Non-lactating: December	1.630 (0.164)	5.10 (3.70–7.03)	<0.001	
Foaled: December	1.483 (0.146)	4.41 (3.31–5.86)	<0.001	
Nulliparous: December	2.260 (0.291)	9.58 (5.41–19.95)	<0.001	

Odds ratios are relative to the reference value, which is 1 (Ref = 1).

mated between 15 and 35 days post-partum would be at their greatest energy deficit (Chin *et al.*, 2020). These modelled results support the TWH, where mares in better condition are more likely to conceive a son.

We also found that nulliparous mares were least likely to produce males. This might be because the capacity of nulliparous mares to accommodate foetal growth is restricted, as nulliparous mares have less foeto-placental area and a limited intra-uterine space, and therefore give birth to lighter foals (Wilsher & Allen, 2003). Additionally, young mares (<5 years) are also likely to be investing energy into their own growth and have recently finished a racing preparation and thus, be in leaner condition. Studies in other ungulates have reported both nulliparous and older females producing more females (i.e. wild asses) (Saltz, 2001; Saltz & Rubenstein, 1995), or, conversely, more males were produced with increasing maternal age (e.g. reindeer; Weladji *et al.*, 2003). Species difference in reproductive biology may predict this variation as fecundity is reported to increase with age in mountain goats (Hamel *et al.*, 2016). In roe deer and white-tailed deer, fertility increased from yearling to adults (DelGiudice *et al.*, 2007; Flajšman *et al.*, 2017) and the allocation of maternal resources into reproduction increased with age (Flajšman *et al.*, 2017). However, the pattern could also be explained by condition resulting from multiple simultaneous influences, as we report here, such that a single variable is inconsistent in predicting

offspring sex. Although results vary between species, maternal age and reproductive biology (e.g. nulli/multiparous) appear to be a consistent index for the capacity for resource allocation by the mother. It is thus vital to understand the biology of the species and what the changes in the measures reflect when investigating sex ratio and incorporate this into predicted sex ratio effects.

Although lactational demand and energy deficit begin to decline after 35 days, conceptions at more than 35 days post-partum also decreased the odds of producing males. Commercial Thoroughbred stallions mate a large number of mares during a heavily condensed commercial breeding season. This provides considerable pressure to achieve pregnancy within one oestrous cycle, using one mating, in an attempt to maintain a high breeding efficiency (Chin *et al.*, 2021a). Therefore, if mares were mated after 35 days it implies either a prolonged post-partum anoestrus or increased number of cycles required to achieve pregnancy, both being indicators of sub-fertility. Alternatively, as mares are still in energy deficit after 35 days, albeit in a smaller magnitude, there can be continued mobilization of body reserves, which combined with lower body condition at parturition may be sufficient to signal limited capacity for maternal investment (Cameron & Linklater, 2007).

Multivariate results and population sex ratio both reflect an increase in proportion of male in later parturition months from August to December. Based on the foaling and conception

**Table 3** Mare level variables retained in the generalized linear model associated with the binomial outcome of foal sex in New Zealand Thoroughbred mares mated in 2005–2015

Variables	Coefficient (SE)	OR (95% CI)	P-value	Wald's test
Parturition month				<0.001
August		Ref		
September	0.364 (0.074)	1.44 (1.25–1.66)	<0.001	
October	0.698 (0.083)	2.01 (1.71–2.36)	<0.001	
November	1.186 (0.095)	3.27 (2.72–3.94)	<0.001	
December	1.630 (0.164)	5.10 (3.70–7.03)	<0.001	
Month of conception				<0.001
September		Ref		
October	−0.268 (0.042)	0.76 (0.70–0.83)	<0.001	
November	−0.678 (0.053)	0.51 (0.46–0.56)	<0.001	
December	−0.972 (0.067)	0.38 (0.33–0.43)	<0.001	
Age (years)				0.028
<5		Ref		
6–14	−0.098 (0.037)	0.91 (0.84–0.97)	0.008	
>14	−0.098 (0.044)	0.91 (0.83–0.99)	0.027	
Status at conception				0.038
Non-lactating		Ref		
Foaled	0.261 (0.087)	1.30 (1.09–1.54)	0.003	
Nulliparous	−0.063 (0.108)	0.94 (0.76–1.16)	0.559	
Parturition month: status				0.027
September: foaled	−0.289 (0.099)	0.75 (0.62–0.91)	0.004	
October: foaled	−0.188 (0.095)	0.83 (0.69–0.99)	0.049	
November: foaled	−0.244 (0.100)	0.78 (0.64–0.95)	0.015	
December: foaled	−0.147 (0.198)	0.86 (0.59–1.27)	0.458	
September: nulliparous	0.056 (0.121)	1.06 (0.84–1.34)	0.643	
October: nulliparous	0.025 (0.118)	1.02 (0.81–1.29)	0.835	
November: nulliparous	0.035 (0.132)	1.04 (0.80–1.34)	0.790	
December: nulliparous	0.630 (0.321)	1.88 (1.00–3.52)	0.049	

Results are presented as odds ratio (OR 95% CI) associated with a colt foal. Odds ratios are relative to the reference value, which is 1 (Ref = 1).

distribution in this study, mares that foaled later have conceived later and therefore experienced longer daylength and warmer temperature at time of conception (Chin *et al.*, 2021a). As the ambient temperature and pasture growth increases and the cold stress decreases from winter to summer months, the environmental cues become more positive as the commercial breeding season progresses from September to December (Chin *et al.*, 2021a). Especially for mares that gave birth in December, where conceptions occurred between November and December which is within the natural breeding season. Therefore, mares would be exposed to optimal environmental cues for resource abundance. This may explain the male bias observed in December (61%). These findings support our initial hypothesis that displacing mating from the natural breeding season can affect the sex ratio. In support of this hypothesis, increase in temperature and solar radiation 1–2 weeks pre-conception increased the proportion of males born in cows (Roche *et al.*, 2006). Cows are non-seasonal breeders indicating the climatic cues for nutrition quality is consistent across some species. As day length increases, the inhibition on hypothalamic–pituitary axis decreases (Nagy *et al.*, 2000) allowing the secretion of luteinizing hormone and leptin to increase during summer months (Altinstaat *et al.*, 2008; Buff *et al.*, 2007; Cebulj-Kadunc & Cestnik, 2008; Hart

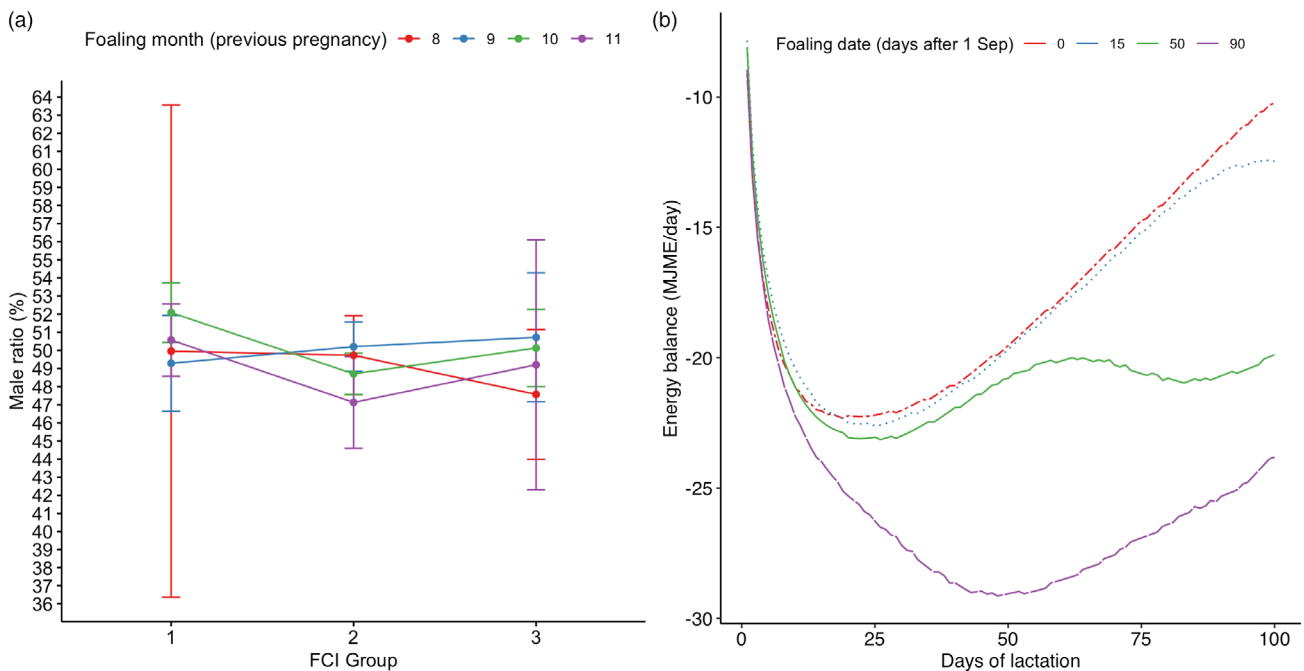
*et al.*, 1984; Thompson *et al.*, 1986). These hormones were reported to enhance the maturation of oocyte (Craig *et al.*, 2004; van Tol *et al.*, 2008; Younis *et al.*, 1989) and maturation stage of oocyte at fertilization were found to influence sex ratio of resulting embryos. Oocytes that were matured *in vitro* for longer before fertilization resulted in significantly more male embryos (Gutiérrez-Adán *et al.*, 1999). The male biased sex ratio in later months observed in this study could be mediated through this mechanism. A lack of season effect in this study also suggests that the entrained response towards periodic environmental cues is a strong modulator as between year variation in weather and pasture growth did not modify the seasonal sex ratio pattern.

Comparing between mares that foaled in the same month, those that foaled later had a longer gestation length and a greater proportion of these mares produced males. The gestation length observed in this study has previously been reported in other Thoroughbred horses (Satué *et al.*, 2011). Likely due to the commercial breeding season being advanced compared to the natural breeding season, the gestation length of Thoroughbred horses in this study appears to be longer than other breeds (Chin, 2022). This is consistent with the evolutionary adaptation theory for horses to foal closer to the period of optimal environmental conditions and abundant food supply as

**Table 4** Mare level variables in the generalized linear model associated with the binomial outcome of foal sex in lactating Thoroughbred mares mated in 2005–2015

Variables	Coefficients (se)	OR (95% CI)	P-value	Wald's test
<b>Parturition month</b>				
August		Ref		<0.001
September	0.034 (0.083)	1.03 (0.88–1.22)	0.683	
October	0.473 (0.097)	1.60 (1.33–1.94)	<0.001	
November	0.964 (0.108)	2.62 (2.12–3.24)	<0.001	
December	1.487 (0.162)	4.42 (3.22–6.08)	<0.001	
<b>Last mating month</b>				
September		Ref		<0.001
October	−0.166 (0.073)	0.85 (0.73–0.98)	0.024	
November	−0.477 (0.091)	0.62 (0.52–0.74)	<0.001	
December	−0.704 (0.110)	0.50 (0.40–0.61)	<0.001	
<b>Previous parturition month</b>				
July		Ref		<0.001
August	−0.531 (0.357)	0.59 (0.29–1.18)	0.137	
September	−0.557 (0.359)	0.57 (0.28–1.16)	0.120	
October	−0.690 (0.363)	0.50 (0.24–1.02)	0.057	
November	−0.888 (0.369)	0.41 (0.19–0.85)	0.016	
December	−0.838 (0.449)	0.43 (0.18–1.04)	0.062	
<b>Foaling to conception interval</b>				
<15 days		Ref		0.019
16–35 days	−0.104 (0.043)	0.90 (0.83–0.98)	0.014	
>35 days	−0.171 (0.062)	0.84 (0.75–0.95)	0.006	

Results are presented as odds ratio (OR 95% CI) associated with a colt foal. Odds ratios are relative to the reference value which is 1 (Ref = 1).



**Figure 3** (a) The percentage (Mean, 95% CI) of males born by New Zealand Thoroughbred mares mated <15 days (FCI group 1), 15–35 days (FCI group 2), >35 days (FCI group 3) post-partum in either August (Red), September (Blue), October (Green) or November (Purple) and (b) the estimated energy balance during 0–100 days of lactation in New Zealand Thoroughbred mares gave birth on either 0 (Red), 15 (Blue), 50 (Green), 90 (Purple) days after 1 Sep. (b) Adapted from Chin *et al.* (2020).

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possible to increase the chance of offspring survival (Monard *et al.*, 1997). The difference in gestation length within conception month were between 7 and 20 days and is longer than the unadjusted gestation length difference between foal sex (2 days) reported within the literature (Chin *et al.*, 2021a; Elliott *et al.*, 2009; Morel *et al.*, 2007; Platt, 1978). This finding indicates presence of sex allocation at the individual level and individuals that were able to increase their gestational investment were more likely to conceive a male. This indicates a difference in ability to invest which could be due to variation in resources available to the individual that is related to social hierarchy and management factors not accounted for in this model. In herd animals, dominance can reflect ability to access resources as well as associated with better maternal condition (Ceacero *et al.*, 2012; Clutton-Brock *et al.*, 1986; Meikle *et al.*, 1984; Vervaecke *et al.*, 2005), and dominant females were found to be more likely to give birth to males (Clutton-Brock *et al.*, 1984, 1986; Meikle *et al.*, 1984). Thoroughbred mares are kept at pasture in groups (typically 14 (range 3–30) horses per paddock (Rogers *et al.*, 2007)) and paddock sizes can vary within a stud farm (Bengtsson *et al.*, 2018; Rogers *et al.*, 2007). Therefore, the stocking density and subsequently pasture available would differ between groups and farms (Bengtsson *et al.*, 2018; Chin *et al.*, 2019). Within a herd, the ability to access resources would differ between individuals depending on each female's level of dominance. Hence, the model could be improved by information at individual and group level which were not available from stud book data used in this study.

A parallel analysis of sex ratio pattern and energy balance dynamics revealed a trend where the direction and extent of sex ratio skew corresponded closely with the estimated energy balance dynamics. The sex ratio skewed towards more males when energy balance increased, and vice versa when energy balance decreased. This pattern was only observed in mares that gave birth in October and November; however, the variation was limited. Whereas the sex ratio remained relatively consistent across different foaling to conception intervals in mares that foaled in August–September mares. It is possible that the effect of energy balance could be restricted by other variables. The smaller, brief, and consistent energy deficit during lactation in mares that gave birth in August–September may mean the effect on sex ratio was smaller and easily counteracted by other variables, or that mares were able to compensate for the deficit with available reserves. Nevertheless, as the sex ratio modelled corresponds to estimated energy balance at conception, this provides some evidence that adjustment can occur quickly.

## Conclusion

Using a large sample size, the multivariate model demonstrated that offspring sex was significantly influenced by a combination of individual level variables related to condition or ability to invest, and that sex determination is multifactorial. Mares were more likely to produce males under less nutritional challenge and when exposed to positive environmental cues. Collectively, the findings support the TWH where females of

better condition are more likely to produce males, and that sex ratio is driven by a diverse range of maternal variables at seasonal, local, and individual levels that represents the underlying ability of mother to invest into reproduction with energy status being the dominant driver. A clear understanding of the biology of the species is important in identifying the potential set of variables and confounders and when drawing predictions to avoid the sex ratio adjustment being overlooked.

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## Conflict of interest

The authors declare no conflicts of interest.

## Author contributions

Conceptualization: All authors; Data acquisition: Y. Y. Chin, Data analysis: Y. Y. Chin, Data interpretation: All authors; Funding acquisition: E. Z. Cameron, K. J. Stafford; Writing of manuscript: All authors.

## Data availability statement

Data used in this study can be found on <https://loveracing.nz/stud-book/search>

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Percentage of male foals grouped by mare age, reproductive status, month of conception, and month of parturition.