Maternal Investment in Kaimanawa Horses

A thesis presented

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*"**THINK, WHEN WE TALK OF HORSES, THAT YOU SEE THEM PRINTING THEIR PROUD HOOFS I' THE RECEIVING EARTH**"*

Shakespeare, circa 1599, 'Henry V'.

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Photo caption:

C-band in August 1994.

Band stallion, Charly (085), is in the foreground. Mares Celia (132) and Cashew (112) are also visible.

Photo by Wayne Linklater

ABSTRACT

Maternal investment (MI) was studied in Kaimanawa horses, a population of feral horses in central North Island, New Zealand. It is predicted that individual mares will vary their maternal behaviour so as to maximise their own and their offspring's reproductive success. Maternal investment is defined and measured by maternal input, proximal maternal costs and reproductive costs to the mother. The primary maternal input is milk. Time spent sucking is frequently used to measure milk intake based on the assumption that more sucking equates with proportionately more milk intake. A review found little support for this assumption and so I tested whether sucking time predicted milk intake by labelling the milk of thoroughbred mares with tritium and measuring its transfer to foals. No significant predictive relationship was found. Therefore sucking time cannot be used as an index of milk intake and conclusions about differential MI based on time spent sucking may be wrong. A mare's social environment is a significant modifier of her MI. Mares were more protective, and suffered reproductive costs, in bands with more than one stallion or in other circumstances where paternity is uncertain or rates of aggression are high. Despite individual differences in maternal style, mare behaviour was modified by experimental manipulations of the number of stallions in a band. In an unusual event when a mare and her adult daughter lived in the same band, both co-operated in the care of a single foal. Mare age and experience modified maternal behaviour. As mares age they become more successful at raising foals through better-targeted input, but no extra investment. I tested the Trivers-Willard hypothesis (TWH) of sex differential MI. The hypothesis predicts that mothers in better condition should produce more sons, and invest more in their sons, whereas mothers in poorer condition should favour their daughters. I argue that horses are an ideal species on which to test the TWH. We found no sex biased MI on a population level in terms of maternal input, proximal costs or ultimate costs. However, the TWH makes specific predictions about individuals, not populations. Individual mares in poor condition gave birth to more daughters and biased their MI towards daughters. Conversely, mothers in good condition gave birth to more sons and biased their investment towards sons, supporting all the predictions of the TWH. Mares alter their maternal investment in ways that conform to predictions based on maximisation of lifetime reproductive success.

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erupting volcano (Mount Ruapehu) and an ash fall during field work! My constant field companion was Uzuri, who found dead horses, kept me company, kept me warm, reminded me to eat and told me in no uncertain terms when the day had been too long.

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The environment in the Ecology Department (now Ecology Group) provided me with much support. Barbara Just kept finances running smoothly, Jens Jorgenson made some wonderful equipment for the study, and all the rest of the technical staff and especially the secretaries, Erica Reid, Jodi Matenga, Petra van Kan and latterly Rachel Wilson, were always helpful and friendly and made working in the Department a pleasure. Head of Department Dave Lambert was a great student advocate and I thank him and Ed Minot for their support. I got a lot out of BEERS and the Annual Student Colloquium – thanks to all those who contributed. I was also lucky to attend several Conferences during my time at Massey, thanks to the financial support of the Graduate Research Fund and the Ecology Department Development Fund.

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Finally, no research is possible without the subjects, and Kaimanawa horses are wonderful to work with. Even those sub-zero days in wind, rain or snow were filled with enjoyment when working with Kaimanawa horses. On a fine clear day, with green grass, fat horses and young foals in the valley, and with an erupting mountain as a backdrop it is just magic. And it doesn't get much better than that.

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NOTE ON THE TEXT

Each chapter is set out largely in the style of the journal to which it has been submitted. Consequently, there is some repetition, particularly in methods sections, and there are stylistic differences between chapters. In addition, other authors are included in the paper reference. For each chapter, my input was the greatest. I planned the research, undertook the field work, analysed the data and wrote the manuscripts. I was, however, helped by my co-authors. Kevin Stafford, Edward Minot and Clare Veltman were my supervisors. Wayne Linklater was studying the Kaimanawa horses for his own PhD and thereby contributed to most aspects of my study.

All research described in my thesis was approved by the Massey University Animal Ethics Committee.

Introduction

"… INDIVIDUALS WHICH GENERATED OR NOURISHED THEIR OFFSPRING BEST WOULD LEAVE… THE GREATEST NUMBER"

Darwin, 1871, 'The Descent of Man and Selection in Relation to Sex'

Photo caption:
Black Bess (074) pregnant in 1996.
Photo by Wayne Linklater

The maternal behaviour of an individual animal may differ from others of the same species. It is predicted that mothers will invest in their offspring so as to increase their own reproductive success and improve their inclusive fitness through increased reproductive fitness of their offspring (Trivers 1972, Trivers & Willard 1973, Maynard Smith 1980, Clutton-Brock 1991). Maternal investment is defined as "any investment by the parent into the current offspring that increases its chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in future offspring" (Trivers 1972). As such, it incorporates both maternal input, proximal maternal costs and costs to future reproduction (Clutton-Brock 1991). Studies frequently do not consider the three components of maternal investment.

In studies of maternal investment there are several often cited confounding factors that complicate the interpretation of measures of variation (eg. future competition with philopatric offspring, Clark 1978, Silk 1983; litter size, Williams 1979). For example, there are several different ways in which a mother can change her maternal investment if there is more than one offspring per litter, or if she can reproduce more than once in a season. Where she has only one offspring, variation is limited to the form and duration of maternal investment in the single offspring (Williams 1979, Gosling 1986).

Studies on sex differential maternal investment suffer from the influence of confounding variables (Clutton-Brock 1991). It is predicted that, in polygynous species, mothers with more to invest should favour sons, as mothers in better condition would be able to raise a successful son who would sire more offspring than a successful daughter who would be limited to a maximum of one offspring per year. Conversely, mothers with less to invest would be less likely to raise a son that would compete well enough to be able to breed, so that a daughter would raise more offspring than a son would. Therefore, these mothers should invest more in daughters (Trivers-Willard Hypothesis, TWH, Trivers & Willard 1973). Although *prima facie* the TWH appears to apply best to species that are sexually dimorphic as well as polygynous, sexual dimorphism can confound the relationship. In sexually dimorphic species Maynard Smith (1980) showed that it could be an evolutionary stable strategy for mothers to invest more in sons on a population wide basis. However, the Trivers-Willard hypothesis predicts that individual mothers would behave differently toward sons and daughters on the basis of their ability to invest. Therefore studies of polygynous yet sexually monomorphic species with a single offspring are particularly valuable; males do not have obligate faster growth so observed differences will be due to differential maternal investment.

In addition, where one sex is philopatric while the other disperses there are added factors that may influence sex differential maternal investment. Philopatric daughters may inherit their mothers rank (Hiraiwa-Hasegawa 1993), compete with their mothers for mates (Hamilton 1967) or resources (Clark 1978, Silk 1987), and any differences in

investment in males early in life may be compensated for by prolonged low levels of investment in daughters (Clutton-Brock 1991).

The horse as a model for testing maternal investment theory.

Horse populations, which are scattered throughout the world (Linklater 1998) have been the subject of much research (eg. Tyler 1972, Feist & McCullough 1976, Gates 1979, Berger 1986, Duncan 1992). However, although maternal behaviour is known to vary between individuals, there has been little research on causes of such variation, and those studies that have looked at differential maternal investment have found conflicting results. For example, sex differential maternal behaviour was described in some studies (Duncan et al. 1984, Berger 1986), but not found in others (Crowell-Davis 1985, 1986).

Horses are ideal for testing theories of maternal investment for several reasons. Confounding variables are at a minimum. Litter size is constrained at one, both sons and daughters disperse from their natal social groups and sexual dimorphism is minimal (Feh 1990, Willoughby 1974). Extensive research on domestic and feral horse populations (eg. Boyd 1979, Boy & Duncan 1980, Carson & Wood-Gush 1983, Duncan et al. 1984, Crowell-Davis 1985, Berger 1986, Crowell-Davis 1986, Crowell-Davis et al. 1986, Duncan 1992, Martin et al. 1992, Monard et al. 1997) shows that the assumptions of hypotheses differential maternal investment apply. In addition, it is easy to validate research methodology used in feral horses on domestic horses.

Horses are a large and easily identifiable animal with a clearly defined period of maternal investment, which facilitates the study of maternal investment from conception to offspring dispersal. Maternal behaviour varies widely between individual mothers.

My study investigated variation in maternal investment in Kaimanawa horses with a view to answering three questions:

Does the social environment influence maternal investment?

How does mare age and experience influence maternal behaviour?

Do mares change their maternal behaviour in relation to the sex of their offspring?

The study population.

Feral horses, known locally as Kaimanawa horses, live in the Kaimanawa ranges and their surrounding plateaux (64000 ha) in the central North Island of New Zealand (Figure 1). Their range includes the New Zealand Army Training Area near Waiouru, where they have lived since the late 1800s (Rogers 1991). The horse population, its range and the study site are described in Linklater (1998), as is the study area and focal population centred in the lower Moawhango river basin. Fifty five mares and their foals born in 1994, 1995 and 1996 are the focus of this study and some other mares in the population, and foals born in 1997, are used in some analyses.

Figure 1. Location of the study site in the North Island of New Zealand.
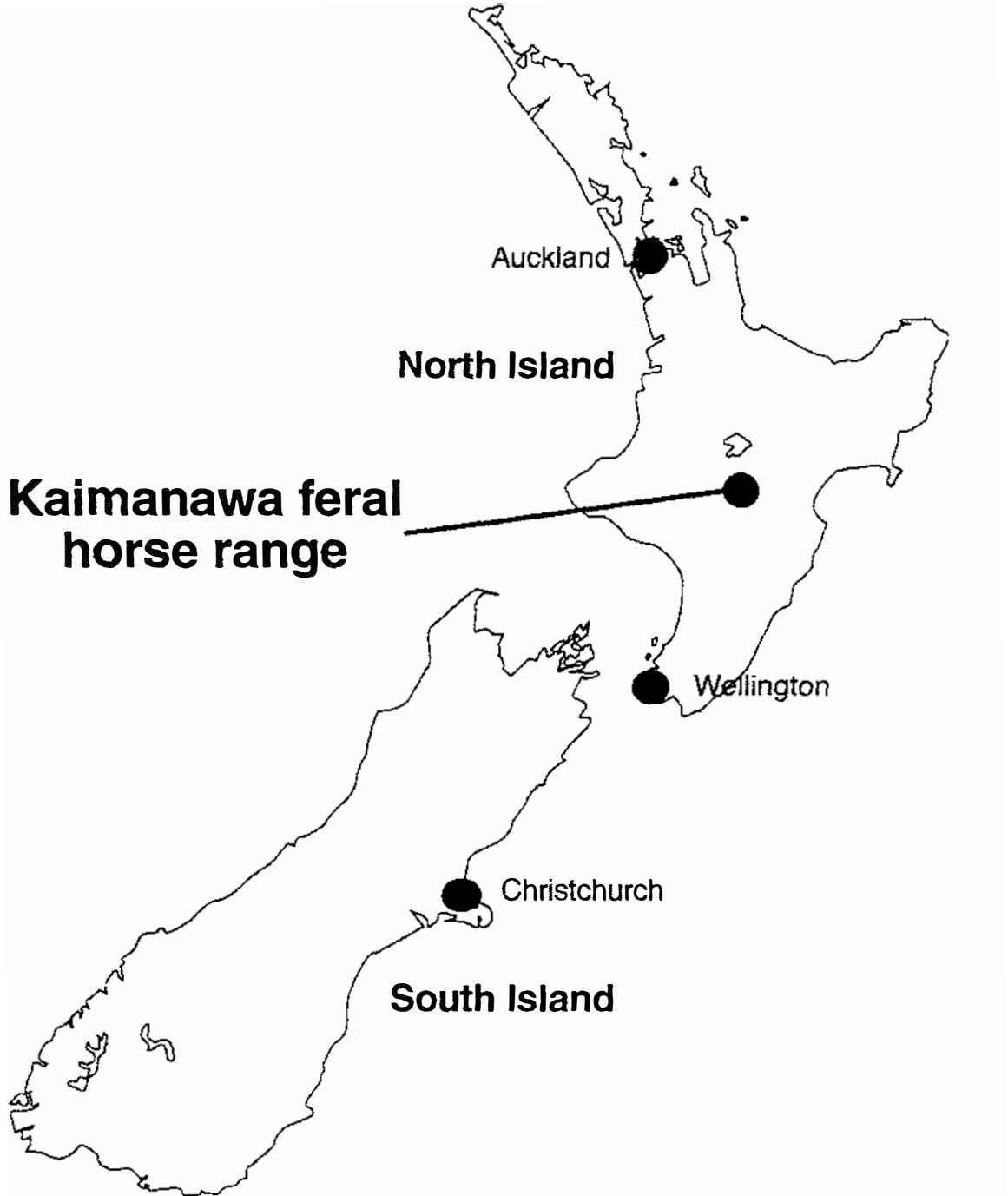
The horses live in stable social groups typically containing one, but sometimes up to four stallions with mares and their offspring. These bands are loyal to a home range that overlaps in part or full with the home ranges of other bands. Both sons and daughters disperse from their natal band, so mares within a band are not closely related. Stallions without bands are found in bachelor groups that have unstable membership. This social structure is similar to that found in all feral horse populations (reviewed in Linklater 1998), and in plains and mountain zebras (Klingel 1972).

The breeding season extends from September to April. Foals are born after an eleven month gestation, with a peak in births between October and December (Fig 2). Foals are suckled for at least 8 months, but are sometimes suckled for up to 2 years and foals continue to live with their mothers until dispersal at around 15 months. Daughters disperse into other bands, whereas sons become bachelors. I studied maternal behaviour from foal conception to dispersal.

Figure 2. Timing of foal births in 1994, 1995, 1996 and 1997.
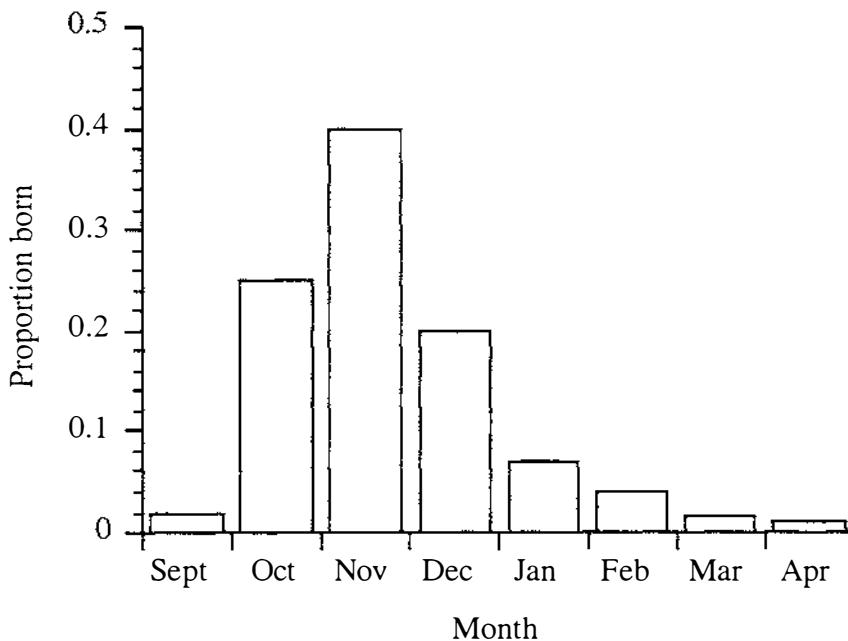
The study.

Studies on maternal investment often use maternal input as an indication of investment. In mammals the primary input is the resources transferred from mother to offspring in the form of milk. However, it is not usually possible to measure milk intake in the field, and time spent sucking is often used as a predictor of milk intake based on the assumption that offspring who suck more get more milk. As recent studies have cast doubt on this

assumption, I begin by reviewing the evidence that time spent sucking can reliably predict milk intake (Chapter 1). I then test whether sucking time predicts milk intake in horses (Chapter 2) by labelling the milk of thoroughbred mares with radioactive isotopes, measuring milk transferred to foals, and sampling their behaviour in a simulation of field measurements.

In the next section I look at the influence of social environment on maternal behaviour. The risk of infant injury or mortality has a significant modifying effect on the behaviour of mothers in many species. In horses, infanticide has been reported, as has increased accidental death rates of foals due to high rate of stallion aggression. I investigate stallion aggression as a modifier of maternal behaviour, particularly in relation to the presence of more than one stallion in a band (Chapter 3). In multi-stallion bands there is a higher rate of aggression, and a lower certainty of paternity. Some mares changed band type and others had their multi-stallion bands experimentally reduced to a single stallion band. Therefore, the influence of the additional stallion on maternal behaviour could be measured.

I document the first reported case of shared suckling in horses (Chapter 4), and investigate the circumstances in which shared suckling or offspring care do or do not occur, and determine if the mares are co-ordinating and co-operating their foal care.

It has been hypothesised that mammalian mothers become more successful at raising offspring as they age due to declining residual reproductive value (Pianka & Parker 1975, Clutton-Brock 1984). An alternative hypothesis suggests that mares become more successful because of greater experience, not because of increased maternal investment (Fairbanks 1996). In Chapter 5, I test predictions from these two hypotheses to determine if mares do become more successful at rearing foals, and whether the success can be attributed to greater effort by older mares, or to the greater experience of older mares.

I then test theories of sex differential maternal investment. In Chapter 6, I outline the assumptions and predictions of the Trivers-Willard hypothesis (TWH) of sex-differential maternal investment. The TWH predicts that mothers in relatively better condition, and therefore with more resources to invest, would be advantaged by producing more sons, whereas females in poorer condition would be advantaged by producing more daughters. I test if there is any variation in birth sex ratios in relation to maternal body condition. In Chapter 7, I extend the hypothesis, as did Trivers & Willard (1973), to determine if mares in better condition invest more in sons and mares in poorer condition invest more in daughters. Other studies of the TWH use population measures of maternal investment. In both chapters I apply the TWH to individual mares, as the TWH predicts that individual mares adjust their strategy in relation to their particular situation.

Finally, I discuss variation in maternal investment in Kaimanawa horses and discuss the implications of these conclusions.

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CHAPTER 1

Suckling Behaviour and Milk Intake

IS SUCKLING BEHAVIOUR A RELIABLE PREDICTOR OF MILK INTAKE? A REVIEW

"THE COW IS OF THE BOVINE ILK,
ONE END IS MOO, THE OTHER MILK"

Ogden Nash, 1931, 'The Cow'.

Photo caption:
Kandy-Floss with foal Popcorn in 1986.
Photo by Elissa Cameron

ABSTRACT

In studies on mammalian parental investment, time spent suckling is often used as a predictor of the milk transferred from mother to infant. It is assumed that the rate of milk transfer is positively correlated with the time spent suckling. However, this assumption has not been tested and empirical studies show conflicting results. Nevertheless, in species in which suckling can readily be observed, time spent suckling is still used to measure milk transfer, although an increasing number of workers recognize that the measure is potentially inaccurate. A meta-analysis on studies that have correlated measures of time spent suckling with milk intake estimates based on weight gain, has revealed a weak positive relationship and significant heterogeneity between studies. Isotope labelling techniques for the measurement of milk transfer independent of behaviour have been in use since the 1970s. The technique has been used particularly to study milk transfer in species in which suckling is difficult to observe. Only one study has attempted to correlate behavioural measures with independent isotope measures, and it found no relationship between the two measures. I suggest that researchers have avoided such a test as it is unlikely that a strong relationship will be found between milk transfer and suckling behaviour, and I discuss the various factors that confound the relationship and contribute to high heterogeneity between studies. Consequently, the assumption that milk transfer can be measured by time spent suckling has inadequate empirical foundation, and needs to be tested using isotope labelling methods.

Chapter reference:
Cameron, E. Z. in press. Is suckling behaviour a reliable predictor of milk intake? A review. *Animal Behaviour*

INTRODUCTION

Although parental investment is defined in terms of costs to the parent in future reproductive success (Trivers 1972), it is usually measured as the resources allocated to the offspring, or parental input (Evans 1990; Birgersson & Ekvall 1994). In mammals, the most obvious parental inputs are the resources transferred during lactation (Pond 1977; Lee 1987), which are more energetically costly than the prenatal costs of gestation (Martin 1984; Clutton-Brock et al. 1989). In studies of mammalian parental investment an estimate of milk obtained by offspring is therefore a fundamental measure of parental input (Martin 1984; Lee 1987; Kretzmann et al. 1993). However, the measurement of milk transferred from mother to offspring is difficult in field situations (Kretzmann et al. 1993), and consequently behavioural measures of suckling have been used to estimate amounts of milk transferred. Such studies assume that all other factors being equal, offspring that suck more obtain more milk (Fletcher 1971; Berger 1979).

The assumption that the time spent suckling is directly proportional to the amount of milk transferred has not been tested (Shackleton & Haywood 1985; Babbitt & Packard 1990b) and has recently been questioned (eg. Anderson & Fedak 1987; Festa-Bianchet 1988; Mendl & Paul 1989; Babbitt & Packard 1990a; Lavigueur & Barrette 1992; Birgersson & Ekvall 1994). Some initial studies found anomalies that suggested that the relationship between suckling and milk transfer may not be as simple as had been assumed (eg. Ewbank 1964; Fletcher 1971; Hall et al. 1978; Berger 1979; Loudon & Kay 1984), and subsequent tests of the relationship have failed to show a significant positive correlation (Mendl & Paul 1989; Birgersson & Ekvall 1994). Consequently, some authors have questioned the reliability and utility of such estimates of milk transfer (eg. Berger 1979; Shackleton & Haywood 1985; Babbitt & Packard 1990 a, b; Alley et al. 1995; Pélabon et al. 1995).

In this study, I review the evidence that suckling behaviour is related to milk transfer in placental mammals and perform a meta-analysis on these data. I suggest reasons why the relationship between suckling behaviour and milk intake might be weak and examine the use of isotope labelling techniques to test the assumption that milk transfer can be estimated by time spent suckling.

TIME SPENT SUCKLING AND MILK INTAKE

A number of studies have correlated time spent suckling with growth rates or weight gain during a suckle bout as indices of milk intake (Table 1). Of the studies that correlated infant growth rates with time spent suckling, only one (5%) found a significant positive relationship between growth and time suckling, three a significantly negative relationship and 16 no significant relationship. However, the relationship between growth and suckling can be obscured when the mother's milk is not the only source of nutrition to the young such as in precocial young that feed on solid food shortly after birth (Martin 1984). Even where milk is the primary source of nutrition other factors such as differences in activity (Duncan et al. 1984; Byers & Bekoff 1990), metabolic rates (Mendl & Paul 1989; Kretzmann et al. 1993), genetic growth potential (Yates et al. 1971) or differences in nutrient assimilation efficiency (Verme 1989), could lead to differences in growth rate despite ingestion of the same quantity of milk (Clutton-Brock 1991). In particular males may be more efficient at the conversion of milk energy into growth (Kretzmann et al. 1993) as testosterone may enhance growth by allowing greater efficiency of nutrient assimilation (Glucksman 1981). In addition, variation in milk quality and composition both between and within individuals (Oldham & Friggens 1989) could cause marked differences in energy intake even if the amounts of milk ingested were similar (Berger 1979).

Studies that have investigated the relationship between time spent suckling and estimates of milk intake by weighing an offspring before and after a suckle bout ('test-

Table 1. Summary of studies that have correlated measures of time spent suckling with estimates of milk intake based on infant weight gain (growth or test-weighing)

Measure	Species	r^a	p^b	n^c	Class^d	Source
GROWTH						
Suckle bout duration	reindeer	**0.04**	NS	7	< 45 d	Lavigueur & Barrette 1992
	human	nd	NS	45		Butte et al. 1985
	human	**-0.28**	NS	46		de Carvalho et al. 1982
Suckle bout frequency	reindeer	**0.54**	NS	7	< 45 d	Lavigueur & Barrette 1992
	reindeer	**0.10**	NS	5	46-100 d	
	sheep	$t_{18}=2.27$	+	19	0-3 wks	Ewbank 1967
	sheep	$t_{18}=0.68$	NS	19	3-6 wks	
	sheep	**0.04**	NS	34	1-2 wks	Fletcher 1971
	sheep	**0.20**	NS	34	3-6 wks	
	sheep	**0.02**	NS	34	7-12 wks	
	human	nd	NS	45		Butte et al. 1985
	human	**0.24**	NS	46		de Carvalho et al. 1982
Total time suckling	reindeer	**0.29**	NS	7	< 45 d	Lavigueur & Barrette 1992
	fallow deer	**0.30**	NS	22	all fawns†	Birgersson & Ekvall 1994
	fallow deer	**0.06**	NS	12	all fawns	Birgersson & Ekvall 1997
	fallow deer	0.63	NS	12	> 2 wks	
	Soay sheep	**0.04**	NS	14	1988	Robertson et al. 1992
	Soay sheep	**-0.64**	-	14	1989	
Scans	mouse	**-0.53**	-	16	all litters	Mendl & Paul 1989
	cat	**-0.43**	-	24	all litters	
TEST-WEIGHING						
Suckle bout duration	fur seal	**0.49**	+	73		Trillmich 1986
	human	**-0.14**	NS	46		de Carvalho et al. 1982
	human	nd	NS	44		de Carvalho et al. 1983
	human	nd	NS	45		Butte et al. 1985
	human	$F_{1.49}=1.9$	NS	52		Drewett et al. 1989
	human	**0.13**	NS	73	to 3 mths	Dewey et al. 1991
Suckle bout frequency	human	**0.19**	NS	46		de Carvalho et al. 1982
	human	nd	+	44	0-2 wks	de Carvalho et al 1983
	human	nd	NS	44	1 mth	
	human	nd	NS	45		Butte et al. 1985
	human	$F_{1.49}=7.8$	+	52		Drewett et al. 1989
	human	**0.58***	+	52		
	human	**0.41**	+	27		Rattigan et al. 1981
	human	**0.13**	NS	73	to 3 mths	Dewey et al. 1991
	red deer	nd	-	19	food type	Loudon & Kay 1984
	red deer	nd	-	17	food type	Loudon et al. 1983
Total time suckling	human	**0.69***	+	52		Brown et al. 1982
	human	**0.57**	+	52		Drewett et al. 1989
	human	**0.47**	+	76		Imong et al. 1989
	human	**0.24**	+	73	to 3 mths	Dewey et al. 1991
	red deer	nd	+	17	food type	Loudon et al. 1983

a: Correlation reported by author, nd = no data supplied; correlation values in bold are those used in the meta-analysis. One study (fallow deer fawns > 2 weeks) was excluded because the authors used data that were a subset of data they used to calculate the correlation for all fawns.

b: Direction of relationship as reported by author; ns no significant relationship, + significant positive relationship, - significant negative relationship

c: Number of individuals in the study

d: Specific age or other classification on which the test was performed

† Improved to r=0.62 when only fawns over 14 days with multiparous mothers used

* Age of infant taken into account

weighing', eg. Butte et al. 1984, or 'weigh-suckle-weigh', eg. Sadleir 1980) have also shown ambiguous results; 10 (48%) found a significant positive relationship, two found a significant negative relationship and nine found no significant relationship (Table 1). In humans test-weighing can provide an accurate measure of milk transfer as the weighing does not disrupt normal suckling patterns (Arthur et al. 1987). Butte et al. (1984) found that estimates of milk transfer by test-weighing were accurate when compared with estimates from the transfer of radio-isotope labelled milk, although Brown et al. (1982) found that test-weighing underestimated milk transfer when measured against known amounts of milk. In animals other than humans, offspring have normally been kept off their mother for a period before the suckle (eg. Loudon et al. 1984; van der Steen & de Groot 1992), so that the test-weighing interferes with normal suckling patterns (Oftedal 1984; Wright & Wolff 1976; Pettigrew et al. 1985). In addition, errors associated with calculating a weight differential that is a small proportion of the animal being weighed can be large (Pettigrew et al. 1985). Consequently, test-weighing procedures tend to provide less accurate estimates of milk intake in animals other than humans.

Despite the problems associated with both methods, these provide the best available estimates of the correlation between time spent suckling and milk intake. Consequently, I combined these data and performed a meta-analysis to identify significant trends within the data set. Meta-analyses are a quantitative technique for combining the results of statistical tests on previous studies that have looked at the same relationship, in this case correlations between weight gain and time spent suckling, taking into account the sample size of each study. The goal of the meta-analysis is to determine if there is an overall significant trend in the studies and to examine the heterogeneity of the data set (Hedges & Olkin 1985). I found that there was a significant positive relationship between all measures of time spent suckling and estimates of milk intake based on weight gain, but that less than 10% of the variation was explained by the relationship between the two variables (Table 2). Of the behaviour sampling methods, only suckle bout frequency and total time suckling were significantly correlated with estimated milk intake, with total time suckling providing better estimates. Similarly, estimates of milk intake based on growth were not significantly correlated with suckling time, but test-weighing and suckling time were correlated. Even when we combined the best behaviour estimate, total time suckling, with the best intake estimate, test-weighing, less than 25% of the variation in milk intake was explained by suckling time ($\rho^2=0.24$, P<0.001, N=4). In addition, there was significant heterogeneity between studies, indicating that the relationship between weight gain estimates of milk intake and time spent suckling varies. Consequently, although there was a significant positive relationship, the power to predict milk intake from time spent

suckling was low and was different between species, between studies and even within studies of the same species (Table 2).

Table 2. Results of meta-analysis on studies that have correlated measures of time spent suckling and estimates of milk intake based on weight gain in infants (growth or test-weighing)

Measure	n^a	ρ (weighted r)	ρ^2	Q (heterogeneity)
By behaviour sampling				
Suckle bout duration	5	0.12	0.01	22.51***
Suckle bout frequency	10	0.24***	0.06	13.89**
Total time suckling	9	0.38***	0.14	35.99***
By intake estimate				
Growth	15	-0.01	0.00	26.58*
Testweighing	11	0.36***	0.13	44.40***
ALL	26	0.24***	0.06	103.22***

a: Number of studies used in meta-analysis
stars refer to degree of statistical significance; *P<0.05, **P<0.01, ***P<0.001

CONFOUNDING FACTORS

Methods Used to Measure Suckling Behaviour

Different measures of suckling behaviour have been used to estimate milk transfer . These measures may not all accurately reflect the amount of time spent sucking (Mendl & Paul 1989; Green 1990), or may measure different aspects of suckling behaviour (Loudon et al. 1983). A meta-analysis of previous studies shows that estimates of milk intake by different methods produce quantitatively different results (Table 2). The accuracy of scan samples (Altmann 1974) depends on the frequency of scans and the accuracy with which scans represent the animal's behaviour (Higgins et al. 1988; Mendl & Paul 1989), and studies to date have found negative correlations with estimates of milk intake (Table 1). Neither suckle bout duration (Festa-Bianchet 1988) nor suckle frequency (Fletcher 1971) appear to be accurate measures of milk transfer alone (Loudon et al. 1983). Extrapolations to larger time periods may also reduce the accuracy of estimates of milk transfer (Woolridge et al. 1985), and measurement of daytime suckling may not reflect differences that occur at night (Duncan et al. 1984).

The definition of measures varies between studies (Carson & Wood-Gush 1983). In particular, suckle bouts have been defined in different ways, sometimes including small breaks during suckles (eg. Tedman & Bryden 1979; Becker & Ginsberg 1990;

Campagna et al. 1992) and sometimes excluding them (eg. Crowell-Davis 1985; Smith-Funk & Crowell-Davis 1992), sometimes defined by nipple contact (eg. Duncan et al. 1984) or by the position of the head (eg. Crowell-Davis 1985); and a definition of what is meant by a suckle bout is not always included (eg. Tyler 1972). Definitions will vary between species with variation in their suckling behaviour, but need to be made explicit if comparisons are to be made.

Variation in the Suckle Ability of Offspring

There can be considerable variation in milk intake and time spent suckling between individual mother-infant dyads in the amount of milk transferred and the amount of time spent suckling (eg. Tyler 1972; Woolridge et al. 1982; Babbitt & Packard 1990b). For example, weaker offspring may take longer to drain teats but receive no more milk (Clutton-Brock 1991). Higgins et al. 1988 found that larger Stellar sea lion, *Eumetopias jubatus*, pups received more milk (measured by isotope transfer), but that the time spent suckling (scan sampling) was similar, suggesting that larger pups were more efficient at getting milk without modification of their sucking time. Similarly, male lambs of bighorn sheep, *Ovis canadensis*, spent less time sucking but grew faster than did female lambs, suggesting that males obtained more milk per second while sucking and emptied the udder more quickly (Festa-Bianchet 1988).

Sucking behaviour may also vary within an offspring. A growing animal can become more efficient at receiving milk without adjusting the time that is spent on the nipple (Pollitt et al. 1981). Consequently, with increasing age offspring suck for a similar amount of time but receive more milk (Stellar sea lions, Higgins et al. 1988; horses *Equus caballus*, Carson & Wood-Gush 1983, humans, Drewett & Woolridge 1979).

The milk intake of human babies varies from day to day such that estimates of milk intake from a single day may be unrepresentative of intake at that age (Woolridge et al. 1985). Even during an individual human suckle bout there is a progressive reduction in intake per suck and increase in the pauses between sucks (Woolridge et al. 1982). In addition, milk intake per suckle bout declines across the day in humans (Butte et al. 1983).

Offspring can vary milk intake either by varying the time spent sucking, or by increasing the frequency and intensity of sucking while on the nipple (Brake et al. 1982a). Young rat, *Rattus rattus*, pups varied their milk intake during a suckle (measured by tongue cannula), but not the time they spent on the nipple, suggesting they were varying their sucking behaviour during the bout (Brake et al. 1982a).

Motivation for Suckling: Hunger and Learning

Suckling behaviour may be indicative of the offspring's motivation for sucking rather than the amount of milk transferred (Mendl & Paul 1989). Offspring of mothers on low

nutrition diets or with low milk production suckled their offspring more frequently (bighorn sheep, Berger 1979; peccary *Tayassa tajacu*, Babbitt & Packard 1990b; red deer *Cervus elaphus*, Loudon et al. 1983; Loudon & Kay 1984; Soay sheep *Ovis aries*, Robertson et al. 1992), suggesting that offspring return to suckle sooner owing to elevated levels of hunger (Loudon et al. 1983; Loudon & Kay 1984). Malnourished rats spent more time in the nest nursing offspring (Hall et al. 1978) and suckling behaviour was enhanced in kittens, *Felis cattus*, whose mothers had been injected with a lactation-inhibiting drug (Martin 1986). These examples suggest that increased hunger led to an increase in suckling frequency, or greater total time on the nipple even though less milk was probably received.

Recent sucking experience can also influence the future suckling behaviour of offspring (Brake et al. 1982b). Early experiences in rat pups can influence the frequency and intensity with which they attach to the nipple. Brake et al. (1982b) found that highly successful feeding experiences lead to increased sucking, and perhaps the ingestion of more milk. Rat pups can adjust their sucking behaviour according to the fullness of their stomach, how long since they last suckled, and whether or not milk was received on the last suckle (Brake et al. 1982a).

Mother's Experience, Physiology and Ability to Release Milk

The experience of the mother and her ability to release milk may vary (Butte et al. 1985; Green 1990; Birgersson & Ekvall 1994). For example, Green (1990) suggested that inefficient milk ejection may in part explain the longer nursing times shown by older bison, *Bison bison*, cows. Conversely, young inexperienced mothers may suckle their offspring longer with no increase in the amount of milk transferred (eg fallow deer *Dama dama*, Birgersson & Ekvall 1994).

Not all sucklings result in the let-down of milk (pigs *Sus scrofa*, Newberry & Wood-Gush 1985), and milk flow does not begin immediately the offspring begins to suckle (cattle *Bos taurus*, Whittemore 1980). Consequently, the milk obtained during one long suckle is likely to be greater than that transferred in two shorter suckles of the same total time and so short suckles are less likely to be primarily nutritive in function (Shackleton & Haywood 1985). Conversely, two suckle bouts that each include a let-down of milk will provide more milk than a single bout of the same total length that included a period of non-nutritive sucking.

The amount of milk released can vary at each nipple. In humans, milk yield can vary between the breasts (Drewett & Woolridge 1981) and piglets sucking anterior nipples receive more milk than posterior end sucklers for no extra time spent sucking (Newberry & Wood-Gush 1985; Pluske & Williams 1996).

Non-Nutritive Suckling

Non-nutritive suckling, where the infant is making nipple contact and sucking but without milk being transferred, is widespread, having been observed in most species in which suckling has been investigated (eg. horse, Tyler 1972; Crowell-Davis 1985; cattle, Lidfors et al. 1994; Rushen & de Pasillé 1995; pig, Rushen & Fraser 1989; Boe & Jensen 1995; snow leopard, *Uncia uncia* McVittie 1978; Japanese macaque, *Macaca fuscata* Tanaka 1992; human, de Carvalho et al. 1982; Jain et al. 1987). Infants of many species will suck regardless of the presence of milk (eg. hand reared, donkey *Equus asinus*, cattle, sheep, goat *Capra hircus*, Wolff 1968a; mother reared, cats, Koepke & Pribram 1971) and in humans a large portion of each suckle bout is non-nutritive (Wolff 1968b). Periods of non-nutritive suckling may be determinable by a change in the rate of sucking per second (Wolff 1968a; Drewett & Woolridge 1979; Rushen & Fraser 1989; Lidfors et al. 1994; Tanaka 1992). For example, in pigs milk is only transferred during fast sucking, regardless of the time spent in slow sucking, so the onset of fast sucking marks the onset of nutritive suckling.

In humans much of the time spent suckling is primarily non-nutritive, and the non-nutritive suckling may be important for stimulating milk production (de Carvalho et al. 1982). Non-nutritive pre-suckling massage of the sow's nipple has been shown to determine the future availability of milk at that nipple in pigs (Boe & Jensen 1995), and pre- and post-suckling stimulation may also occur in beef cattle (Lidfors et al. 1994).

In addition, it has been hypothesized that some suckles are primarily social rather than nutritive (Adler et al. 1958; Robbins & Moen 1975; McVittie 1978; Gauthier & Barrette 1985; Shackleton & Haywood 1985), because offspring often seek their mothers and suck when distressed or alarmed (Lent 1971). Therefore, it is likely that suckling satisfies emotional as well as nutritional needs (Adler et al. 1958; Carson & Wood-Gush 1983), and nipple contact does not necessarily mean that milk is being transferred. Furthermore, in cattle non-nutritive suckling rates are higher in calves receiving a lower ration of milk (Rushen & de Pasillé 1995).

The accuracy of estimates of milk transfer from suckling behaviour may be enhanced by measuring nutritive suckling only (Tanaka 1992), particularly in those species where nutritive suckling can be distinguished by differences in local sucking rate (Wolff 1968a; Tanaka 1992) or suckling posture (Drewett et al. 1974).

Variation in Milk Composition

Even if the amount of milk actually transferred is known, the energy content of milk must be known before the resources transferred from mother and offspring can be estimated (Berger 1979; Kretzmann et al. 1993). In horses, offspring of mothers fed a high quality diet sucked more than did offspring of mothers on a low quality diet, but the mares on the high quality diet produced greater quantities of more dilute milk (Pagan & Hintz 1986).

Milk yield, quality and composition is known to vary between individuals (Dodd 1957; Johansson & Claesson 1957; Oftedal 1985; Gittleman & Oftedal 1987), in response to environmental conditions, and with age, experience and time (reviewed in Oldham & Friggens 1989). Consequently, an analysis of milk composition is required if energy transfer from mother to offspring, rather than amount of milk, is to be determined.

TESTING IF SUCKLING TIME MEASURES MILK INTAKE

Isotope methods for measuring milk transfer were developed in the late 1960s (MacFarlane et al. 1969), and the accuracy of the measure has been established by measuring the transfer of known amounts of milk (Wright & Wolff 1976; Dove & Freer 1979; Green & Newgrain 1979; Fjeld et al. 1988). Isotope labelling techniques have been used increasingly since the early 1980s, particularly in pinnipeds, marsupials and species that raise their young in dens and underground (Fig. 1). The frequency of studies that assume that milk intake can be estimated by time spent suckling has been static over the last 15 years, although the number of studies that mention that suckling may not be an accurate measure of milk transfer have steadily increased (Fig. 2). Therefore, as recognition that a test of the relationship between time spent suckling and milk intake is required has grown, the technique to test the efficacy of estimating milk transfer from suckling behaviour has been in regular use.

Only one study, however, has attempted to correlate estimates of milk transfer by behavioural and isotope labelling methods (Stellar sea lion: Higgins et al. 1988). No relationship between time spent suckling, measured by scan sampling, and milk intake was found for the four individuals in the study. It has been suggested that scan sampling may not accurately reflect time spent suckling (Mendl & Paul 1989) and when combined with the small sample size it is difficult to draw a conclusion from this study. Further studies correlating time spent suckling and isotope estimates of milk intake are required. In addition, an analysis of milk energy content will make an estimate of energy transfer between mother and offspring, the actual currency of parental cost, possible.

Researchers appear to be avoiding a test of the relationship between time spent suckling and milk intake, and I suggest that there is unlikely to be a significant relationship between time spent suckling and milk transfer owing to variations in suckle ability and motivation of the offspring, rates of non-nutritive suckling, the ability of the mother to release milk and milk composition. I conclude that there is insufficient evidence that time spent suckling provides an index of milk intake. Results obtained from studies assuming that suckling time is proportional to milk intake can only be regarded as tentative until such time as the relationship is empirically tested.

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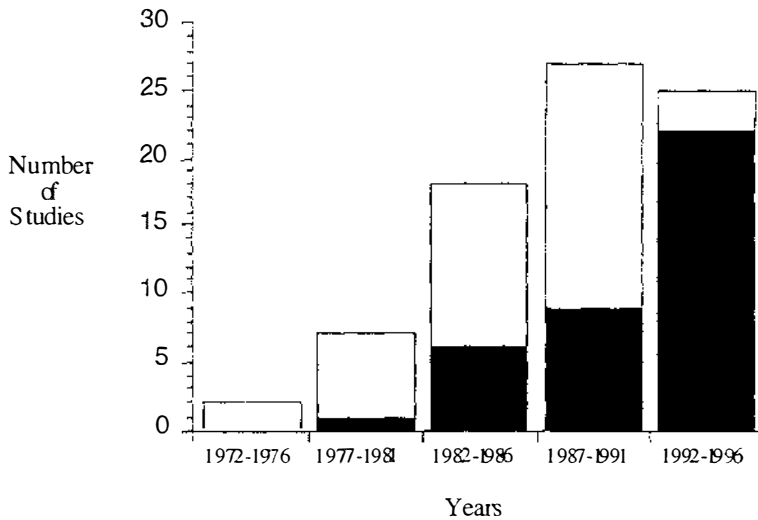
Figure 1. Studies between 1972 and 1996 that have used isotopes to measure milk intake. Filled bars are studies on pinnipeds, marsupials and species that rear their young in dens or underground, mammals in which suckling is difficult to observe. Open bars are studies on easy-to-observe species. Studies used to construct the graph are in Appendix 1.
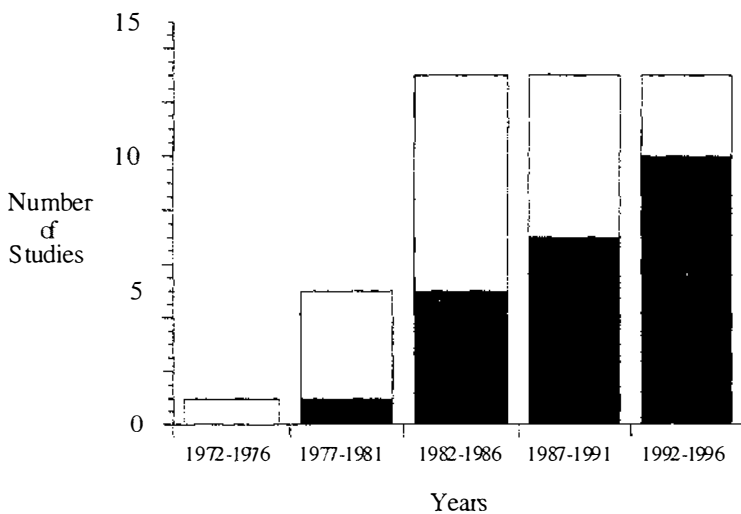

Figure 2. Studies between 1972 and 1996 that have used suckling behaviour to estimate milk intake. Open bars are studies that did not recognize any potential problems with the methods. Filled bars are studies that noted that the measure might not be accurate. Studies used to construct the graph are in Appendix 1.

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APPENDIX ONE

Studies measuring milk intake (MI) by isotope labelling of milk (I) or by time spent suckling (S). Studies using time spent suckling that noted that there were potential problems with the measure are listed as Sp

Common name	Species	MI	Source
African lion	*Panthera leo*	Sp	Pusey & Packer 1994
spotted hyaena	*Crocuta crocuta*	Sp	Pusey & Packer 1994
dog	*Canis domesticus*	I	Green & Newgrain 1979
		I	Oftedal 1984
black bear	*Ursus americanus*	I	Oftedal et al. 1993a
		I	Farley & Robbins 1995
grizzly bear	*Ursus arctos horribilis*	I	Farley & Robbins 1995
polar bear	*Ursus maritimus*	I	Arnould & Ramsay 1994
vervet monkey	*Cercopithecus aethiops*	S	Lee 1984
		S	Lee 1987
rhesus macaque	*Macaca mulatta*	Sp	Gomendio 1990
human	*Homo sapiens*	I	Butte et al. 1983
		I	Coward 1984
		I	Woolridge et al. 1985
		I	Lucas et al. 1987
		I	Fjeld et al. 1988
		I	Butte et al. 1988
		S	Drewett et al. 1989
		I	Bocquier et al. 1991
		I	Butte et al. 1991
		I	Infante et al. 1991
		I	Butte et al. 1992
striped skunk	*Mephitis mephitis*	I	Gittleman & Oftedal 1987
American mink	*Mustela vison*	I	Gittleman & Oftedal 1987
meadow vole	*Microtus pennsylvanicus*	I	Tamarin et al. 1983
		I	Sheridan & Tamarin 1986
prairie dog	*Cynomys ludivicianus*	I	Hoogland et al. 1989
mouse	*Mus musculus*	I	Rath & Thenen 1979
		I	Thornburn & Bailey 1983
		I	Knight et al. 1986
rat	*Rattus norvegicus*	I	Romero et al. 1979
guinea pig	*Cavia porcellus*	I	Weaver et al. 1988
		I	Weaver & Roberts 1990
		S	Künkele & Trillmich 1997
cattle	*Bos taurus*	I	Holleman et al. 1975
zebu cattle	*Bos indicus*	S	Reinhardt & Reinhardt 1981

bison	*Bison bison*	Sp	Green 1986
		S	Wolff 1988
		Sp	Green 1990
muskoxen	*Ovibos moschatus*	I	Holleman et al. 1988
		I	Parker et al. 1990
moose	*Alces alces*	I	Reese & Robbins 1994
red deer	*Cervus elaphus*	S	Kelly & Drew 1976
		S	Clutton-Brock et al. 1981
		S	Clutton-Brock et al. 1982
elk	*Cervus elaphus nelsoni*	I	Robbins et al. 1981
white-tailed deer	*Odocoileus virginianus*	Sp	Gauthier & Barrette 1985
mule deer	*Odocoileus hemionus*	I	Carl & Robbins 1988
fallow deer	*Dama dama*	Sp	Gauthier & Barrette 1985
		Sp	Birgersson & Ekvall 1994
caribou	*Rangifer tarandus*	I	McEwan & Whitehead 1971
		Sp	Lavigueur & Barrette 1992
		I	Parker et al. 1990
pronghorn	*Antilocapra americana*	Sp	Byers & Moodie 1990
scimitar-horned oryx	*Oryx dammah*	S	Pfeifer 1984
dorcas gazelle	*Gazella dorcas*	I	Maltz & Scholnik 1984
Saharan arrui	*Ammotragus lervia*	S	Cassinello 1996
bighorn sheep	*Ovis canadensis*	Sp	Berger 1979
		Sp	Festa-Bianchet 1988
		S	Hass 1990
		Sp	Hogg et al. 1992
domestic sheep	*Ovis aries*	I	Wright & Wolff 1976
		I	Dove & Freer 1979
		I	Villette & Theriez 1983
Soay sheep	*Ovis aries*	Sp	Robertson et al. 1992
ibex	*Capra ibex*	I	Maltz & Scholnik 1984
goat	*Capra hircus*	S	Pickering 1983
		Sp	Alley et al. 1995
mountain goat		I	Carl & Robbins 1988
pig	*Sus scrofa*	I	Pettigrew et al. 1985
		I	Pettigrew et al. 1987
collared peccary	*Tayassa tajacu*	Sp	Babbitt & Packard 1990a
		Sp	Babbitt & Packard 1990b
horse	*Equus caballus*	I	Doreau & Dussap 1980
		I	Oftedal et al. 1983
		Sp	Duncan et al. 1984
		S	Crowell-Davis 1985
		Sp	Berger 1986
		I	Doreau et al. 1986
		I	Doreau et al. 1990
		I	Martin et al. 1991
		I	Martin et al. 1992
		Sp	Smith-Funk & Crowell-Davis 1992
Grevy's zebra	*Equus grevyii*	Sp	Becker & Ginsberg 1990
African elephant	*Loxodonta africana*	S	Lee & Moss 1986
harbor seal	*Phoca vitulina*	I	Bowen et al. 1992
ringed seal	*Phoca hispida*	I	Lydersen et al. 1992
		I	Lydersen & Hammill 1993
harp seal	*Phoca groenlandica*	S	Kovacs 1987b
		I	Lydersen & Kovacs 1996
		I	Oftedal et al. 1996
hooded seal	*Cystophora cristata*	I	Oftedal et al. 1993b
		I	Lydersen et al. 1997
bearded seal	*Erignathus barbatus*	I	Lydersen et al. 1996

crabeater seal	*Lobodon carcinophagus*	I	Green et al. 1993
grey seal	*Halichoerus grypus*	S	Kovacs 1987a
		I	Iverson et al. 1993
		S	Smiseth & Lorentsen 1995
		I	Lydersen et al. 1995
		I	Reilly et al. 1996
Weddell seal	*Leptonychotes weddellii*	S	Tedman & Bryden 1979
		I	Tedman et al. 1981
		I	Tedman & Green 1987
California sea lion	*Zalophus californianus*	I	Oftedal et al. 1987
		I	Ono et al. 1987
		I	Iversen et al. 1991
		Sp	Ono & Boness 1996
		I	Ono & Boness 1996
Stellar sea lion	*Eumetopias jubatus*	I	Higgins et al. 1988
Northern elephant seal	*Mirounga angustirotris*	S	Reiter et al. 1978
		S	Ortiz et al. 1984
		I	Ortiz et al. 1984
		I	Costa et al. 1986
		I	Kretzmann et al. 1993
Southern elephant seal	*Mirounga leonina*	S	Campagna et al. 1992
		I	Fedak et al. 1996
northern fur seal	*Callorhinus ursinus*	I	Costa & Gentry 1986
Galapagos fur seal	*Arctocephalus galapagoensis*	S	Trillmich 1986
New Zealand fur seal	*Arctocephalus forsteri*	Sp	Chilvers et al. 1995
		S	Lea & Hindell 1997
Antarctic fur seal	*Arctocephalus gazella*	I	Arnould et al. 1996
		I	Arnould 1997
echidna	*Tachyglossus acculeatus*	I	Green et al. 1985
eastern quoll	*Dasyurus viverrinus*	I	Green et al. 1997
brush-tailed bettong	*Bettongia penicillata*	I	Merchant et al. 1994
northern brown bandicoot	*Isodon macrourus*	I	Merchant et al. 1996a
tammar wallaby	*Macropus eugenii*	I	Green et al. 1988
		I	Cork & Dove 1989
		I	Dove & Cork 1989
rock wallaby	*Petrogale assimilis*	I	Merchant et al. 1996b

CHAPTER 2

Suckling Behaviour and Milk Intake

IS SUCKLING BEHAVIOUR A RELIABLE PREDICTOR OF MILK INTAKE? A TEST

"THERE IS NO FINER INVESTMENT FOR ANY COMMUNITY THAN PUTTING MILK INTO BABIES"

Winston Churchill, 1943, Radio Broadcast.

Photo caption:
Thoroughbred mares at Flock House Thoroughbred Research
Farm. Miss Illusion (suckling her foal) and Tamah.
Photo by Elissa Cameron

ABSTRACT

Studies of parental investment in mammals have frequently used suckling behaviour to estimate energy transfer from mother to offspring, and consequently to measure maternal input. Such studies assume that the more an offspring sucks, the more milk it will receive. This assumption has been questioned, and a review of the literature found little support for it. To test if suckling behaviour provided an accurate index of milk or energy intake we used a radioactive isotope technique to label the milk of thoroughbred horse (*Equus caballus*) mares and to measure milk transfer to foals. We found no significant linear relationship between usual measures of suckling behaviour and milk or energy intake. No behaviours associated with suckling nor with characteristics of mares and foals improved the relationship; only the number of bunts associated with each suck episode and foal age even approached significance. If we had used suckling behaviour to test theories on differential maternal investment our conclusions would have been in error. For example, female foals tended to suck for longer than did males but there was no difference in the amount of milk transferred. Consequently, we show that measures of suckling behaviour do not adequately predict milk intake in the domestic horse and we suggest that conclusions about differential maternal investment based on suckling behaviour are likely to be in error.

Chapter reference:

Cameron, E. Z., Stafford, K. J., Linklater, W. L. & Veltman, C. J. in press. Suckling behaviour does not measure milk intake in horses. *Animal Behaviour.*

INTRODUCTION

Measures of parental input (Evans 1990) are frequently used to quantify parental investment (Birgersson & Ekvall 1994). In mammals, the milk transferred from mother to offspring is the most energetically costly form of parental input (Martin 1984, Clutton-Brock 1991). Milk transfer is difficult to measure in the field (Kretzmann et al. 1993) and so milk transfer has been estimated by measures of suckling behaviour based on the assumption that offspring that suck more will receive more milk (Fletcher 1971, Berger 1979).

Although the validity of this assumption has been questioned (eg. Berger 1979, Mendl & Paul 1989, Babbitt & Packard 1990a&b, Pélabon et al. 1995), suckling time is still frequently used to estimate maternal input (eg. Cassinello 1996, Lea & Hindell 1997, Künkele & Trillmich 1997; reviewed in Cameron in press [chapter 1]). An analysis of previous studies suggests that there may not be a predictive relationship between time spent suckling and milk intake (Cameron in press [chapter 1]). Factors that may confound the relationship between time spent suckling and infant milk intake include variation in

milk composition (Berger 1979, Kretzmann et al. 1993), sucking ability of offspring (Babbitt & Packard 1990), hunger (Mendl & Paul 1989), experience and physiology of the mother (Shackleton & Haywood 1985, Birgersson & Ekvall 1994) and periods of non-nutritive suckling (Rushen & de Pasillé 1995; reviewed in Cameron in press [chapter 1]). Some of these factors may be controlled for by measuring different aspects of suckling behaviour. For example, bunting during suckling might indicate lack of milk flow (Lent 1971). Similarly, if the mother ends a suckle bout, it may indicate that the offspring did not feed to satiation. In addition, analysis of milk samples allows the calculation of energy transfer, the currency of maternal input.

Radio-isotopes are regularly used to measure milk transfer from mother to offspring, independent of behaviour (eg. Arnould 1997, Green et al. 1997, Lydersen et al. 1997; reviewed in Cameron in press [chapter 1]). The use of radioactively labelled isotopes to measure the transfer of milk from the mother to the offspring combined with analysis of milk energy content has been suggested as a test of the relationship between time spent suckling and milk intake (Babbitt & Packard 1990b, Cameron in press [chapter 1]). Only one study has previously attempted to correlate milk transfer, measured using radio isotopes, with behavioural measures of time spent suckling, but no significant relationship was found (Stellar sea lion, *Eumetopias jubatus*, Higgins et al. 1988).

We tested the relationship between time spent suckling and milk intake in domestic horses using tritiated water (^3H_2O). We also analysed milk energy content to test the relationship between measures of suckling behaviour and energy intake by the foal. We then measured a number of behaviours associated with suckling, and characters associated with mares and foals to determine if a strong predictive relationship between suckling behaviour and milk energy intake can be obtained.

METHODS

Fifteen multiparous thoroughbred mares and their foals in a research herd at Flock House, New Zealand were studied from November 1996 to January 1997. Our experimental procedure was approved by the Massey University Animal Ethics Committee, and mares and foals were monitored throughout by veterinarians for any evidence of adverse effects. We aimed to measure milk transfer around peak lactation, 39 days postpartum (Oftedal 1985). The 15 horses were divided into three groups of 5 based on foal birth dates (Table 1). Each group grazed in company with non-experimental mares and their foals which were of a similar age.

Milk intake was estimated using the procedures of Martin et al. (1992). As foals had been observed to drink water we could not assume that milk was the only source of water. Therefore, we used a double isotope dilution technique to measure milk and water intakes during a 14-day period. On day 0 each foal was weighed, a blood sample was taken and the foal was injected intramuscularly with 148kBq ^3H_2O per kg body weight

Table 1. Details of mares and foals

Mare	grp*	mare age (yrs)	last year's foaling	mare wt† (kg)	foal age‡ (days)	foal sex	foal wt‡ (kg)
Genies Gypsy	a	13	no foal	556	45	f	129.0
Hillary Queen	a	8	no foal	638	40	m	119.0
Tamah	a	8	foal	588	58	m	132.0
Veronica Belle	a	8	no foal	550	55	f	123.5
Western Queen	a	10	no foal	580	43	f	112.0
Brown Jug	b	9	foal	642	34	m	113.5
Decimate	b	7	no foal	568	24	f	95.0
Kate	b	11	foal died	642	26	f	104.0
Sarmajaz	b	9	foal died	556	31	m	104.0
Starry Halo	b	10	foal	616	25	f	93.5
Bella Charma	c	8	foal	549	45	m	108.5
Flight to Glory	c	15	foal	504	31	f	95.0
Lewita	c	14	foal	515	35	m	117.5
New Leaf	c	15	foal	551	58	f	128.0
Omakere Star	c	10	foal	594	55	f	127.5

* Group a: 4 to 18 November 1996; Group b: 25 November to 11 December 1996; Group c: 6 to 20 January 1997.
† At day 7 of trial
‡ At day 0 of trial

(bwt). Foals were muzzled to prevent intake of food or water for two hours from the time of isotope administration to allow for equilibration of the isotope in the body water pool, after which a further blood sample was taken. Blood samples were taken 2, 4 and 7 days later. The total body water of the foal and the fractional loss of ^3H_2O was calculated from these measures and used to estimate the foal's total water intake from milk and non-milk sources.

Milk intake was determined from day 7. On day 7, mares were weighed and injected intravenously with 370 kBq $^3H_2O.kg^{-1}$ bwt to label the milk. Mares were held in yards and withheld from food and water, and their foals were muzzled so they could not suck for 6 hours after isotope administration to allow for ^3H_2O equilibration. Blood samples were taken from the mares and foals before and six hours after injection, and on days 9, 11 and 14. During both periods when foals were muzzled for two and six hours the mares and foals were kept together in yards with shade and monitored throughout for any adverse effects. We choose to muzzle foals rather than separate them from their mothers to allow them to solicit comfort from their mothers.

Blood samples were taken by jugular venupuncture into plain vacutainers (10ml). No more than 10mls was taken at any one sample. During blood sampling the horses were held by a handler while a veterinarian took the blood samples. Both mares and foals had been extensively handled so the procedures caused no unnecessary stress to them. The samples were centrifuged at 3000 rpm for 10 minutes. The serum was removed and frozen at -20°C until analysis. Water was recovered from the samples by dioxane precipitation (Springell & Wright 1976) and isotope was counted using liquid scintillation counting. 1 ml of serum was mixed with 1 ml of 1, 4-dioxane and left to stand for 30 minutes. The sample was then centrifuged at 5600rpm for 5 minutes. The supernatant was drawn off and allowed to stand for a further 30 minutes and was again centrifuged at 3000g for 5 minutes. 1 ml of the resultant supernatant was mixed with 9 mls of PCS Scintillation liquid (Amersham) for liquid scintillation counting. Samples were counted 3 times for 5 minutes and the average rate of disintegrations per minute (DPM) was used to determine isotope content of blood samples.

Milk intake by the foals was calculated by the equation

$$M \text{ (milk intake)} = [Q \text{ (kb-ka)}] / [\alpha_0 (e^{-kat} - e^{-kbt})]$$

where Q = total body water of foal (dose injected/equilibrated isotope in blood-amount in blood before injection) x quantity of tracer in foal at time t; α_0 = concentration of tracer in milk at zero time; ka = fractional loss of tracer from the mare; kb = fractional loss of tracer from the foal (Holleman et al. 1975). It was assumed that milk and water intakes remained constant over the 14 day period.

Milk samples were taken from each of the mares on day 14. Mares were hand-milked and remained calm and apparently unstressed throughout the procedures. These were analysed for percent dry matter (freeze dried) and energy content of milk (kJ/g freeze dried material). Values were multiplied by milk intake to calculate the energy intake by each foal. In one mare the milk sample was insufficient to calculate milk energy content. Consequently this mare and foal were used only for the behavioural measures of suckling and for milk intake and they were excluded from any analysis that involved energy intake.

Foal behaviour was sampled during focal animal samples (Altmann 1974) of between 1 and 3 hours on 3 different days at different times of the day during the final 7 days of the procedure. Between 5.5 and 6.5 hours of data were collected on each foal. Our behaviour sampling was based on methods used in earlier field studies of maternal investment. The samples were longer than those used in other studies of suckling behaviour in equids (eg. Crowell-Davis 1985, Berger 1986, Becker & Ginsberg 1990, Smith-Funk & Crowell-Davis 1992), except Duncan et al. (1984), who collected scan samples throughout 24 hour periods. We recorded the length of each suckle bout excluding breaks in nipple contact during the bout. Bursts of sucking within a bout, separated by breaks in nipple contact, were termed episodes. Episodes were separated by

breaks of less than 60s, whereas bouts were defined as being separated by breaks of greater than 60s, although in practice all bouts were separated by more than five minutes. We recorded the time between suckle bouts from the end of one suckle bout to the beginning of the next apparently successful suckle. Total time spent suckling per day was calculated from duration and time between suckles as described by Becker & Ginsberg (1990). The behaviour of foals was scanned every four minutes during a focal sample in order to calculate the percent time spent sucking, as well as time spent grazing, lying down and in social or play interactions.

In addition we recorded the number of suck episodes during a suckle bout, the number of episodes during which the foal bunted, the proportion of suckles that were ended by the mother and the proportion of suck attempts that were unsuccessful. Unsuccessful sucks were those that lasted for less than 5 seconds.

We performed multiple regressions using an automatic stepwise selection procedure with a p<0.2 entry and exit criteria using SAS (SAS Institute, 1990). The results of all statistical tests are 2-tailed.

RESULTS

Estimated milk intake by foals averaged $X \pm$ SE $= 14.72 \pm 0.94$ kg/day (range 10.63 to 23.09), the energy content of freeze-dried milk was $X \pm$ SE $= 19.27 \pm 0.20$ kJ/g (range 18.189 to 21.028) and milk energy intake averaged $X \pm$ SE $= 30631 \pm 2262$ kJ/day (range 21152 to 49270). Measures of time spent suckling are shown in Table 2.

Table 2. Time spent sucking by 15 thoroughbred foals

Behavioural measure	mean ± sem	range
Total time suckling (mins.24hrs^{-1})	46.14 ± 3.63	26.67 to 77.77
Suckle duration (s)	64.40 ± 1.49	57 to 79
Time between suckles (s)	2153 ± 130	1333 to 3142
Percent time suckling (scans)	3.67 ± 0.59	1.00 to 9.46

There was no significant effect of group membership on milk energy intake (one-way ANOVA, $F_{2,11}=2.50$, NS) and the factor was therefore excluded from further calculations.

There was no significant linear relationship between milk energy intake and suckle bout duration (regression: $t_i=-0.13$, $N=14$, NS; Fig 1a), suckle bout frequency
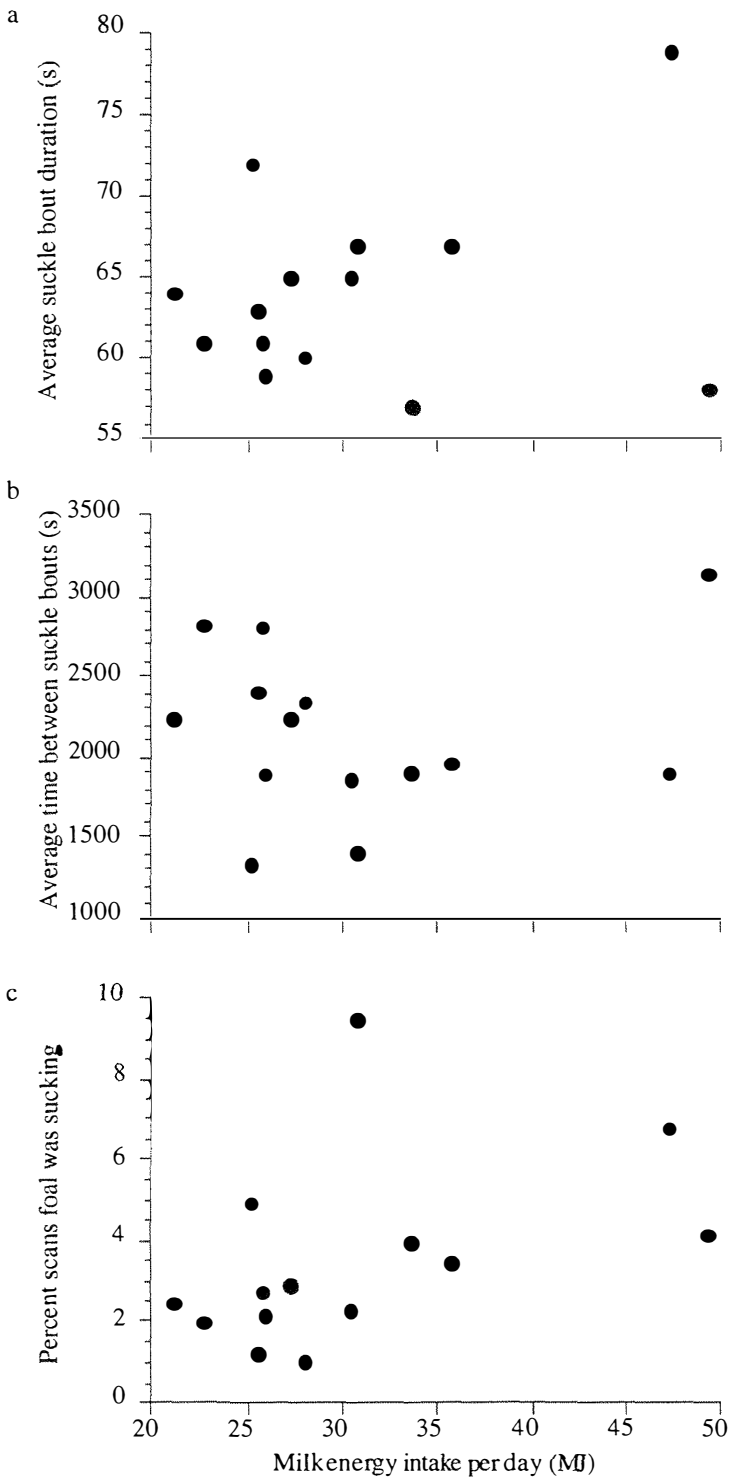
Figure 1. The relationship between milk energy intake and a) suckle bout duration, b) suckle bout frequency, and c) percent scans during which the foal was sucking.

(regression: t_1=0.23, N=14, NS; Fig 1b), or proportion of scan samples during which the foal was sucking (regression: t_1=1.67, N=14, NS; Fig 1c). There was no significant relationship between total time spent suckling and milk energy intake (regression: t_1=0.41, N=14, NS; Fig 2). No significant model could be constructed from these four measures of time spent suckling (regression: $F_{5,8}$=1.87, NS)

None of mare age, mare weight, foal age, foal sex or foal weight was a significant predictor of milk energy intake either alone or when all combined with time spent suckling in a stepwise regression.

When all factors (time spent suckling, episodes per bout, proportion episodes with bunts, proportion episodes terminated by mare, proportion unsuccessful attempts, scans of proportion of time spent suckling, grazing, lying and in social and play activities) were analysed in a stepwise regression, no significant model could be found that would predict the amount of milk energy a foal received. The most significant model used a single factor (bunts) and was only approaching significance (regression: $F_{1,12}$=4.21, p=0.063;Fig 3)

DISCUSSION

The mean and variance of foal milk intake and the energy content of milk were similar to that found in other studies (reviewed in Doreau & Boulot 1989, Martin et al. 1992). Consequently, daily milk energy intake was also similar (eg. Martin et al. 1992, 33800 ± 11500 kJ.day^{-1}). Estimated time spent suckling, using the variety of measures that have been used in previous studies on both feral and domestic horses, yielded similar results to these studies on both feral and domestic horses (eg. total time, 40-75 min.day^{-1}, Tyler 1972, Carson & Wood-Gush 1983, Crowell-Davis 1985; suck bout duration, 55-75s Carson & Wood-Gush 1983, Crowell-Davis 1985, Smith-Funk & Crowell-Davis 1992; suck bout frequency, 2-2.5.hr^{-1} Carson & Wood-Gush 1983, Crowell-Davis 1985, Smith-Funk & Crowell-Davis 1992; scan sampling, 2-3%, Duncan et al. 1984).

Measures of foal sucking behaviour using sampling periods characteristically used in field studies of wild mammals did not significantly predict milk energy intake. If all foals had been exactly the same age the relationship may have been improved. However, even with the addition of foal age, weight and sex, and mare age and weight into a stepwise regression no significant model was constructed. Although there appeared to be three outliers confounding the relationship, two that sucked frequently for low energy intake and one that sucked least but had the highest energy intake, there were no distinguishing factors that would have enabled the identification of these three dyads *a priori*..

The strength of the relationship was not enhanced by including behaviours related to suckling in the model. Of all variables tested, only the proportion of suckles during which bunting occurred approached significance. Other factors which we did not measure
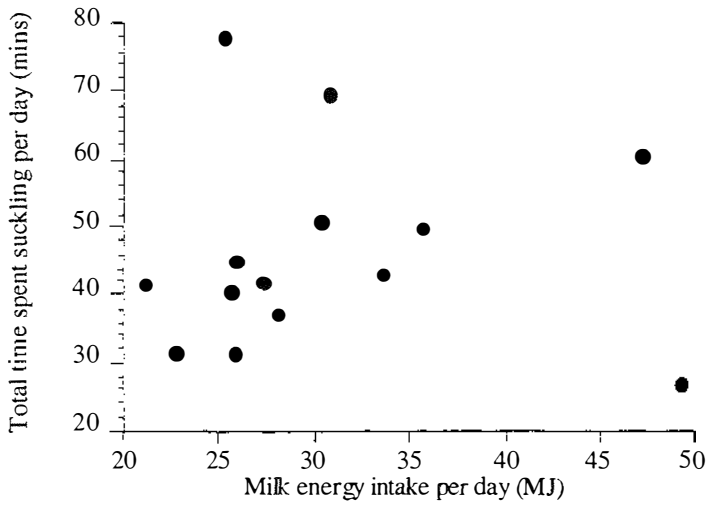
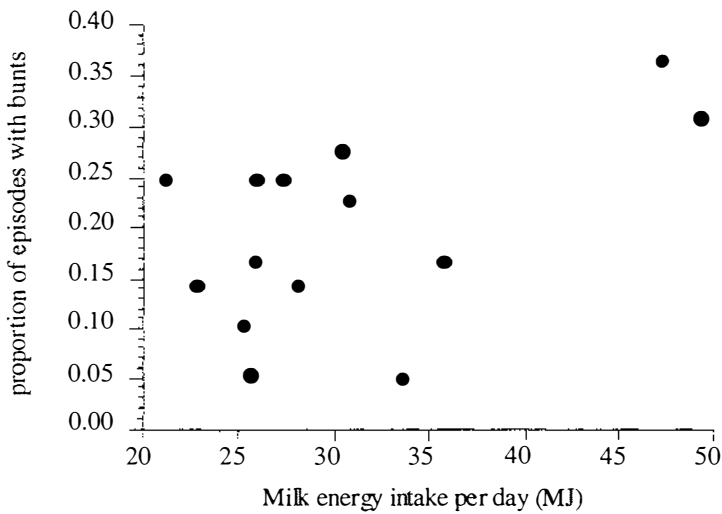Figure 2. The relationship between milk energy intake and total time spent sucking.

Figure 3. The relationship between milk energy intake and the proportion of suck episodes during which bunting occurred

may have provided more accurate estimates of milk intake, but would be difficult to measure in most field situations. The rate of sucking during a bout has been shown to indicate when nutritive sucking is occurring (Drewett et al. 1974) and estimates of milk intake may be improved if only nutritive sections of bouts are recorded (eg. Tanaka 1992). Similarly, the number of swallows during a bout may more accurately reflect milk intake (Lau & Henning 1989). In humans, longer records of suckling behaviour, particularly when age of the infant is taken into account, improved the relationship with milk transfer, but at least 50% of the variation was still unaccounted for (eg. Brown et al. 1982, Drewett et al. 1989).

If measures of time spent suckling had been used as an index of milk energy intake for hypothesis testing, results would have been erroneous. For example, females sucked more often than males suggesting differential maternal input (t-test: t=-2.31, df=13, p<0.05), but they did not actually receive any more milk energy (t-test: t=-0.14, df=12, NS).

As our horses did not vary from other reported values for horses in time spent suckling, milk intake or milk composition, the relationship between suckling behaviour and milk energy intake was probably similarly obscured in previous studies on horses. It seems likely that studies on other species may also fail to show a relationship between behavioural measures of suckling and milk energy intake. Therefore, our results suggest that studies using time spent suckling to infer milk energy intake are probably not accurate and may have led to misleading conclusions, particularly regarding differential maternal investment .

ACKNOWLEDGMENTS

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CHAPTER 3

Social Environment

THE INFLUENCE OF SOCIAL BAND TYPE ON MATERNAL BEHAVIOUR

"THERE HAVE BEEN
OR I AM MUCH DECEIVED, CUCKOLDS ERE NOW"
Shakespeare, circa 1600, 'The Winter's Tale'.

Photo caption:
Yearlings Pose (94122) and Penny (94130) with band stallion
Ally (078). Pose was fathered by Ally, but Penny was not.
Photo by Elissa Cameron

ABSTRACT

The risk of infant mortality influences maternal behavior, particularly protectiveness, in species in which infanticide is known to occur. Equids are unique amongst ungulates in that infanticide and feticide have been reported where paternity has been uncertain, and they have a similar social structure to other species in which infanticide has been reported. Stallions benefit from infanticide as the mare has greater reproductive success in the subsequent year. Mares are found in bands with a single stallion or bands with more than one stallion in which paternity is less certain. We investigated maternal behavior in relation to band type. Mares in bands with more than one stallion were more protective of their foals, particularly when stallions and foals approached one another. The rate of aggression between the stallion and foal was a significant predictor of maternal protectiveness. Mares which changed band types with a foal at foot were more protective of their foal in multi-stallion bands than they were in single stallion bands, and mares were more protective in the year after losing a foal. In addition, of four foals that were born in one band, the two that were not fathered by the single band stallion had more protective mothers, even when the mare had been a stable band member. Stallion aggression is a significant modifier of mare behavior and maternal effort, probably due to the risk of infanticide.

Chapter reference:

Cameron, E. Z., Linklater, W. L., Stafford, K. J. & Minot, E. O. Social environment and maternal behaviour in feral horses: experimental manipulations. *Submitted to Behavioral Ecology.*

INTRODUCTION

The risk of infant injury or mortality influences maternal care (eg. Berger, 1979; Harrington et al., 1983; Hauser, 1988; Lycett et al., 1998) and in habitats with a high risk of infant mortality mothers are more protective (Johnson and Southwick 1984). Similarly, mothers have been reported to be more protective when they receive high rates of aggression (Maestripieri 1998) and when a new male is introduced into the social group (Fairbanks 1996, Fairbanks & McGuire 1987).

Infanticide by males occurs in a variety of taxa and species, particularly amongst carnivores and primates (eg. alpine marmot *Marmota marmota*, Coulon et al., 1995; arctic ground squirrel *Spermophilus parryii,* McLean 1983; blue monkey *Cercopithecus mitis stuhlmanni*, Leland et al., 1984; brown bear *Ursus arctos*, Swenson et al., 1997; hanuman langurs *Presbytis entellus*, Hrdy, 1977; lions *Panthera leo*, Packer & Pusey, 1983; red howler monkeys *Alouatta seniculus*, Crockett & Sekulic, 1984; red-tailed monkey *Cercopithecus ascahius schmidti*, Leland et al., 1984; savanna baboons *Papio*

cyncocephalus, Collins et al., 1984). Infanticide is most common where there are long term male-female associations with a high degree of certainty of paternity for a male (Leland et al. 1984). An infanticidal male benefits from a female returning to a reproductive state following lactational anoestrus, and therefore bearing his offspring sooner, in comparison with non-infanticidal males (Hrdy 1977). When there is no lactational anoestrus, breeding is highly seasonal, or time between offspring is long, benefits to an infanticidal male may include reduced lactational costs and thus increased condition in the female. Thus the inter-birth interval is reduced or the probability of successful breeding in the subsequent year is increased (Borries, 1997; Coulon et al., 1995; Hrdy and Hausfater, 1984; Packer and Pusey, 1984). Consequently, infanticide has been proposed as a viable male reproductive strategy wherever the time to a female's next offspring is shortened by the by the death of her current young (Packer and Pusey 1984). The reproductive benefits for infanticidal males have been shown in several seasonal breeding species (eg sifaka *Propithecus diadema* Wright, 1995; ring-tailed lemur *Lemur catta* Pereira and Weiss, 1991; hanuman langur *Presbytis entellus* Borries, 1997). Feticide is thought to have similar effects (Agooramoorthy et al 1988, Berger 1983).

Females, however, are advantaged by increasing their own reproductive success (Trivers 1974), not that of the males and they show a variety of behaviors to counter male infanticide. Female protectiveness of offspring (Collins et al., 1984; Fairbanks and McGuire, 1987; Hrdy, 1977) and defence of attacked offspring (Mallory and Brooks, 1978; Packer and Pusey, 1983) are the most obvious counter-strategies. Other strategies include female grouping (Grinell and McComb, 1996; Hrdy, 1977), avoidance or intolerance of new males (Hrdy, 1977; Packer and Pusey, 1983), intense aggression toward unfamiliar conspecifics (Parmigiani et al., 1994), 'friendships' with males (Palombit et al., 1997) and pseudo-oestrus (Hrdy, 1977). Recently, it has been suggested that patterns of primate social organisation are explained better by infanticide than by resource distribution, sexual selection or predation (Kappeler, 1997; Van Schaik and Kappeler, 1997).

Equids are unique amongst ungulates in that they have year round stable social groups with a male present, a social system similar to those carnivore and primate species in which infanticide is known to occur (Leland et al., 1984; Van Schaik and Kappeler, 1997). To our knowledge evidence of infanticide has not been reported in any ungulate except the harem dwelling equids (mountain zebra: *Equus zebra* Penzhorn, 1984, Burchell's zebra: *Equus burchelli* Joubert, 1972; przewalski horse: *Equus przewalskii* Kolter and Zimmerman, 1988; Ryder and Massena, 1988; Boyd, 1991; horses: *Equus caballus* Duncan, 1982). Feticide following band take-over has also been reported (Berger, 1983), but remains controversial (Kirkpatrick and Turner, 1991). In horses, evidence of infanticide has been reported in captivity (Duncan, 1982), and is thought likely to occur in feral populations (Berger 1986; Duncan, 1982; Tyler, 1972). Incidences

of infanticide are associated with unfamiliar males in a new group and in many cases the same stallion subsequently killed none of his own foals in the following season (Boyd, 1991; Duncan, 1982; Ryder and Massena, 1988). Mares will vigorously defend their foals from such attacks (eg. Boyd, 1991; Joubert 1972; Penzhorn, 1984; Ryder and Massena, 1988; Tyler, 1972), and act protectively even when the threat is not overt (eg. Crowell-Davis and Houpt, 1986). Stallions which have lived with a female group are also protective of their foals (Berger, 1986; Boyd, 1991).

We investigated variation in maternal behavior in relation to whether there was one or several stallions in a band, and the rate of stallion-foal aggression. As mares have a maximum of one foal a year (Platt, 1978), in multi-stallion bands at least one stallion was not the father of each foal. Subordinate stallions obtain significantly less paternity than dominants so the dominant is most likely to be the foals' father (Bowling and Touchberry, 1990; Eagle et al., 1993). To determine if males benefit from infanticide we measured foaling rates by mares in the year after their foal died, including simulated infanticide. Furthermore, we investigated mare-foal behavior in mares that changed band types or whose band was experimentally changed to a single stallion by the removal of the subordinate stallion. We also compared foals within the same band in which the band stallion was or was not the foal's father to determine if mares were more protective of foals that were not the resident stallion's offspring.

METHODS

Study site

Feral horses, known locally as Kaimanawa horses, live in the south western Kaimanawa ranges in the central North Island of New Zealand. Horses have been feral in the Kaimanawa ranges since the mid to late 1800s and the study population was largely unmanaged and not confined by artificial barriers or fences. The population, its range and the study site are described in Linklater (1998). Kaimanawa horses live in year round stable social groups known as bands. These bands typically contain one stallion and one or more mares, but may contain up to 4 adult stallions. The mare-foal dyads are from 12 single stallion and 6 multi-stallion bands that are sympatric in the study area.

Potential advantages to infanticide

We recorded the reproductive success of focal mares, and whether they foaled in the subsequent year. Because few mares foaled as 3-year-olds, and no 3-year-old mare who foaled ever foaled as a four-year-old, regardless of the first foal's survival, we excluded 3-year-old mares from our analysis. To determine if mares which lost their foal became pregnant before or after losing their foal we estimated conception date of their subsequent foal by backdating average gestation (336 days; Kiltie, 1982) from the date of birth. Four

mares had their foals illegally shot when the foals were 1-4 months old, resulting in a fortuitous simulation of infanticide.

Sampling regime

Mares and their foals born in the 1994 (n = 26) and 1995 (n = 35) seasons were studied from birth to dispersal. Mares and their foals born in the 1996 season were studied between birth and 110 days of age (n = 39). The birth date of all focal foals was known to within ± 5 days, all foals were sexed, and the band type in which they were born was recorded (single stallion band n=55, multiple stallion band n=29). Mares that changed bands during the gestation or lactation of their current foal (n=16) were called changers and were excluded from analysis of differences between band types. Because sex differential maternal investment in horses has been reported in some studies (eg. Berger, 1986; Duncan et al., 1984), although not in others (eg. Crowell-Davis, 1985; Smith-Funk and Crowell-Davis, 1992), we analyse measures from sons and daughters separately.

Mare condition was calculated from the mode of body condition scores taken in the month before foal birth. Body condition scores were estimated by visual body fat distribution based on an 11 point scale from 0-5 with 0.5 gradations (Carroll and Huntingdon, 1988; Rudman and Keiper, 1991) with the aid of 10-15x binoculars or a 15-60x telescope whenever horses were sighted, provided visibility was good. Mares with scores of 0 were very thin and with scores of 5 were obese. Inter-observer reliability was high (EZC, WLL, r = 0.91, Wilcoxon matched-pairs signed ranks test with correction for ties, $Z_{129}=1.35$, NS). In horses, body condition scores correlate with body fat percentage (r=0.81; Henneke et al., 1983). We recorded foaling success in the subsequent year for all mares.

Foals were divided into six categories based on the following parameters:
1. 0-20 days: 50% of all foal mortality to one year of age occurs before 20 days of age.
2. 21-50 days: period surrounding peak lactation (39 days, Oftedal, 1985).
3. 51-110 days: 85% of foal mortality to one year of age occurs before 110 days. In addition, the investment before 110 days is essential to foal survival. No foal orphaned before 110 days survived; the youngest survivor was orphaned at 132 days (Cameron, 1998 [chapter 4]). Captive foals are weaned at 120-180 days (Crowell-Davis and Houpt, 1986).
4. 111-200 days. All foals that were not orphaned suckled to around 200 days; the youngest weaned foal was 196 days.
5. 201 days to weaning.
6. weaning to dispersal. Both male and female foals disperse from their natal bands (Monard et al., 1996). However, most foals, except those born in 1994, had not dispersed by the end of the study.

Bands were located and mare foal dyads were sampled using focal animal samples until at least 3 suckle bouts, and therefore two inter-suck periods, had been recorded, or both individuals moved out of view. Mare-foal dyads were sampled from birth to weaning, except foals born in 1996 which were studied to 110 days of age. The day was divided into three periods of equal length based on the time of sunrise and sunset, and mare-foal dyads were sampled from all of these three periods.

Behavioral measures

It is unlikely that the time spent suckling provides an index of maternal investment by representing milk intake (Cameron, in press [chapter 1]). Furthermore, a test on domestic horses showed no relationship between time spent suckling and energy intake (Cameron et al., in press [chapter 2]). Suckling behavior, however, can be used to determine the degree of conflict between the mother and offspring over the allocation of resources (Byers and Bekoff, 1992). Consequently, we do not use suckling behavior as an index of energy intake, but as an indication of conflict between mare and foal over energy intake.

We measured the length of suckle bouts to the nearest second, excluding small breaks in nipple contact during a bout. Within a bout the sucking bursts (Carson and Wood-Gush, 1983) separated by breaks in nipple contact were called sucking episodes. We recorded the number of episodes per bout, the number of episodes during which the foal bunted and the terminator of each sucking episode. Unsuccessful suck attempts were those that lasted less than 5 seconds, during which time the milk would probably not have been released (Whittemore, 1980). We calculated the proportion of suck attempts that were unsuccessful.

The spatial relationship between mares and their foals is particularly variable and therefore likely to reflect differences between individuals and influence infant survival (Estes and Estes, 1979; Green, 1992). Spatial relations appear to be particularly sensitive to proximal threats and reflect maternal protectiveness (eg Fairbanks, 1996).

During the focal sample, instantaneous samples were taken every four minutes and the distance between the mare and foal in adult body lengths, and their behavior was recorded. Four minute intervals are close enough together to accurately represent behavior but far enough apart to enhance independence of data (Rollinson et al., 1956), and other studies have used similar time intervals (eg Becker and Ginsberg, 1990; Crowell-Davis, 1986a; Duncan et al., 1984). Because of decreasing accuracy of estimates with increasing distance between mare and foal, distances were estimated to the nearest body length up to 10 body lengths, to the nearest 2 body lengths to 20, and the nearest 5 body lengths thereafter. Foals that were closer than 1 body length to their mother were recorded as 0.5 body lengths. For analysis of distance between mare and foal we used only samples where at least one member of the dyad was active (that is not lying down or standing still).

All approaches across a 2-body-length boundary around the mare and the foal were recorded. For analysis we used the percentages of approaches that were by the foals. Consequently, lower scores indicate more mare effort into maintaining contact with her foal.

Social interactions between the mare and foal, and the stallion(s) and foal were recorded on an all-occurrence basis. We recorded the initiator of the interaction, and whether the interaction was affiliative or aggressive. For the purposes of analysis we used social interactions that were not associated with suckling; unsuccessful suck attempts and interactions that occurred during a suckle bout or an unsuccessful suckle attempt were not included.

Weaning dates were calculated as the mid point between the last sample during which the foal had a successful suck bout and the first sample during which no suck attempts were made. Similarly, age at dispersal was calculated as the mid-point between the last sighting of the foal with its mother and the next sighting of the foal without the mother.

Mare-foal dyads in both band types

The subordinate stallion from two bands each with two stallions were removed for three weeks in the 1997 breeding season (Linklater et al., 1998). Three mare foal dyads were thereby manipulated from a multi-stallion band to a single stallion band. We compared the pre-removal behavior of these dyads with the single stallion phase. In addition, two mares each with a foal-at-foot changed band types, and we compared these mare-foal dyads in single and multi-stallion bands.

Response of mares to foal loss in previous year.

Six mares lost a foal in one year and had a foal in the subsequent year. We compared the behavior of these mares in the two years during the first 20 days after foaling and during the period of peak lactation (21-50 days post-partum).

Comparison of foals within a band by paternity

In one single stallion band six foals were born; five males and one female. Of these, four males and one female had blood samples taken as yearlings which were analysed for paternity by equine bloodtyping (Ian Anderson, unpublished data) and the sixth foal died before samples were taken. One male foal was born after its mother shifted into the band four months before it was born. All other mothers were present in the band throughout gestation. The foal whose mother shifted could not have the band stallion as a father nor could one other male foal. Consequently, we had four male foals, two of which were almost certainly fathered by the band stallion and two that could not have been. One mare whose foal was not fathered by the band stallion was a loyal band member who had lived

in the band throughout the previous year, while the other shifted into the band prior to the birth of her foal. We compared maternal behavior towards these foals.

Statistical analysis

Where appropriate, data were log-transformed to correct for positive skew and squared to correct for negative skew. Multivariate analyses of variance (MANOVA) were performed using general linear models on SAS (SAS Institute Inc, 1989). The results of all statistical tests are 2-tailed. Results presented are means ± one standard error. We considered $p<0.05$ as statistically significant and $0.05<p<0.1$ as a trend.

RESULTS

Potential advantages of infanticide

If a foal died or was killed during the first four months after birth, the foal's mother was significantly more likely to foal in the subsequent year than either mares who raised a foal in that year, or even mares that did not foal in that year (foaling rate: foal in previous year 67.5%, n=80, no foal in previous year 85.71%, n=21, foal died or killed in previous year 100%, n=11; $\chi^2=7.27$, 2df, $p<0.05$). Of the 11 mares that lost a foal, less than half (5/11) were pregnant when the foal died or was killed, and all these non-pregnant mares became pregnant after the death of their foal. Therefore, if the foals had been killed by infanticidal stallions, these stallions could have fathered new foals with over half of these mares.

Of the four mares whose foals were shot (simulating an infanticidal event), only one was pregnant at the time of foal death, and all of the other mares became pregnant on the first oestrus following foal death. Therefore, an infanticidal male need only remain proximal to a mare whose foal he has killed until she enters oestrous if he is to father the subsequent offspring.

Differences between band types

Between band types, there was no significant difference in mare age for mares whose age was known (single stallion (ss) 7.41 ± 0.31, n=51; multi-stallion (ms) 6.63 ± 0.76, n=19; $t_{68}=1.14$, NS), or timing of foal births (days from start of season foals born ss 72.07 ± 5.58, ms 70.40 ± 6.94, $t_{83}=0.18$, NS). However, mares in multi-stallion bands were in significantly poorer condition in the month before foal birth (ss 2.76 ± 0.06, ms 2.48 ± 0.07, $t_{83}=2.75$, $p<0.01$), and had significantly more female foals (% male foals, ss 58, ms 33, n=85, $\chi^2=4.75$, 1df, $p<0.05$).

Mare costs and foal quality

There was no significant difference in foaling rates between multi-stallion and single-stallion bands during the three years of the study for focal mares that were over three years of age (ss 75% foal, n=102, ms 68% foal, n=63, χ^2=0.74, 1df, ns). However, there was a trend for more foal deaths in multi-stallion bands (survival to one-year-old, ss 94% n=50, ms 80% n=30, χ^2=3.66, p<0.1). In addition, yearlings that had been raised in single stallion bands were in significantly better condition than were multi-stallion yearlings (combined sexes, ss 2.61 ± 0.04, ms 2.39 ± 0.06, t_{41}=2.85, p<0.01).

There was no difference in weaning dates for either sons or daughters in relation to band type (weaning sons: ss 320 ± 24 days, ms 301 ± 44 days t_{22}=0.4, NS; daughters: ss 286 ± 15 days, ms 294 ± 35 days, t_{22}=0.48, NS).

There was no difference in condition change during the period of peak lactation (which coincides with peak foal death) although multi-stallion mares tended to lose more condition when they had daughters (sons: ss, -0.1 ± 0.1, ms, 0.06 ± 0.06, t_{33}=-0.89, NS, daughters: ss, 0.13 ± 0.07, ms, -0.21 ± 0.1, t_{38}=2.75, p<0.1). Multi stallion females also had less probability of foaling in the year after successfully raising a foal (ss 80% foal, ms 50% foal, n=74, χ^2=7.53, 1df, p<0.01).

Table 1. Results of Multivariate Analyses of Variance of behavioral measures and band type

	0-20	21-50	51-110	111-200	201-wean	weaned
Daughters	$F_{7,25}$=0.89	$F_{7,24}$=0.39	$F_{7,21}$=1.97	$F_{7,9}$=0.96	$F_{7,7}$=1.59	$F_{2,15}$=0.99
Sons	$F_{7,27}$=0.55	$F_{7,24}$=1.53	$F_{7,25}$=1.74	$F_{7,13}$=1.24	$F_{7,9}$=0.78	$F_{7,17}$=8.80**

** $P<0.01$

Maternal behavior

The results of the mutivariate analysis of variance are shown in Table 1. There was little variation in any measure of suckling behaviour between band types for sons or daughters (Fig. 1). Between 200 days of age and weaning multi-stallion mothers ended significantly more sucking episodes for both sons and daughters (Fig 1c). Aspects of the spatial relationship did vary. Mothers in multi-stallion bands contributed significantly more to contact by approaching both their sons and daughters more often than did mothers in
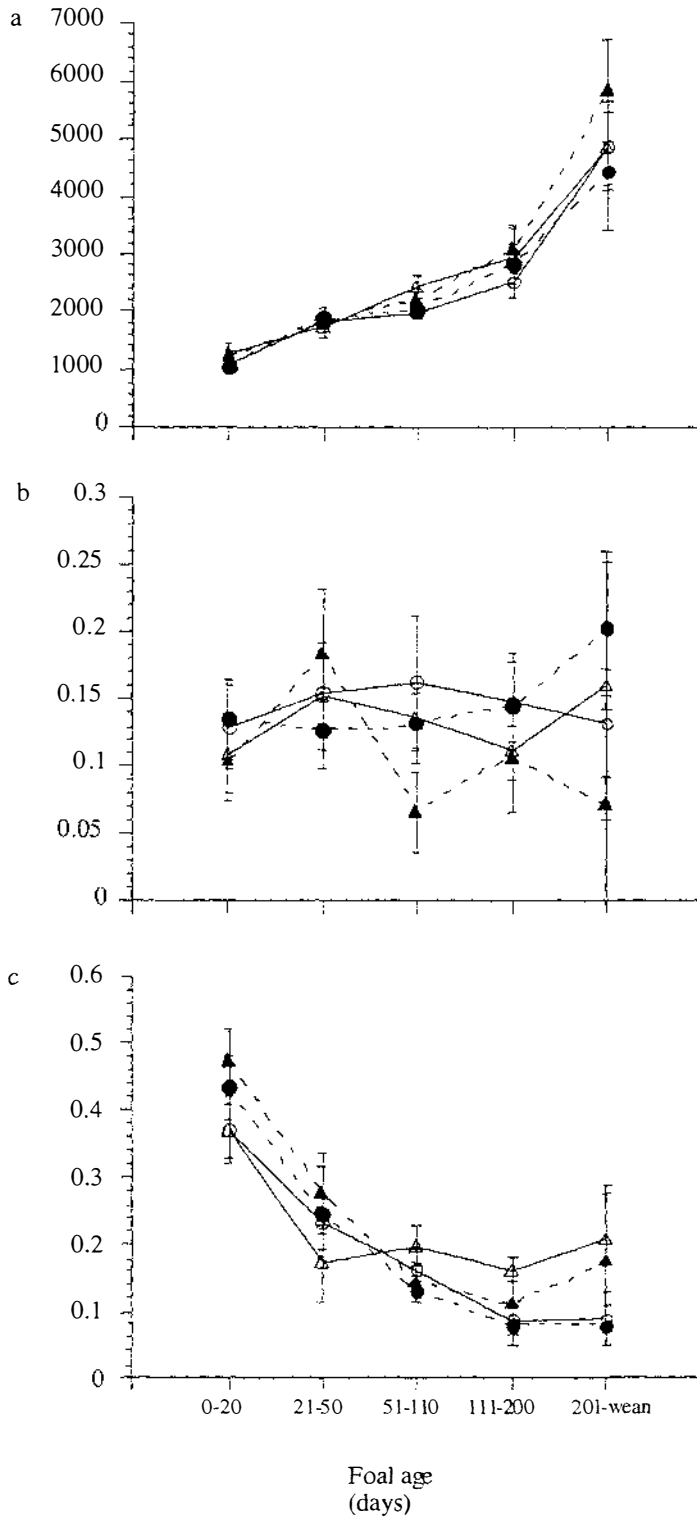
Figure 1. Sucking behavior in foals in single or multi-stallion bands as foals age. 1a interval between suckle bouts, 1b proportion of suck attempts that were unsuccessful, and 1c the proportion of suckle episodes that were ended by the dam. Circles indicate foals raised in single stallion bands with triangles multi-stallion bands; Lines are sons and dashed lines daughters.
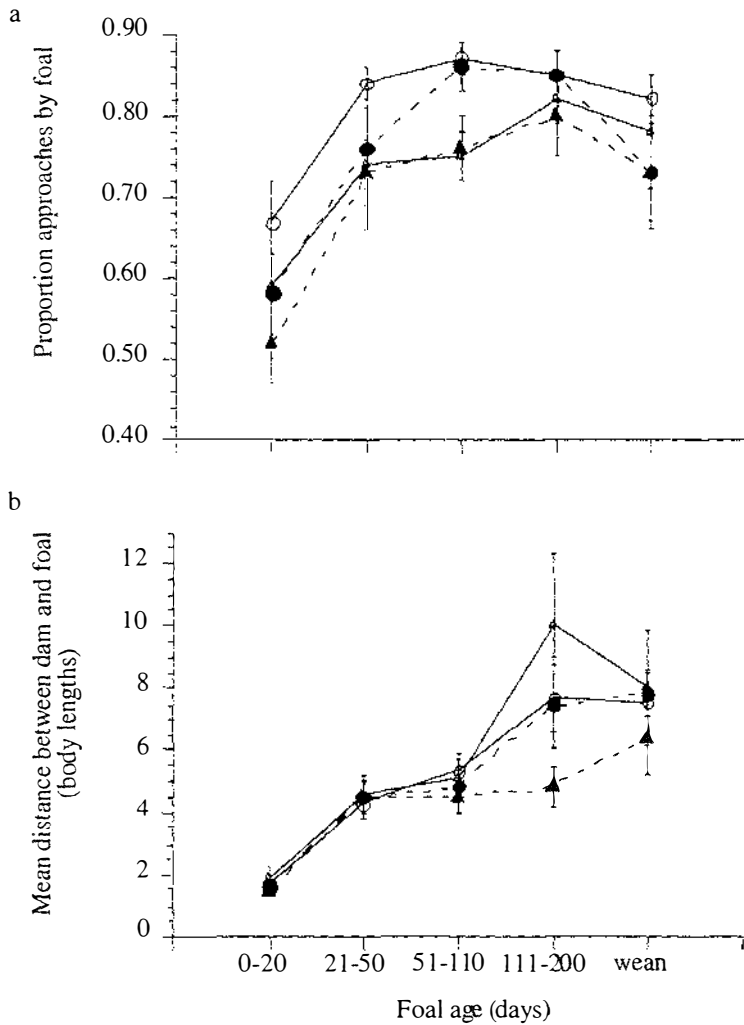
Figure 2. Spatial relationship of mares and foals in single and multi-stallion bands as foals age: a) proportion of the approaches between mare and foal due to the foal (lower scores therefore indicate more mare effort), and b) the mean distance between the mare and foal. Circles indicate foals raised in single stallion bands with triangles multi-stallion bands; Lines are sons, dashed lines daughters.

single stallion bands (Fig 2a). However, the mean distance between mare and foal did not vary between band types (Fig 2b).

When samples between birth and 110 days of age were combined as the period of essential investment for foal survival the MANOVA was significant for daughters (Wilk's Lambda=0.56, $F_{7,33}$=3.7, P<0.005) and showed the same trend for sons (Wilk's Lambda=0.68, $F_{7,29}$=2.0, P<0.1). In both cases the percent of approaches by the foal was the only significant single variable (daughters: $F_{1,39}$=15.22, P<0.0005; sons: $F_{1,35}$=9.55, P<0.005), with mothers in multi-stallion bands contributing more to contact maintenance with their foal than mares in single stallion bands.

There was no difference in the rate of interaction between a mare and her foal from birth to 110 days of age in relation to band type, although mares in single stallion bands tended to interact more with sons and mares in multi-stallion bands tended to interact more with daughters (log transformed; sons: ss 0.43 ± 0.07, ms 0.20 ± 0.05, t_{37}=1.68, p=0.1; daughters: ss 0.20 ± 0.04, ms 0.29 ± 0.05, t_{40}=1.75, p<0.1). However, foals in multi-stallion bands interacted significantly more often with a stallion than did foals in single stallion bands (log transformed; sons: t_{37}=4.6, p<0.0001; daughters: t_{40}=2.71, p<0.01, Fig 3). In particular, the rates of negative interactions between stallions and foals were higher in multi-stallion bands for both sexes of foals (log transformed; sons: t_{37}=4.35, p<0.0001; daughters: t_{40}=3.08, p<0.005, Fig 3).

The hourly rate of stallion-foal aggressive interaction (x) was a significant predictor of the contribution of the mare to contact maintenance (log x, y^2 transformed, $F_{1,79}$=14.16, p<0.001, r=0.39).

Mares in multi-stallion bands approached their foals after an approach event between a stallion and the foal significantly more often than did mares in single stallion bands (log transformation of % approaches between males and foals that were followed by a dam approach; son, t_{37}=9.1, p<0.0001; daughter, t_{40}=5.23, p<0.0001, Fig 4). The hourly rate of stallion-foal aggressive interaction was a significant predictor of mare approach following stallion-foal approach in all band types; where stallions were more aggressive to a foal, mares were more likely to approach their foal after a stallion-foal approach event (logx, logy transformed, $F_{1,79}$=8.63, p<0.005, r=0.31).

Foals in both band types

The same mares put significantly more effort into maintaining contact (paired t-test, t_4=3.2, p<0.05) and were significantly closer to their foals (paired t-test, t_4=4.07, p<0.05) when they were in a band with multiple stallions than in a single stallion band. Mares also tended to end more suckle bouts (paired t-test, t_4=2.33, p=0.08) which led to a trend for more episodes per suckle bout when in multi-stallion bands (Fig 5). In addition, when there was a single stallion none of the mares ever approached their foal
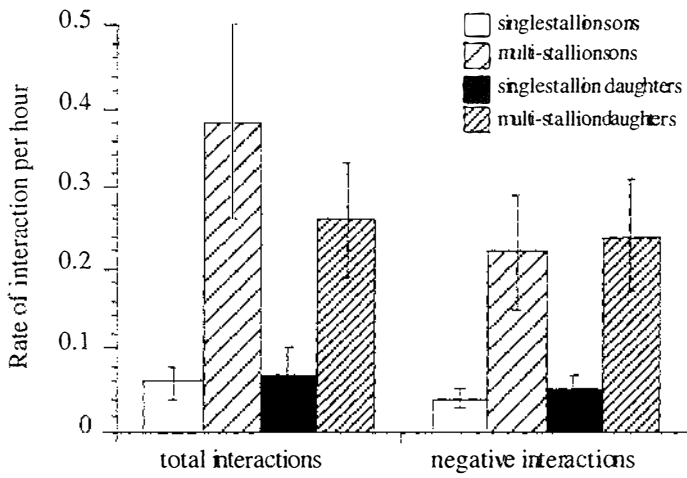
Figure 3. The rate of total and negative interactions between stallions and foals in relation to band type.
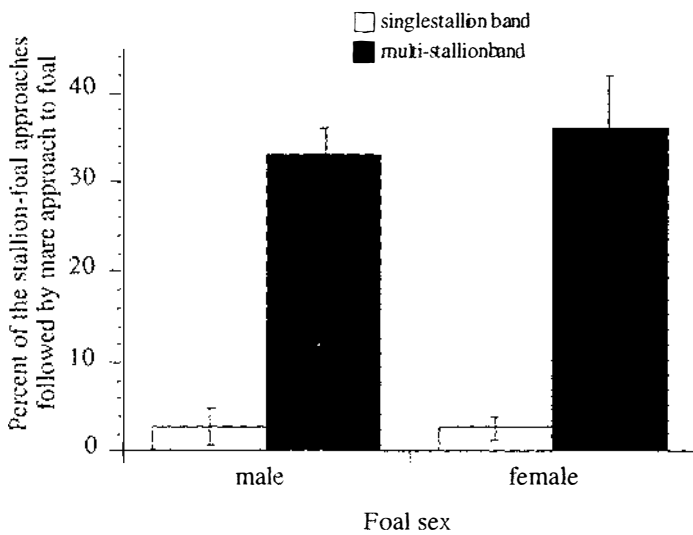

Figure 4. The proportion of approach events between the foal and a stallion that were followed by an approach to the foal by the dam in relation to band type.

after a stallion-foal approach event, but when there was more than one stallion they all did (paired t-test, $t_4=4.073$, $p<0.05$).

All mares had their own consistent maternal style. They all showed greater effort when in a band with more than one stallion, but the differences between mares were greater than changes within a mare.

Response to foal loss in previous year

There were no significant differences in any measure between a foal that died and the foal born in the subsequent year to the same mare. However, in the subsequent year there was a trend for mares to be closer (paired t-test $t=1.96$, $0.05<P<0.1$) and approach their foal more often (paired t-test $t=2.07$, $0.05<P<0.1$) during the first 20 days (Figure 6).

Paternity within one single-stallion band

During a foal's first 20 days mares put more effort into contact maintenance (proportion of approaches between mare and foal due to foal: band stallion sire 0.86, extra-band sire 0.63) and were closer to their foals that were not sired by the band stallion (mean distance between mare and foal in body lengths: band stallion sire 3.43, extra-band sire 1.11), and the difference was greatest for the mare that shifted into the group. There was no difference once the foals reached 50 days. However, foals not sired by the band stallion dispersed later (dispersal age in days; sired: 426, 573; not sired: 646, 659) and were in poorer condition as yearlings (sired: both 3.0, not sired: both 2.5).

DISCUSSION

Although no instance of infanticide was observed in our population, infanticide may have occurred, and it has been reported in other populations of equids (eg. Boyd, 1991; Duncan, 1982; Penzhorn 1984; Ryder and Massena, 1988). Even in lions, where infanticide is considered common following pride take-over by a new male or coalition of males, few infanticidal instances have been documented (Pusey and Packer, 1994). Mares that lose foals have a significantly higher foaling rate the following year than mares that do not foal or mares that raise a foal successfully. Consequently, horses are similar to other species in which infanticide occurs in that infanticide provides an advantage to infanticidal males. Furthermore, mares live in year round stable groups, so that paternity of foals is predictable. Inter-birth intervals are shortened by foal death, and there is a high probability that the subsequent foal will be sired by the male the mare is with during the first oestrus after foal death.

Maternal care patterns varied between bands with a single stallion and those with multiple males. Mares in multi-stallion bands put more effort into approaching their foals, and this was predicted by the rate of aggressive interaction between stallions and foals,
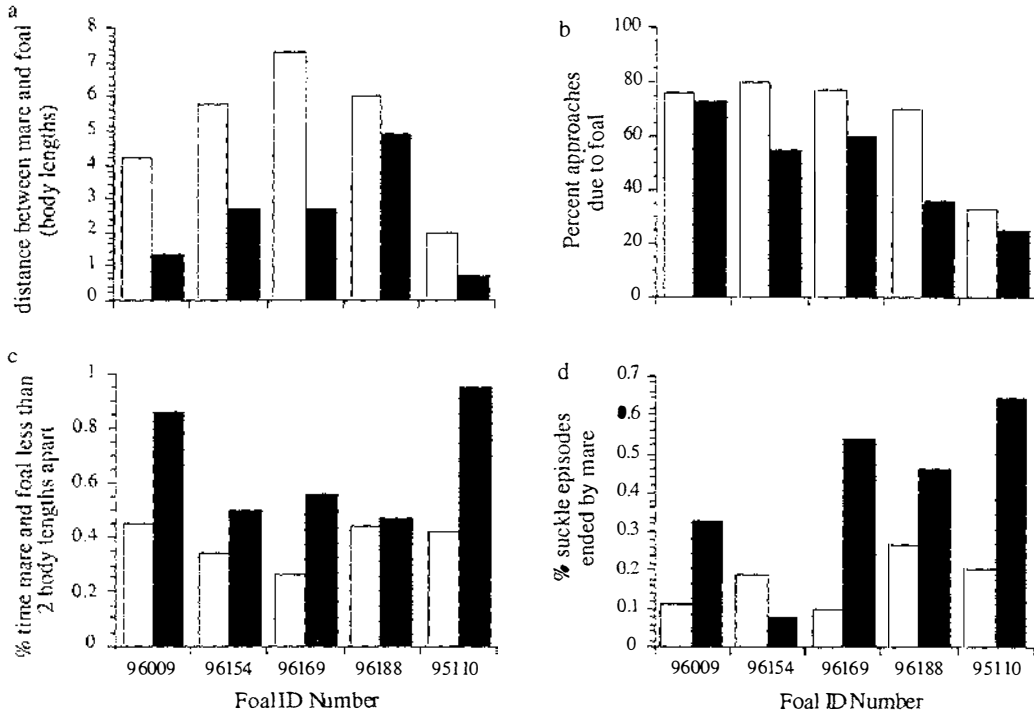
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Figure 5. Change in behavior of mare-foal dyads that had their band reduced to 1 male (96009, 96154, 96169) and which changed band types during lactation (96188 and 95110). a) distance between mare and foal (p<0.05), b) percent approaches due to the foal, where lower scores indicate more mare effort (p<0.05), c) percent time the mare and foal were proximal (p<0.05), and d) percent suckle episodes ended by the mare (p<0.1). Clear bars indicate a single stallion in the band and filled bars indicate more than one stallion in the band.
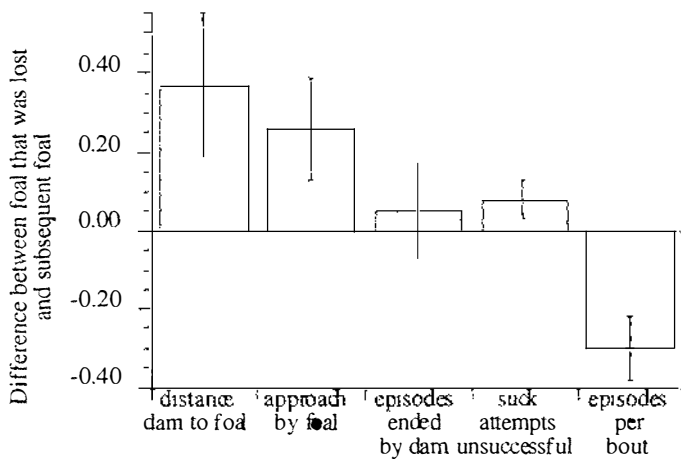

Figure 6. The effect of foal loss on subsequent maternal behavior. Bars indicate the difference in 5 maternal behaviors between the foal that died and the subsequent foal. The subsequent foal was closer to its mother, its mother approached it more often, it had fewer unsuccessful sucks and more episodes in each suck bout.

which was greater for foals in multi-male bands. In particular, mares in multi-stallion bands were more likely to approach their foals after an approach by a stallion (see Crowell-Davis & Houpt, 1986 for photo series of similar protectiveness in domestic horses). Mares in multi-stallion bands were more protective toward their foals, and they seemed to be responding to the possible risk of injury to the foal, as predicted by stallion aggression to the foal. Foal rearing was also more costly to mares in multi-stallion bands; they lost more condition and were less likely to foal in the subsequent year.

Mares that shifted bands or had their band experimentally reduced by the removal of the subordinate male were more protective when there was more than one male in the band. Social disturbance and the presence of unfamiliar males induces similar protective behaviour in other species (eg. Collins et al., 1984; Fairbanks and McGuire, 1984) and protectiveness reduces the likelihood of infants being killed (Collins et al., 1984). Therefore mares in single and multi-stallion bands do not have inherently different maternal strategies but adjust their behavior to the proximal potential risk. Each mare had her own maternal style, as has been found previously in a variety of species (eg. Fairbanks, 1986), including horses (Crowell-Davis, 1986a), but each was more protective in multi-stallion bands. Similar results have been found in primate groups where an unfamiliar male is present (Fairbanks, 1996).

In addition, within a single-stallion band mares were more protective of foals that were not sired by the band stallion. In horses, reports of stallion-foal aggression have been confined to circumstances where there is uncertainty of paternity (Boyd, 1991; Duncan, 1982; Ryder and Massena, 1988). Protective behavior is induced in the mothers of many species with the presence of non-paternal males (Fairbanks, 1997; Fairbanks and McGuire, 1987; Hrdy, 1977; Packer and Pusey, 1983), or other individuals that are a threat to offspring (Maestripieri, 1995, 1998; Parmigiani et al., 1994). Experimental manipulations of social environments are particularly valuable, as few studies have been able to separate cause and effect (Maestripieri, 1998). We show that mares are both assessing and responding to their current social environment, particularly differences in stallion behaviour, as well as assessing risk in relation to foal paternity.

Mares in multi-stallion bands seemed to have more costs associated with foal rearing, which may be due to the higher rates of aggression in multi-stallion bands that result in extra effort in the form of protective behavior (Linklater, 1998). Multi-stallion band mares were less likely to foal in the subsequent year, lost more weight during the period of peak lactation, and raised offspring who were in poorer condition as yearlings. Although we found no difference in foal survivorship in our focal population, Linklater (1998) shows that in the greater population of Kaimanawa mares, multi-stallion band females are more likely to lose their foal from conception to independence than are single stallion mares. In addition, Duncan (1992) suggested that social instability and stallion aggression contributed to high rates of neonatal death during some years.

Previous studies have found that mares who fail to form stable consort relationships or change groups regularly have lower reproductive success (Kaseda et al. 1995, Rutberg and Greenberg 1990), and suggest that aggressive interactions may interfere with successful reproduction indirectly (Rutberg and Greenberg 1990), or directly by feticide (Berger 1983) or infanticide (Duncan 1982). Linklater (1998) showed that mares that were not stable group members behave similarly to mares in multi-stallion bands. Therefore, aggression by stallions, and the potential for infanticide are important factors that modify maternal behavior and influence mare reproductive success. Mares in multi-stallion bands and in other situations where paternity is uncertain expend greater maternal effort in the form of protective behaviors, and consequently have lower reproductive success.

ACKNOWLEDGMENTS

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CHAPTER 4

Social Environment

SHARED OFFSPRING CARE BY A MOTHER AND HER DAUGHTER

"BLOOD'S THE WORD. NOTHING LIKE BLOOD,
IN HOSSES, DAWGS, AND MEN"
Thackeray, 1847-1848, 'Vanity Fair'.

Photo caption:
Toronto, with Banff (066). Banff is mother of Saskatchewan
(Sassy, 021), and both mares shared suckling and care.
Photo by Elissa Cameron

ABSTRACT

Non-offspring nursing is characteristic of species that have large litter sizes (polytocous) and live in small stable kin groups. In monotocous species this extreme form of communal parenting is associated with stealing and exclusive adoption. Wild horses (*Equus caballus*) are monotocous and live in small, stable non-kin groups. We report a mother and daughter who raised a foal together, sharing and coordinating all offspring care. The foal received the same total input as foals reared singly but each mare provided less input. An examination of the communal suckling that occurs in such unusual circumstances helps to explain its evolution. Our results are similar to other reported cases of cooperative communal suckling in monotocous species, which seems more common than previously thought. In all cases the cooperating individuals were close relatives, indicating that high degrees of relatedness are a pre-requisite for the occurrence of communal suckling in monotocous species, and implicating kin selection in the evolution of cooperative communal care.

Chapter reference:

Cameron, E. Z., Linklater, W. L., Stafford, K. J. & Minot, E. O. Shared suckling and offspring care by mother and daughter feral horse mares. *Submitted to Equine Veterinary Journal.*

INTRODUCTION

Amongst mammals non-offspring nursing is the most extreme form of communal parenting. This is because lactation is the most energetically costly part of parental investment (Clutton-Brock, 1991; Packer, Lewis & Pusey, 1992). Non-offspring nursing is most common in species characterised by large litters and small kin groups (Packer *et al.*, 1992; eg. lions *Panthera leo* Pusey & Packer, 1994). Although non-offspring nursing has also been reported in monotocous species (eg. water buffalo *Bubalus bubalus*, Murphey *et al.*, 1995; African elephant *Loxodonta africana*, Dublin, 1983; Lee 1987; Indian elephant *Elaphus maximus*, MacKay, 1973; Rapaport & Haight, 1987; fallow deer *Cervus dama*, San José & Braza, 1993; bighorn sheep *Ovis canadensis*, Hass, 1990) it is almost always associated with reproductive errors (Riedman, 1982) such as milk theft or exclusive adoption (Packer *et al.*, 1992). Simultaneous non-offspring nursing in monotocous species has been reported in African elephants (Lee, 1987), in some bat species that live in large colonies (eg. Mexican free-tailed bat *Tadarida brasiliensis*, McCracken, 1984; pipistrelles *Pipistrellus pipistrellus*, Eales, Bullock & Slater, 1988) and in captive Indian elephants (Rapaport & Haight, 1987). Recent research suggests that nutritive non-offspring nursing in African elephants is rarer than previously thought as

most reported instances were probably non-lactating juveniles allowing infants to suckle (Lee & Moss, 1986; Lee, 1987; Lee, 1989).

Horses are a monotocous species (Platt, 1978) and non-offspring nursing is rare both in captive (Crowell-Davis, 1985) and feral populations (Tyler, 1972; Packer *et al.*, 1992). Mares are typically intolerant of non-offspring foals and of other mares that approach their own young foals. Foals, however, may attempt to suck from mares that are not their mother (Crowell-Davis, 1985), from sub-adult females and even from maturing or adult males (Tyler, 1972), mares are typically intolerant of non-offspring foals and of allowing other mares to approach their own young foals (Tyler, 1972; Feist & McCullough, 1976; Berger, 1986). The occasional instances of non-offspring nursing are characterised by short suckles and involve mistaken identity, stealing or attempted stealing (Tyler, 1972; Crowell-Davis, 1985; Berger, 1986). Adoption or swapping of offspring are rarer still and only observed in domestic horses where the mothers are confined and parturition is highly synchronised (Tyler, 1972; Crowell-Davis & Houpt, 1986; Huntingdon & Cleland, 1992), or with human intervention (Tyler, 1972). All these instances are regarded as cases of reproductive error (Riedman, 1982). Shared care and nursing of an offspring by mares has not before been reported in equids.

We measured the behaviour of a mother and daughter Kaimanawa horse who raised a single foal between them, and compared their behaviour to mares who raised their foals singly. We also recorded instances where shared suckling could have occurred, such as orphaning and foal loss, to determine the circumstances in which shared care occurs.

METHODS

We have been studying the maternal behaviour of wild horses in the Kaimanawa ranges, New Zealand since 1994, and have recorded over 3000 suckle bouts involving 113 foals. Of these only one foal (F) has been observed to suck from more than one mare. F consistently nursed from two mares, who were themselves mother (M, born 1986, estimated from tooth wear) and daughter (D, born 1993). It is unusual in feral horse populations for mothers and daughters to live together because daughters disperse around sexual maturity (Berger 1986; Rutberg & Keiper, 1993; Monard, Duncan & Boy, 1996). In the situation described M and D joined their current band in late 1994 when D was a yearling still sucking from M. Consequently the stallion in the new band is not D's father, and M and D have remained in this band together.

Both M and D were judged pregnant from assays of faecal estrone sulphate concentration in samples taken between March and September 1996 (Henderson *et al.* 1997). However, parturition by M and D was not seen and they were observed with only one foal between them when it was around 3 days old.

To determine if the behaviour of M, D and F was different from those of normal mare-foal dyads, each mare was paired with two control mares. The mares were paired on the basis of their age, parity and the sex of their offspring. Band size was also similar for each of the six mares. All six mares and their foals were sampled between 6 and 11 times from birth to 100 days of age in focal samples of 30 to 220 minutes. The total observation time is shown in Table 1.

Table 1. Details of focal mares and foals.

Symbol	ID.	Name	Born[a]	Band size[b]	Experience	d.o.b.	Obs
D	021	Sassy	1993	8	Primiparous	2.11.96	1190
D1	009	Libby	1993	5	Primiparous	6.11.96	865
D2	169	Celeste	1993	3	Primiparous	1.10.96	1060
M	066	Banff	1986	8	Last foal 1994	2.11.96	1190
M1	127	Ulysses	1986	7	Last foal 1996	18.10.96	570
M2	100	Darcy	1987	7	Last foal 1993	22.11.96	710

a. Date of birth estimated by tooth wear patterns during a muster in 1994 except for D2 who was known to be a yearling in 1994.
b. Number of adult females in group.
c. Date of birth accurate to ± 2 days.
d. Total time that behaviour was sampled (minutes) from birth to 100 days of age.

During a sample we recorded every suck attempt; those lasting less than 5 seconds were denoted unsuccessful as no milk is likely to have been transferred in the first 5 seconds (Whittemore, 1980). The length of successful sucks and the interval between sucks was recorded to the nearest second. Small breaks in nipple contact during a bout were subtracted from measures of suckle bout duration. We used average duration (excluding breaks in nipple contact within a bout) and average inter-suck time to calculate total time spent suckling (Becker & Ginsberg, 1990). Bursts of sucking within a bout separated by breaks in nipple contact were termed episodes (termed bursts by Carson & Wood-Gush, 1983), and the number of episodes per bout was recorded. We also recorded whether or not a foal bunted (violently pushed nose into udder) during an episode and whether the mare of the foal terminated each episode. Breaks in nipple contact and bunting may indicate loss of milk flow or hunger (Lent, 1971; Gomendio, 1989), and the terminator of an episode may indicate levels of conflict between mare and foal and whether the foal fed to satiation (Byers & Bekoff, 1990).

We recorded every approach or leave event between mare and foal across a 2 body length boundary around each individual, and the instigator of these movements. These were used to calculate an index of the effort each put into maintaining contact (difference between % approaches and % leaves due to foal; Hinde & Atkinson, 1970). The index ranges between -100 and +100, with lower scores indicating more effort by the mother.

At four minute intervals we recorded instantaneous samples of the distance between mare and foal, and whether or not the mare and foal were nearest neighbours. We recorded the behaviour of the mare and foal, and for analysis of mare-foal distance and nearest neighbours we used only samples where at least one of the mare and foal were active (ie not standing or lying).

Throughout the study we have also recorded orphaning of foals and the result of the orphaning, and foal loss by mares to determine if shared suckling occurred.

RESULTS

F sucked M and D alternately; 83% of suckles were followed by a suck from the other mare ($\chi^2 = 10.60$, p<0.01). The total time F spent sucking per day (breaks in nipple contact between episodes of sucking within a bout were excluded), from M or D was less than the time control foals sucked from their mothers. Consequently, M and D each suckled F for less than half the time other mares suckled their foals, well outside the spread of times for all mares with female foals in 1996 (M = 19.36 mins/day, D = 13.38 mins/day; range for 20 other mares with female foals in 1996 = 39.73-146.09mins/day). For F, each sucking bout contained fewer breaks in nipple contact (F, 48% 1 episode only, controls, 25% 1 episode only, χ^2=6.58, 1 df, p<0.02), fewer sucking episodes were associated with bunts (χ^2=6.15, 1 df, p<0.02, figure 1a), the proportion of unsuccessful sucks was not significantly different but tended to be less (χ^2=0.624, 1 df, ns, figure 1b) and fewer sucking episodes were ended by the mother (χ^2=7.54, 1 df, p<0.01, figure 1c).

The mean distance between F and the mares M and D was greater than the mean distance between each of the control mares and their foals (Mann-Whitney U-test, p<0.0001), but the mean distance to the closest mother was similar (Mann-Whitney U-test, ns, figure 2a). Similarly, the proportion of time that either of the mares and F spent within 2 body lengths (figure 2b), or were each other's nearest neighbour (figure 2c), was lower for M and D than for the other dyads (χ^2=30.52, 1 df, p<0.001; χ^2=142.68, 1 df, p<0.001), but similar when calculated as the proportion of time spent within 2 body lengths of, or nearest neighbour of either mother (χ^2=1.22, 1 df, ns; χ^2=1.46, 1 df, ns). Consequently, each mare spent less time close to the foal, but the foal had at least one of the mares near as often as did singly reared foals. Moreover, each mare put similar effort into contact maintenance with F as did the control mares (figure 2d). Consequently, the differences in association patterns between F, M and D were not due solely to differences in F's behaviour; each mare still put a similar effort into maintaining contact with F as did control mares.
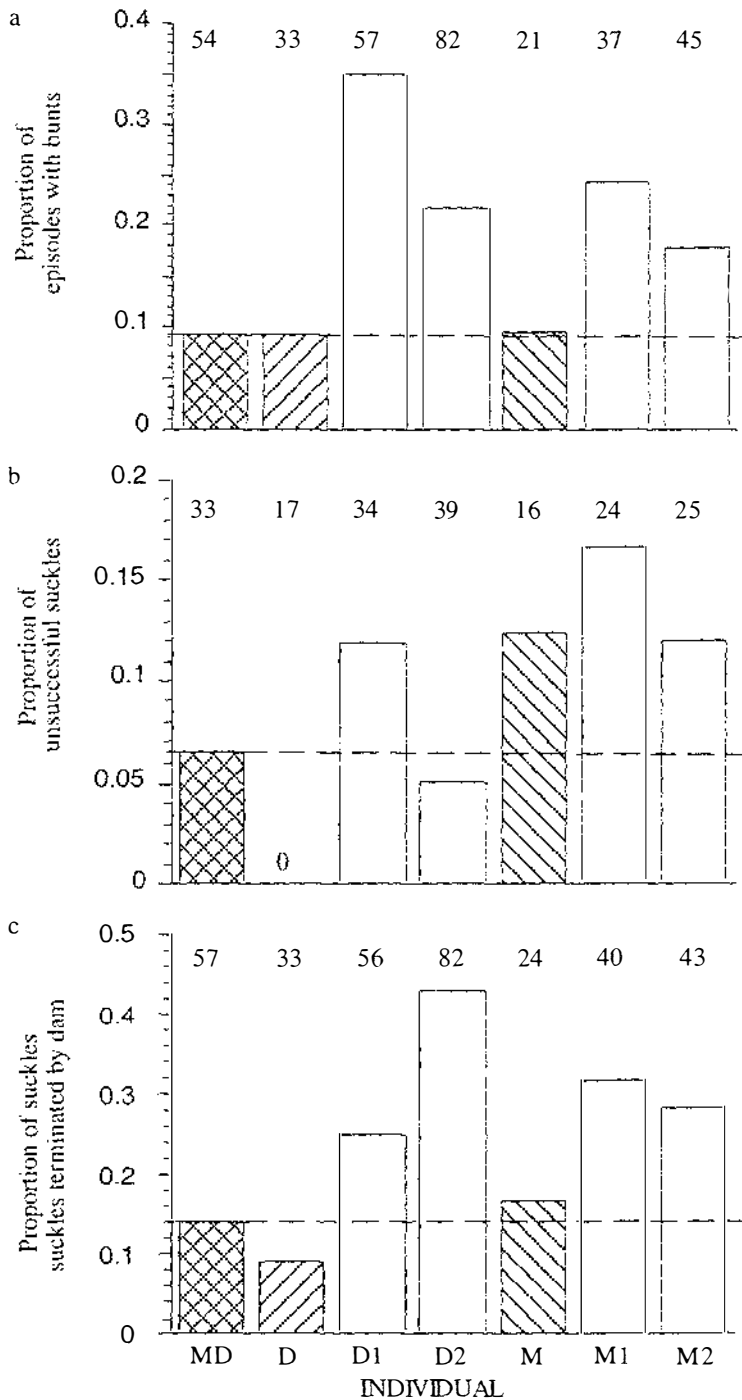
Figure 1. The proportion of a) suck episodes during which the foal bunted, b) suck attempts that were unsuccessful, and c) suckles ended by the dam of the shared foal compared to similar control foals that were raised singly from birth to 100 days. M refers to the elder mare who is mother of D, the younger mare and MD refers to the M and D combined (ie. input from the foals perspective), which is shown across the graph as a dashed line. Control mares, alike in all respects to either D or M except that they raised their foals singly are designated D1, D2, M1 or M2. Samples sizes are along the top.
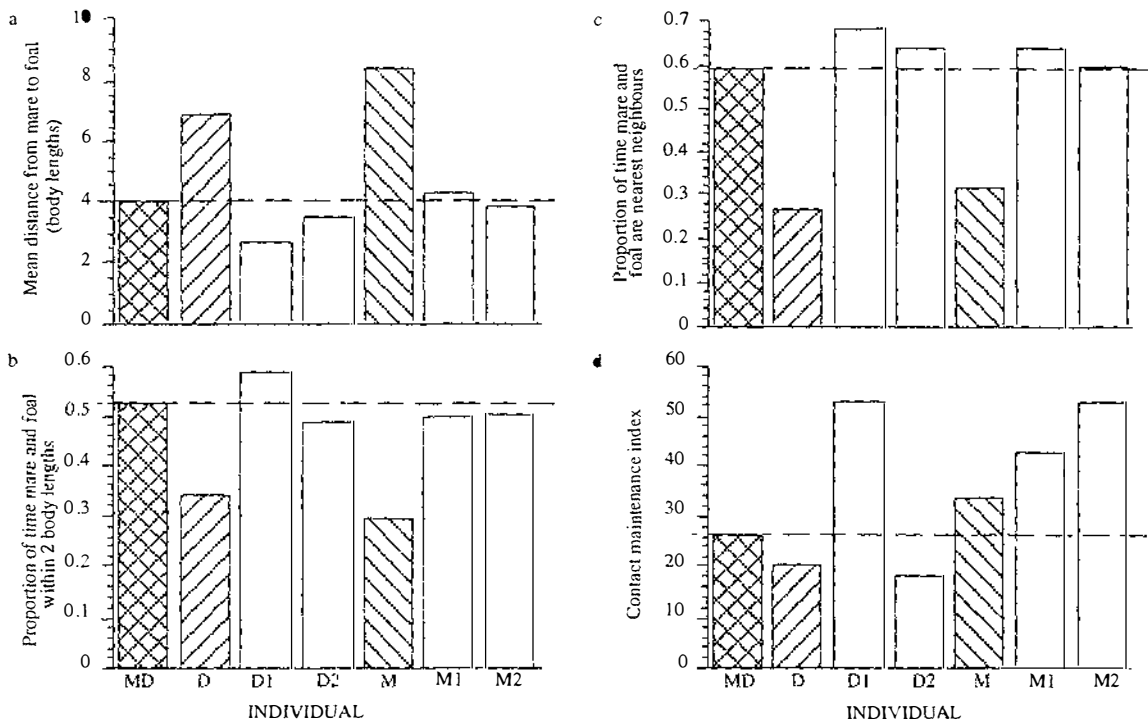
Figure 2. Spatial behaviour of the shared foal and control foals raised singly from birth to 100 days of age showing a) mean distance between mare and foal, b) proportion of time that mare and foal were proximal, c) proportion of time mare and foal were nearest neighbours, and d) contact maintenance where lower scores indicate more mare effort.

Other foals were never observed to suck from mares that were not their mothers even when they become temporarily separated from their mother (3-12 hours, 5 foals). Five foals orphaned since 1994 have not successfully sucked from any mare after the death of their mother. Two foals orphaned at a young age (6 and 21 days) attempted to suck from other mares but were prevented by aggressive responses. The youngest also attempted to suck from its dead mother, another dead mare and an observer (EZC). Both foals died within a month of becoming orphans. Foals orphaned at an older age (4, 5 & 9 months) were never observed to attempt to suck from any other horse, and all survived to adulthood. In addition, mares that lost their foal when other foals of a similar age were in their band never allowed those foals to suckle (n = 8).

DISCUSSION

Time spent sucking is an inaccurate measure of milk transfer (Babbitt & Packard, 1990; Cameron, in press [chapter 1]) and we suggest that F received as much milk as control foals, as indicated by fewer bunts, terminations by the mother and breaks in nipple contact, each of which indicate hunger or break in milk flow (Lent, 1971; Gomendio, 1989) and fewer unsuccessful bouts or bouts ended by the mothers, indicating that F fed to satiation (Byers & Bekoff, 1990). Consequently, although each mare was transferring less energy to F than she would a singly raised foal, F received at least as much milk as other foals. In only 10% of cases of non-offspring nursing do non-mothers provide the same quantity of milk as do mothers (Packer *et al.*, 1992). The spatial patterns we describe suggest that the females are not only sharing nursing of F, but that they are sharing other aspects of foal rearing. The degree of sharing suggests that each mother contributes approximately half and the foal receives the same total input. This indicates a high level of coordination and cooperation.

We report an unusual event in that a mother and daughter wild horse lived in the same social group with only one having a current foal. In this instance cooperative suckling and offspring care occurred. Although this is the first documented case of cooperative offspring nursing and care, we believe that it is probably not an isolated event. Shared offspring care and suckling have both been observed in managed semi-captive Icelandic horses (van Dierendonck, personal communication). Therefore, horses do have the capacity to cooperate in offspring care.

Previously reported studies of shared suckling in free living monotocous species have all involved closely related individuals. In both Mexican free-tailed bats (McCracken, 1984) and pipistrelles (Eales *et al.*, 1988) communal suckling appears to be associated with relatedness. African elephants that have suckled non-offspring have been either sisters or grandmothers of the infant (Lee, 1989). In captive Indian elephants a grandmother not only suckled her grandson simultaneously with its mother but came into milk to do so. In addition, the grandmother would let no other calf suckle (Rapaport & Haight, 1987). Except for in bats, a single shared offspring has been involved, supporting the hypothesis that monotocous species are constrained to care for only one offspring (Packer *et al.*, 1992). Previous studies (eg. Packer *et al.*, 1992) have suggested that cooperative nursing is associated with polytocous species but not monotocous species. The exceptions on both sides of this division suggest that it is not useful and it lacks explanatory power. Kin selection, however, seems to have an important role to play in the occurrence of cooperative offspring care.

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CHAPTER 5

Mare Age and Experience

AGING, EXPERIENCE AND INCREASING REPRODUCTIVE SUCCESS

" EXPERTE CREDITE"
(TRUST ONE WHO HAS GONE THROUGH IT)

Virgil, circa 19BC, 'Aeneid'.

Photo caption:
Experienced mother Musty (067) and Shiro,
her foal born in 1995 (95067).
Photo by Elissa Cameron

ABSTRACT

In many mammalian species females become more successful at raising offspring as they age. It is predicted that as a mother ages her residual reproductive value decreases and consequently each individual offspring will be more valuable as there is less conflict with potential future offspring. Therefore as mothers age their maternal investment should increase. Empirical evidence of the influence of declining residual reproductive value on maternal investment is unconvincing in many studies. Alternatively, mothers may not be investing any more, but may be more successful due to greater experience. Most studies do not preclude either hypothesis. In horses, a highly polygynous but only marginally sexually dimorphic species, mare age did influence maternal input. Older mares, who were more successful at raising foals, were more protective for the first twenty days of life, but less diligent thereafter. Total maternal input by older mothers did not seem to be any greater, but it was better targetted. In addition, older mothers were more likely to foal in two consecutive years, supporting the hypothesis that they are investing less in their offspring than younger mares. Therefore, older mothers seem to be more successful due to experience, not a decreasing residual reproductive value.

Chapter reference:

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INTRODUCTION

It has been predicted that reproductive effort should progressively increase with age in animal species in which reproductive value declines with age (Williams 1966, Pianka and Parker 1975, Clutton-Brock 1984, Clutton-Brock 1991). Reproductive effort in the current offspring should increase and parent-offspring conflict over allocation of resources decrease as residual reproductive value decreases. Each offspring should become more valuable to a mother as the number of future potential offspirng declines over her lifetime (Residual Reproductive Value Hypothesis, RRV, Trivers 1974, Pianka and Parker 1975, Clutton-Brock 1991). Many mammalian species appear to support this hypothesis as success at raising offspring increases with age (eg. langurs *Presbytis entellus* Dolhinow et al. 1979, white-tailed deer *Odocoilus virginianus* Ozoga and Verme 1986, bighorn sheep *Ovis canadensis* Festa-Bianchet 1988a, pigs *Sus scrofa* Meikle et al. 1996).

However, to show that reproductive effort increases with age requires a demonstration that older mothers devote a greater proportion of their available resources

to offspring, and the mother must experience reduced survival or reproductive success (Clutton-Brock 1991). An increase in reproductive success with age could also be explained by maternal experience rather than increasing maternal effort; older mothers may be more successful at rearing young because of experience gained from previous offspring. Consequently, they may invest no more in their offspring, but may target their care to the periods in which it is most valuable (eg. Green 1990, 1993, Fairbanks 1996). We call this hypothesis the Targeted Reproductive Effort hypothesis (TRE). The role of experience appears to be most obvious in females that have lost their previous offspring (Fairbanks 1988, Cameron 1998 [chapter 3]). These hypotheses (RRV and TRE) generate opposing predictions; the first that reproductive effort increases with age and the second that reproductive effort decreases with age (Fairbanks 1996).

Green (1990) found that as bison mothers age their offspring suckle more but that the costs do not increase. This suggests an advantage to greater experience. Contrary to the prediction of greater investment in individual offspring with declining residual reproductive value, younger mothers, particularly primiparae, often incur the greatest reproductive costs (eg. rats *Rattus norvegicus* Künkele and Kenagy 1997, bighorn sheep Festa-Bianchet 1988b).

Many studies use time spent suckling as an index of maternal investment based on the assumption that more suckling equates with more milk received (eg. Green 1986). However, suckling behaviour is not a reliable indicator of milk intake (Cameron, in press [chapter 1]) and can lead to incorrect conclusions about maternal investment (Cameron et al. in press [chapter 2]). Therefore, in the absence of direct measurements of milk transfer, maternal behaviour is an important measure of effort (Reiter et al. 1981, Clutton-Brock 1984, Festa-Bianchet 1988b). Suckling can only be used as a measure of conflict between mother and offspring (Berger 1979, Byers and Bekoff 1986). More importantly, costs to the mother, particularly in future reproduction are an implicit part of maternal investment (as defined by Trivers 1972) and we measure costs to the mother both proximally in terms of condition loss and costs to her future reproductive success.

In horses, mares become more successful at raising foals as they age, and this could be because of declining RRV (Rubenstein 1982). However, this hypothesis has not been tested and Cameron (1998 [chapter 3]) showed that the loss of an offspring causes increased maternal input in the next foal, but only during the early part of its life, 0-20 days, during which time 50% of foal mortality occurs. Therefore maternal behaviour in horses is modified by mare experience, and individual mares have individual maternal styles (Cameron 1998 [chapter 3], Crowell-Davis 1986a). In addition, horses are limited to a maximum of one foal per year, have a long period of maternal dependence, which seems to vary markedly, and not all mares foal in every year.

The two hypotheses both predict that mares change their maternal behaviour as they age. However, as maternal investment is ultimately defined in terms of costs to a

mother's future reproduction (Trivers 1972), the RRV hypothesis and its consequent increased maternal investment would predict that older mares would be less likely to foal in the year after raising a foal. However, the TRE hypothesis would predict that a old mare was at least as likely as a younger mare to foal in consecutive years.

We investigated maternal investment in Kaimanawa horses in relation to mare age to determine if increasing reproductive success with age is due to increased maternal investment in individual offspring, or whether mares are more successful because they are more experienced.

METHODS

We studied maternal behaviour and maternal costs in relation to mare age in a population of feral horses that inhabit the area surrounding the Kaimanawa ranges in central North Island, New Zealand. The population has been feral since the late 1800s. Horses live in stable year-round social groups (bands) which contain between one and four stallions and one or more females and their immature offspring. The study site, study population and its social organisation are described in Linklater (1998).

Mares were aged by tooth eruption and wear patterns during a muster in 1994; additional mares were known to be yearlings in 1994, and one mare was aged as belonging to the oldest age category based on published photographs showing her to be adult in 1989. Mares do not usually foal until their 3rd year and do not reach their full reproductive potential until 5 years. We therefore classified mares between the ages of 3 and 5 as young (Y), and all these mares were raising their first or second foal. The next 3 year age category (6-8) included no primiparous mares, and we classified these as mid-aged mares (M). For 10 year olds or older, aging by tooth wear is inaccurate (Richardson et al. 1995), and we therefore grouped all mares 9 or older into the oldest category (O). We knew the age of 61 mares, and 47 of these mares had at least one of her foals born in 1994, 1995 or 1996 studied as a focal foal (Y=31, M=26, O=26). 16 mares had two focal foals, 10 had three focal foals and 21 contributed only one foal.

Pregnancy rates were calculated from analysis of oestrone conjugates in faeces or blood (Henderson et al. 1996). Foal birth dates were known to within ± 5 days. Foetal losses were calculated for the 61 focal mares from pregnancy and foal at foot rates. Consequently some losses that were claasified as foetal losses may have been neonatal foal losses. Foal losses were calculated from birth to one year of age. Foetal and foal losses were combined to determine total rate of offspring loss.

Measures of suckling behaviour and the spatial relationship between mares and their foals were measured. The first twenty days, during which foal mortality was highest, were grouped in the first time category; thereafter the categories were based on life history milestones. The second category was 21-50 days, the period surrounding peak lactation. 51-110 days includes the period of investment that is essential to foal

survival. No foal weaned younger than 110 days survived (Cameron 1998 [chapter 4]). All foals received maternal input in the form of milk to around 200 days of age if they were not orphaned. All samples between 200 days and weaning were grouped together and samples from weaning to dispersal were the final category.

Mare-foal dyads were sampled at all times of the day. Bands were located and mare foal dyads were sampled in focal animal samples either until both individuals moved out of view or until at least 3 suckle bouts had been recorded. No samples were taken at night.

During the focal sample, instantaneous samples (Altmann 1974) were taken every four minutes at which time the distance between the mare and foal, and the behaviour of the mare and foal were recorded. Four minute intervals were chosen as they have been shown to accurately represent behaviour but be far enough apart to enhance independence of data (Rollinson et al. 1956). In addition, other studies have used similar time intervals (eg Boy and Duncan 1980, Crowell-Davis1986a, Duncan et al. 1984). Distance between individuals was estimated in adult body lengths. Foals that were closer than 1 body length to their mother were recorded as 0.5 body lengths. For analysis of distance between mare and foal we used only samples where at least one of the dyad was active (that is not lying down or standing still).

All approaches across a 2 body length boundary around the mare and the foal were recorded, and in 1995 and 1996, all leaves across the boundary were also recorded. On the rare occasion where it was not possible to tell whether the mare or the foal was the instigator of the approach or leave, the event was recorded, but not used in analysis of approaches and leaves. For analysis, we used % of approaches that were by the foal, % leaves by the foal, and an index of contact maintenance which incorporates both approaches and leaves calculated as the differences between % approaches by foals and % leaves by foal (Hinde and Atkinson 1970). The resultant score varies between +100 and -100. Positive values occur when the mother is primarily causing separation, and negative values occur when the mare is primarily responsible for contact. Consequently, lower scores indicate more mare effort into maintaining contact with her foal. During 1994 only approaches were recorded and we therefore used the percentage of approaches that were by the foal as an index of contact maintenance.

We do not use suckling behaviour as an index of energy intake, but as an indication of maternal behaviour and rate of conflict between mare and foal over energy intake. We measured the length of suckle bouts to the nearest second, excluding small breaks in nipple contact during a bout. Within a bout the sucking bursts separated by breaks in nipple contact were called suck episodes (c/f bursts, Carson and Wood-Gush 1983). We recorded the number of episodes per bout, the number of episodes during which the foal bunted, who ended each episode and the time between bouts. Mares were judged to have ended an episode when the mare's activity or aggression from the mare

forced the foal to withdraw its nose. When the foal withdraw its nose with no noticeable change in mare behaviour, the foal was judged to have ended the episode. Unsuccessful suckle attempts were those that lasted less than 5 seconds, during which time the milk would probably not have been released (Whittemore 1980). We calculated the proportion of suck attempts that were unsuccessful. We converted duration and frequency of suckling into a measure of time spent suckling per day (Becker and Ginsberg 1990).

Social interactions between the mare and foal were recorded on an all-occurrence basis. In each instance the type of interaction was recorded, and the instigator of the interaction. For the purposes of analysis we used social interactions that were not associated with suckling; unsuccessful suck attempts and interactions that occurred during a suckle bout were not included.

Multivariate analyses of variance (MANOVA) were performed using the general linear models procedure in SAS (SAS Institute 1989). The results of statistical tests presented are 2-tailed. We considered p<0.05 as statistically significant and 0.05<P<0.1 as a trend.

RESULTS

Of all mares in the focal population whose ages were known (n=55), older females had higher foaling rates (% foaling: Y=54, M=71, O=77, χ^2=9.32, 2df, P<0.01). In addition, foals of young mares tended to die more often than foals of mid-aged or old mares (% foals die before 1 year: Y=18, M=3, O=9, χ^2=5.49, 0.1<P <0.05).

There was no difference in foal sex with mare age (% male: Y 47, M 58, O 54, χ^2=0.70, 2df, NS). However, there was a significant difference in mare age in the different band types, predominantly because young mares were less likely to be in a stable group (Fig 1, χ^2 = 12.39, 4df, P <0.01).

Old mares put more effort into maintaining contact with or approaching their foals for the first 20 days of life, but less thereafter (Fig 2a). Old mothers also spent less time close (<2 body lengths) and more time far (>15 body lengths, Fig 2d) from their foals after the first 20 days. However, the mean distance between mare and foal was similar for all ages during the first 50 days, with older mares being further from their foals thereafter (Fig 2b).

There was no significant difference with mare age in any suckling behaviour except the proportion of suckle attempts that were unsuccessful; older mothers had the least and mid-aged mothers the most unsuccessful suckles (Fig 2c). Therefore, older mares showed the least conflict with their foals over the milk supply.

There was no difference in rate of interaction between mares and their foals in relation to mare age although old mares tended to interact less (mean ± standard error: Y 0.33 ± 0.6 n=31, M 0.31 ± 0.44 n=26, O 0.24 ± 0.05 n=22, ANOVA $F_{2,76}$=0.77 NS).
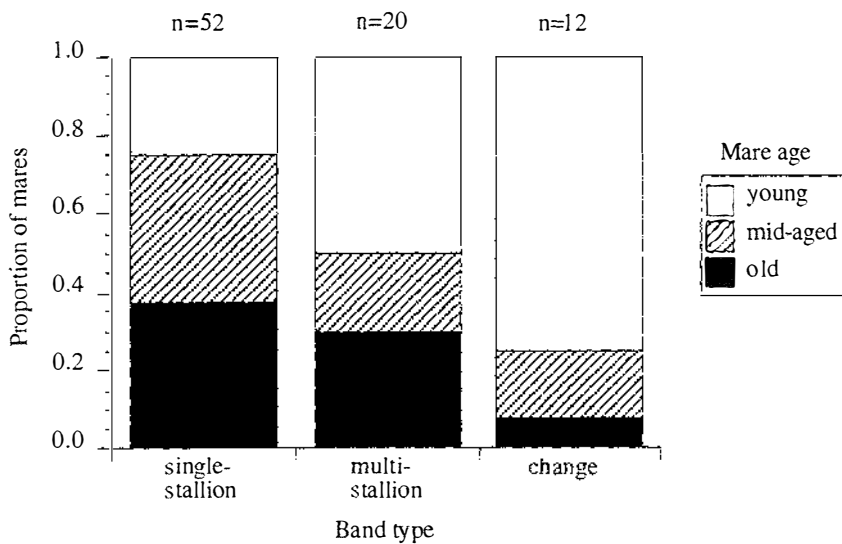
Figure 1. Band type in which mares raised their foals in relation to mare age.

There was no significant difference in weaning age between age categories (ANOVA $F_{2,38}$=0.85, Fig 3a). However, both young and mid aged mares weaned foals younger if they had a foal in the subsequent year than if they did not foal (young, t_{15}=3.8, P <0.01; mid, t_{13}=2.43, P <0.05) whereas there was no significant difference in foal weaning age in relation to the mother's future reproduction in old mares (t_9=1.47, NS; Fig 3b). Old mothers generally weaned their foals before one year of age regardless of whether they were pregnant, whereas younger mothers continued to suckle their yearlings if they did not foal.

Mare age had no significant effect on differences in mare weight change either during gestation (mid gestation condition minus pre-birth condition, ANOVA $F_{2,57}$=1.12 NS) or during from birth to after peak lactation (pre-birth condition minus condition during 3rd month post-partum (ANOVA $F_{2,74}$=1.37 NS).

Of all the mares that produced a foal in one year only a proportion also foaled in the subsequent year. We found no significant difference in this proportion between young, mid and old mares, though there was a tendency for more mares to foal as they aged (χ^2= 5.05, 2df, 0.05< P <0.1, Fig 4). Mare age was a significant predictor of whether a mare foaled in the year after successfully raising a foal (Logistic regression χ^2= 6.45, 1 df, P <0.01).
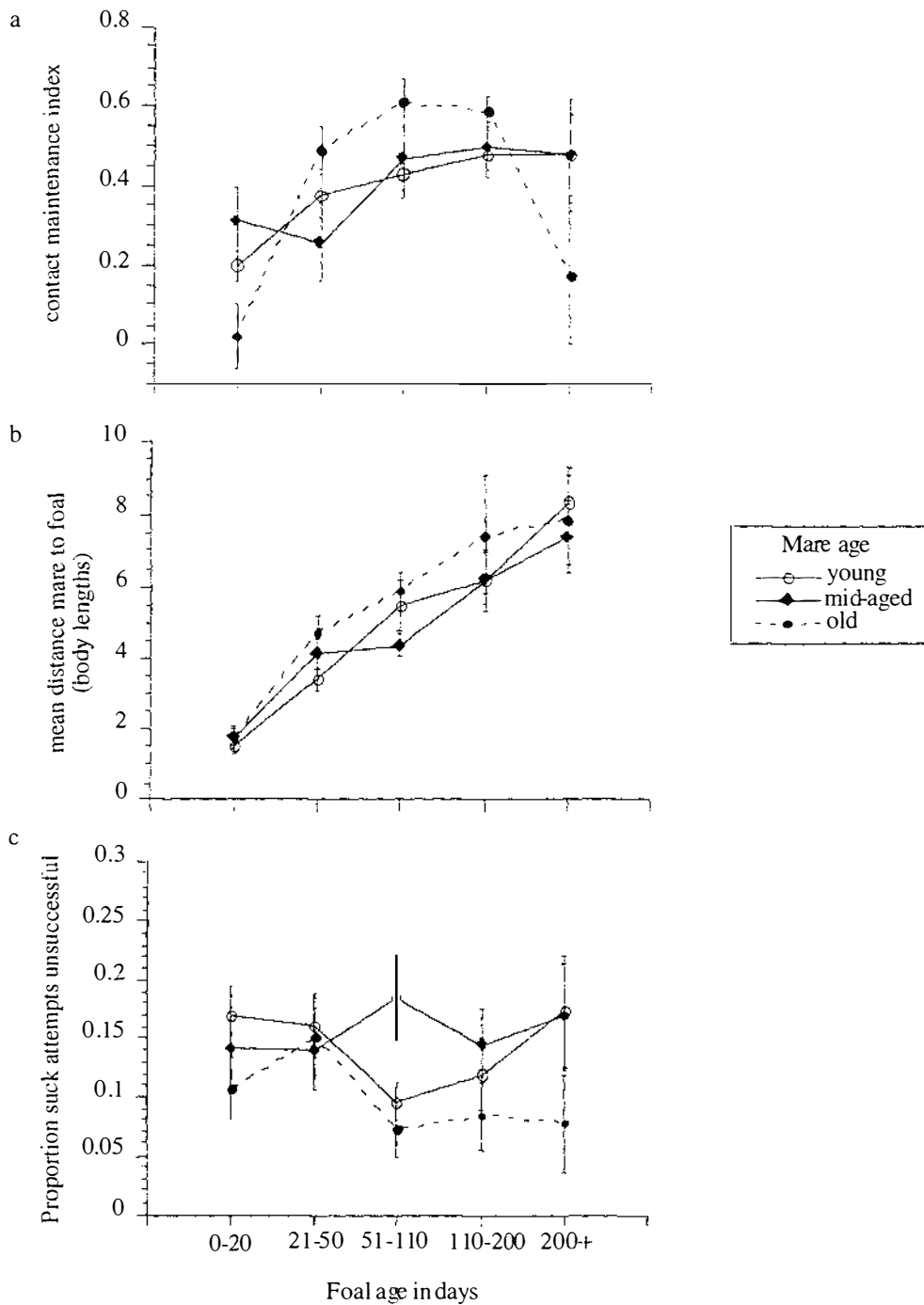
Figure 2. Mare effort with increasing age up to weaning in terms of a) maintaining contact with her foals (% approaches-% leaves by foal, Hinde and Atkinson 1970), b) the mean distance between mare and foal, and c) the proportion of suckle attempts that were unsuccessful. Values presented are means ± standard error

n= 17 15 9   7 6 3   10 9 6

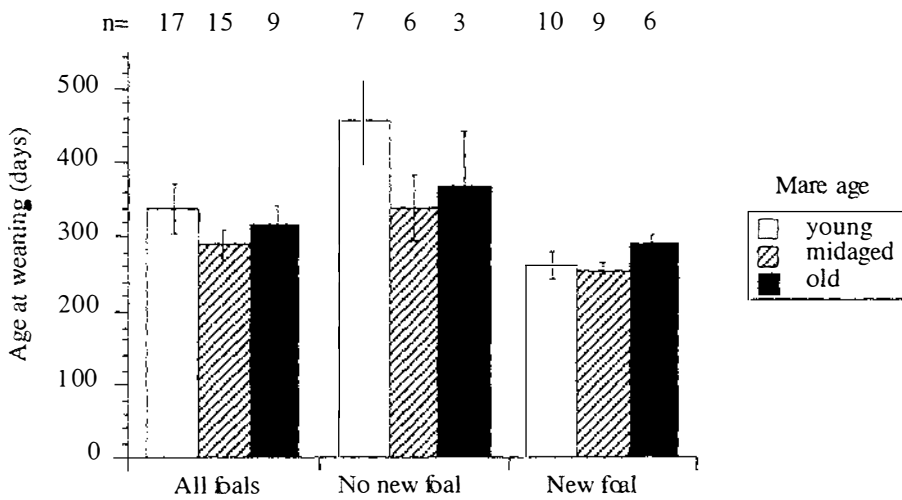Figure 3. Weaning age of foals by the age of its mother for all foals, foals whose mothers did not foal in the subsequent year, and foals whose mothers foaled in the subsequent year. Values are means ± standard error.
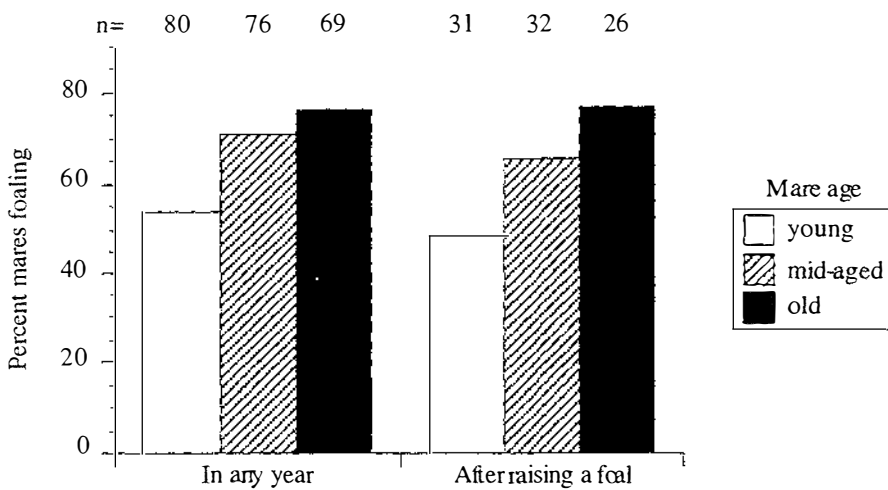
n= 80 76 69   31 32 26

Figure 4. Reproductive success of mares of different ages in any year, regardless of reproduction in the previous year, and in the year following successful foal rearing.

DISCUSSION

Older females are more successful at raising offspring because they have a higher probability of giving birth in any year and lower death rates of neonates (eg. Ozoga and Verme 1986; Festa-Bianchet 1988a). Our results show that Kaimanawa mares were consistent with this pattern. Old Kaimanawa mares show greater effort in the first 20 days after birth, during which time most foal deaths occur, but less effort thereafter. Other studies have found similar results (eg. bison, Green 1986, 1990; vervet monkeys, Fairbanks 1996). In addition old mares, with the lowest residual reproductive value show the least conflict with their foals over suckling, whereas mid aged mares show the most conflict.

These results are consistent with greater experience leading to better targeted investment; older mothers appear to be better mothers due to their past experience. Older mares appear to be investing no more and possibly less, in total. However, the nutritional demands of lactation are by far the greatest cost to mothers, and we did not assess these here. Nonetheless, there was some indication that older mares were more tolerant of foal suckling, consistent with their decreasing reproductive value. These results are consistent with the influence of foal loss on subsequent reproductive effort in Kaimanawa horses; mares that lost a foal increased their effort during the first 20 days of foal life (Cameron 1998 [chapter 3]).

Maternal investment is defined in terms of costs to future reproduction. Therefore, we can distinguish between the increased maternal investment predicted by RRV models and the lack of increased investment that would be predicted from TRE models of increasing maternal experience. We found no difference in costs in terms of weight change between the three age categories. Old mares, however, were more likely to foal in the year following the successful rearing of a foal. In contrast, only about half of the young mares foaled after successfully rearing a foal in the previous year. This result should be treated with caution. However, due to the inaccuracy of aging techniques (Richardson et al. 1995) means that the range of ages in the old category was greater than in the other categories, and the very old mares may have suffered some reproductive costs. In domestic horses a decline in reproductive success occurs after around 15 years of age, though it is not clear whether this is an effect of age or multiparity (Ginther 1992). Similar declines have been noted in some feral populations (eg. Berger 1986, Garrott et al. 1991), but not in others (eg. Duncan 1992). Nevertheless, the hypothesis of increased maternal effort with declining residual reproductive value predicts that maternal investment increases as females age, not just in very old mothers (Clutton-Brock 1991), although most strong evidence of increased maternal investment with age occurs in mothers who do not survive to reproduce again (eg. Clutton-Brock 1984, Green 1990).

Therefore, our results support predictions based on increasing experience rather than declining residual reproductive value. This is consistent with other studies that have

shown that reproduction costs more to young mothers and less to old mothers (eg. Green 1986, Festa-Bianchet 1988b, Künkele and Kenagy 1997). Older mothers are more successful mothers because they target their investment to the periods of high foal death and invest less thereafter. They wean all foals around the same age regardless of their pregnancy status, and suffer lower reproductive costs. They do not appear to invest more, but are 'older and wiser'.

ACKNOWLEDGEMENTS

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CHAPTER 6

Offspring Sex

BIRTH SEX RATIOS RELATE TO MARE CONDITION AT CONCEPTION

"I FORMERLY THOUGHT THAT WHEN THE TENDENCY TO PRODUCE TWO SEXES IN EQUAL NUMBERS WAS ADVANTAGEOUS TO THE SPECIES IT WOULD FOLLOW FROM NATURAL SELECTION."
Darwin, 1871, 'The Descent of Man and Selection in Relation to Sex'.

Photo caption:
Grommett (026) mates with Libby (009), with Libby's foal
Palace (96009) in the foreground.

Photo by Elissa Cameron

ABSTRACT

Several hypotheses have been proposed to explain variation in birth sex ratios, based on the premise that variation is expected when the profitability of raising sons and daughters varies between individual parents. We test the Trivers-Willard hypothesis that mothers in better condition produce relatively more sons and that mothers in poorer condition produce relatively more daughters when male reproductive success is more variable. We examined birth sex ratios in relation to mare body condition at conception in horses. Horses meet the assumptions of the Trivers-Willard hypothesis better than many species on which it has been tested and in which sex ratio biases are not confounded by sexual size dimorphism such that one sex is more likely to be lost *in utero* by females in poor condition. Mares that had a female foal were in poorer condition at conception than those which had a male foal, and mares that had foals of different sexes in different years were in significantly poorer condition when they conceived their female foal. There was no relationship between offspring sex and mid-gestation condition and there was no difference in foaling rates in relation to body condition at conception. Consequently, sex ratio deviations are not explained by foetal loss *in utero*. Furthermore, differential foetal loss of the less viable sex cannot explain the greater proportion of males produced by mares in better condition. Therefore our results suggest that sex ratio modification occurs at conception in wild horses.

Chapter reference:

Cameron, E. Z., Linklater, W. L., Stafford, K. J. & Veltman, C. J. Birth sex ratios relate to mare condition at conception in Kaimanawa horses. *submitted to Behavioral Ecology.*

INTRODUCTION

Sex ratios at birth and hatching can vary (Clutton-Brock, 1985; Clutton-Brock et al., 1986; Clutton-Brock & Iason, 1986; Frank, 1990; Hardy, 1997). Adaptive theories predict variation in birth sex ratio when the profitability of raising sons and daughters varies between individual parents (Trivers & Willard, 1973). Large sex ratio variations were recently reported in Seychelles warbler eggs, indicating differential conception of male and female offspring in relation to offspring profitability (Komdeur et al., 1997). In mammals, although variations in birth sex ratios tend to be relatively small, some estimates have been based on inadequate evidence and there is a positive publishing bias (Festa-Bianchet, 1996), a few studies have shown significant variation (Clutton-Brock & Iason, 1986).

The Trivers-Willard model suggests that where one sex has more variable reproductive success, such as males in polygynous species, mothers in good

condition will be advantaged by producing more of that sex, whereas mothers in poor condition would be advantaged by producing more of the reproductively stable sex (Trivers & Willard, 1973). The hypothesis was formulated for species with a litter size of one, and depends on three premises (Trivers & Willard, 1973):

a) that the condition of the young at the end of parental investment will tend to be correlated with the condition of the mother during parental investment.

b) that these differences in condition tend to endure into adulthood.

c) that adult males will be differentially helped in reproductive success by slight advantages in condition, such as through intense male-male competition in polygynous species.

Wild horses meet these assumptions better than most species on which the hypothesis has been tested previously. Horse litter size is fixed at one (Platt, 1978). Differential investment in male and female offspring has been reported (Berger, 1986; Duncan et al., 1984), and there is a correlation between maternal rank and reproductive success in both sons (Feh, 1990) and daughters (Duncan, 1992), suggesting a correlation between female condition and offspring condition and reproductive success as adults. In some other species, one sex may be easier to invest in than the other. For example, where sons disperse and daughters are philopatric, it may be easier to influence the reproductive success of daughters for relatively little extra investment. This is not the case in horses, where both male and female offspring disperse, and so there can be no direct inheritance of social rank (Berger, 1986; Rutberg & Keiper, 1993). In addition, sexual size dimorphism at birth is minimal (Duncan, 1992), so males are not markedly more costly to raise *in utero* due to accelerated growth rates alone, which has been a frequently cited reason why mothers in poor condition lose more male offspring in other species (Clutton-Brock, 1991).

Previous studies have examined offspring sex ratios in mammals in relation to indices of condition such as food availability or diet (eg. Monard et al., 1997; Smith et al., 1996), maternal ranking (eg. Cassinello & Gomendio, 1996; Clutton-Brock et al., 1984; Festa-Bianchet, 1991), whether mothers were primiparous or multiparous (eg. Cassinello & Gomendio, 1996), reproductive success in the previous year (eg. Green & Rothstein, 1991; Rutberg, 1986), interval since last offspring (eg. Wiley & Clapham, 1993), or maternal body condition at slaughter when foetuses are mature enough to be sexed (eg. Reimars & Lenvik, 1997; Wauters et al., 1995). In Camargue horses, Monard et al. (1997) found that sex ratios were female biased in years following a season of poor food availability, during which mares were in poorer body condition. However, they were unable to determine if differences were due to differential conception or to differential loss during gestation. Estimates of female body condition around (Kojola & Eloranta, 1989) or before (Moses et al., 1995)

conception provide more appropriate estimates at the time when sex ratio adjustment can occur (Krackow, 1995).

We aim to determine if birth sex ratios vary according to the predictions of the Trivers-Willard hypothesis in relation to condition at conception both within and between individual mares. We examine differences in foaling rates of mares of different condition to determine if sex ratio variation is likely to be due to differential loss of offspring or differences in conception sex ratio. Furthermore, we investigate variation in sex ratio in relation to the previously used indices of whether mares are primiparous or multiparous, previous year's foaling success and mid-gestation condition.

METHODS

Feral horses (*Equus caballus*), inhabit the Kaimanawa mountains and surrounding plateaus and valleys of central North Island, New Zealand. Since August 1994 we have been studying a population of approximately 400 of these horses which inhabit the Moawhango river basin and surrounding plateau. Individual horses were reliably identifiable by either freeze brands on their rumps or by natural markings.

Body condition scores were estimated by visual body fat distribution based on an 11 point scale from 0-5 with 0.5 gradations (Carroll & Huntingdon, 1988; Huntingdon & Cleland, 1992; Rudman & Keiper, 1991) with the aid of 10-15x binoculars or a 15-60x telescope whenever horses were sighted, provided visibility was good. Mares with scores of 0 were very thin and with scores of 5 were obese. Interobserver reliability was high (EZC, WLL, $r = 0.91$). In horses, body condition scores correlate with body fat percentage ($r=0.81$, Henneke et al., 1983).

We calculated body condition at conception for Kaimanawa mares by backdating from the date of foaling (accurate to ± 5 days) by the average gestation length ± 1 standard deviation (336 ± 10 days; Kiltie, 1982) and taking the mode of visual body condition scores during this period for mares with foals born in 1995, 1996 and 1997, and at mid gestation (168 ± 10 days) for foals born in 1995 and 1996. All mares used in the analysis were scored at least twice during these 20 day periods, and mares were only scored once on any one day. Modal body condition scores were also calculated for mares in the month before birth, and for foals in their 12th month to determine if female condition during investment was reflected by foal condition near the end of the period of investment.

Body condition scores at conception approximated a normal distribution with the modal scores of most mares being 2.5 and with a spread from 1 to 4. 33 mares foaled once, 30 foaled twice and 14 foaled three times between 1994 and 1997.

Mares were classified as primiparous if they had not foaled before and were known to be too young (≤ 2 years) to have foaled prior to the study (n=22). Mares

were classified as multiparous only if they were known to have foaled previously (n=103; unknown n=10). We also classified mares in relation to their previous year's reproductive success (no foal or lost foal n=67, successfully raised foal to 6 months n=67, unknown n=1)

We recorded the sex of every foal born to a mare within the focal population in the foaling season (September to February) starting in 1995 (n=42), 1996 (n=55) and 1997 (n=38) by sighting genitalia, which are visible in both sexes from birth.

To determine if foaling rates were different between mares of different condition at conception we recorded the condition of all mares during the mean conception date ± 1 standard deviation (15 November to 16 January 1994-95 and 1995-96) and recorded which of these mares foaled in the subsequent season.

The results of statistical tests presented are two-tailed.

RESULTS

There was a significant difference between the condition at conception of mares who had a female foal and mares who had a male foal (Mann-Whitney U-test, U=2794, N_1=69, N_2=66, p<0.05). Improved mare condition at conception was a significant predictor of offspring sex (logistic regression, df=1, χ^2=7.89, p<0.01, figure 1). Furthermore, we compared the condition at conception of mares that had foals of different sexes and found that mares were in significantly poorer condition when they conceived their female foal (Paired t-test, t_{24}=-2.45, p<0.05).

There was no difference in birth dates throughout the season for male or female offspring (Mann-Whitney U-test, U=2205, N_1=69, N_2=66, ns). There was no significant variation in sex ratio between primiparous and multiparous mares, whether a mare had foaled in the previous year or the year the foal was born in (Table 1).

Although mare condition at conception was correlated with mare condition at mid-gestation (r_s=0.36, n=72, p<0.05), there was no difference in mare condition mid-gestation between mothers who had a male or female foal (Mann-Whitney U-test, U=777, N_1=41, N_2=36, ns). The correlation was stronger between condition at conception and condition during the month before birth (r_s=0.64, n=80, p<0.001). There was also a correlation between mare condition before birth and foal condition as yearlings (r_s=0.62, n=72, p<0.05).

There was no significant difference in foaling rates with condition at conception (Mann-Whitney U-test, U=2579, N_1=50, N_2=110, ns). Around 30% of all mares did not foal. In mares that foaled in subsequent years there was a no difference in time between birth of the current foal and the conception of the subsequent son or daughter (Mann-Whitney U-test, U=514, N_1=37, N_2=35, ns), though there was a tendency for males to be conceived after a longer period of time.

Figure one. Birth sex ratios in relation to condition at conception in Kaimanawa horses. Note that no mares of condition less than 1 at conception conceived, and that no mare scored over 3.5 at conception.
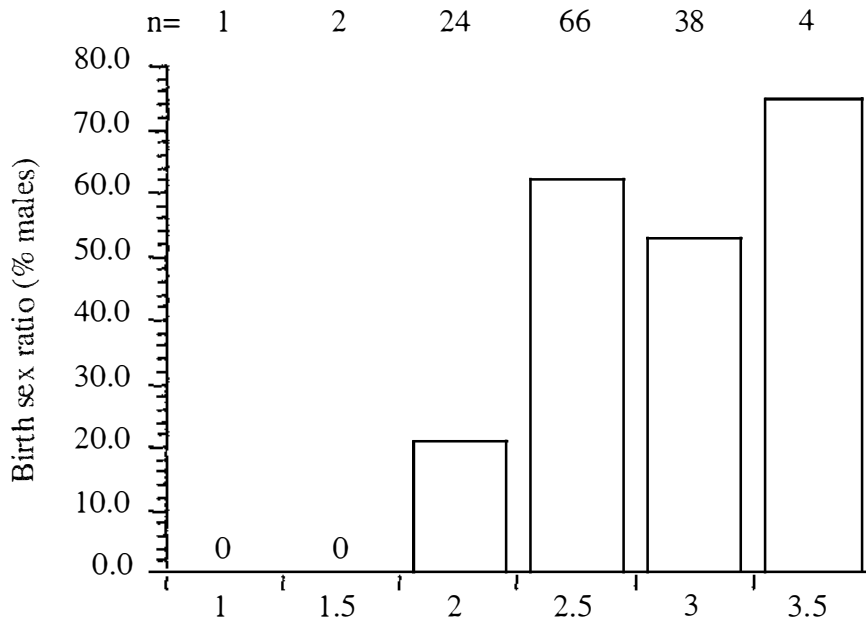
Table one. Variation in sex ratio in relation to mare experience, previous years foaling success and condition mid-gestation.

Category[a]	% male	n	unity[b]	G-test[c]
Experience				NS
primiparous	50	22	NS	
multiparous	50	103	NS	
Previous year				NS
foal	51	67	NS	
no foal	49	67	NS	
Year				NS
1995	55	42	NS	
1996	55	55	NS	
1997	42	38	NS	

a 10 mares were of unknown parity and previous year's reproduction was unknown in 1 case
b Deviation from an expectation of unity is calculated from a binomial distribution
c RxC G-test with Williams correction (Sokal & Rohlf 1981)

DISCUSSION

Our study confirms the applicability of Kaimanawa horses as an ideal test of the Trivers-Willard hypothesis. Mares only ever had one foal per breeding attempt, and there was a correlation between mare body condition during investment and foal condition as yearlings when maternal investment was ending. Although we do not show that these advantages endure into adulthood, previous studies show increased reproductive success in the offspring of dominant mares (Duncan, 1992; Feh, 1990). Theoretically, males will be differentially helped by condition advantages in a species as highly polygynous as the horse.

Our results strongly support the predictions of the Trivers-Willard hypothesis. In the Kaimanawa horse population, mares in poor condition at conception gave birth to predominantly female foals, as do Camargue mares in the year following a season of poor food availability (Monard et al. 1997). There was no significant difference in foaling rates in relation to condition at conception and around 30% of all mares did not produce a foal. Our 70% live foal rate is similar to rates found in other studies of both domestic (50-80%; Rossdale & Ricketts, 1980) and wild (45-74%; Keiper, 1979; Keiper & Houpt, 1984) horses. Some unrecorded loss of neonates may also have occurred as neonatal mortality can be high in horses (Berger, 1986; Duncan, 1992; Waring, 1983). However, live foal rates were similar regardless of condition at conception and loss of neonates is likely to be similar irrespective of the mother's earlier condition. Therefore, it seems unlikely that the birth sex ratios we report are due to differential loss of foetuses or neonates by mares in poorer condition. In addition, those mares who had a male foal in one year and a female in another were in significantly poorer condition when they conceived their female foal.

Furthermore, mares in better condition produced more male foals than expected from an assumption of parity. Where sex ratios differ significantly from parity in both directions it is not possible to argue that the difference is due solely to differential loss of less viable or more costly foetuses, usually males in mammalian species (Clutton-Brock, 1991). For sex ratios that differ from parity in both directions to be explained by differential loss of less viable or more costly foetuses, a requirement would be that daughters be less viable in good condition mares and sons less viable in poor condition mares (Clutton-Brock, 1991). Alternatively, mechanisms that favour the differential conception of males or females have been postulated (Krackow, 1995; eg. timing of insemination Guerrero, 1974; Paul & Kuester, 1987; embryonic mortality Huck et al., 1988).

We found no difference in sex ratio between multiparous and primiparous mares or whether a mare had foaled in the previous year. Furthermore, by mid-gestation maternal condition was no longer a predictor of offspring sex ratio, supporting the premise that sex ratios vary due to differential conception of male and female

offspring. Indeed, although condition during gestation has been frequently used to examine sex ratio variation (eg. supporting Trivers-Willard: Burke & Birch, 1995; Hewison & Gaillard, 1996; Kucera, 1991; Rutberg, 1986; Wauters et al., 1995; not supporting: Reimars & Lenvik, 1997), such studies must assume either sex ratios are adjusted after conception or that condition has not changed significantly since conception. In species with long gestation like horses (11 months), condition may change significantly during gestation. If males do cost more than females *in utero*, as is suggested for several sexually dimorphic species (Clutton-Brock, 1991), then mothers carrying male offspring may lose more weight during pregnancy than mothers carrying female offspring. Measures of body condition at or around conception (eg. Kojola & Eloranta, 1989; Moses et al., 1995) provide a more accurate test of the Trivers-Willard hypothesis than measures taken during gestation if, as our data suggest, biases are caused by differential conception of males and females.

Previous studies have documented variation in sex ratio in relation to female weight around conception time in line with the prediction that females in good condition produce more male offspring (reindeer Kojola & Eloranta, 1989; bushy-tailed woodrats Moses et al., 1995), suggesting differential conception may be occurring. In Kaimanawa horses the birth sex ratio differs from parity in both directions depending on mare condition at conception and variations in the birth sex ratio are too large to be accounted for by differential foetal loss alone. In addition, there is no variation in sex ratio in relation to condition during gestation. Therefore, our data suggest that mares are conceiving male and female offspring differentially.

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CHAPTER 7

Offspring Sex

SEX DIFFERENTIAL MATERNAL INVESTMENT

"THE MALES ARE MUCH BETTER THAN THE FEMALES...
BECAUSE [the females] ARE NOT SO WELL ABLE TO LEAPE,
OR ENDURE THE WOODES..."
Edward Topsell, 1607, 'Historie of Foure-Footed Beastes'.

Photo caption:

Mary (050, centre) with her daughter, Magdalene (95050, in front of Mary) and yearling son, Jessy (94050, behind Mary).

Photo by Elissa Cameron

ABSTRACT

The Trivers-Willard hypothesis (TWH) predicts that a mother will treat a son or daughter differently depending on her ability to invest and the impact of her investment on offspring reproductive success. Although many studies have investigated the hypothesis, few have definitively supported or refuted it due to confounding factors or an inappropriate level of analysis. We studied sex differential maternal investment in feral horses, which meet the assumptions of the TWH with a minimum of confounding variables. Population level analyses revealed no differences in maternal behaviour toward sons and daughters. When we incorporated mare condition we found that sons were more costly to good condition mares, whereas daughters were more costly to poor condition mares, although no differences in maternal behaviour were found. However, the TWH makes predictions about individual mothers. Therefore, we examined investment by mares who raised both a son and a daughter in different years of the study. Mares in good condition invested more in their son in terms of maternal input, proximal maternal costs and costs to future reproduction. Conversely, poor condition mares invested more in daughters. Therefore in a model species, predictions of the Trivers-Willard hypothesis are supported.

Chapter reference:

Cameron, E. Z., Linklater, W. L. Mothers prefer sons AND daughters: testing the Trivers-Willard Hypothesis in a model species. *Submitted to Proceedings of the Royal Society London B.*

INTRODUCTION

Mothers should invest in their offspring so as to increase their own reproductive success (Trivers 1972, Trivers & Willard 1973, Maynard Smith 1980, Clutton-Brock 1991). Maternal investment is defined as "any investment by the parent in an individual offspring that increases that offspring's chance of surviving (and hence reproductive success) at the cost of the parent's ability to invest in other offspring" (Trivers 1972). Therefore, by definition, maternal investment includes maternal input and maternal costs, both those incurred immediately as well as costs to future reproduction. Studies of maternal investment need to consider all these elements (Evans 1990, Clutton-Brock 1991).

The most tested hypothesis has been on sex-biased maternal investment, specifically that in polygynous species, mothers in good condition (i.e. that have more to invest) would be advantaged by producing more sons and mothers in poorer condition would be advantaged by producing more daughters (Trivers & Willard 1973). Trivers & Willard (1973) extend their hypothesis to maternal investment such that females in good condition should invest more in sons and females in poor condition should invest more in daughters.

Most studies purportedly testing the Trivers-Willard Hypothesis (TWH) have tested the hypothesis that mothers invest more in sons than daughters and that sons are more costly to mothers (e.g. invest more in sons: Duncan *et al.* 1984; Hogg *et al.* 1992, Bérubé *et al.* 1996; Birgersson & Ekvall 1997; no differential investment: Green & Rothstein 1991; Smiseth & Lorentsen 1995; Lunn & Arnould 1997). However, such studies test Maynard Smith's (1980) model that, where a species is polygynous and sexually dimorphic, greater investment in sons by all mothers could become an evolutionary stable strategy. The TWH, however, predicts that individual females will invest differently in sons and daughters depending on their ability to invest. Therefore, in some circumstances daughters will be favoured over sons. Furthermore, maternal investment may vary more between mothers than within mothers (Maestripieri 1998, Fairbanks 1996, Crowell-Davis 1986). Therefore, even if mothers are behaving differentially toward male and female offspring individually, this may be obscured if the study is conducted on a population level. Therefore, longitudinal studies of the same individuals with different sexed offspring are the most appropriate way testing for maternal discrimination in investment on the basis of offspring sex (McCann *et al.* 1989).

Studies investigating differential investment by individual mothers in relation to their condition and the potential future reproductive success of her offspring are less common than population level studies. Bereczkei & Dunbar (1997) showed that parents adjusted their investment in relation to the fitness payoffs of sons or daughters in Hungarians and gypsies, and Meikle *et al.* (1984, 1996) and Meikle & Vessey (1988) tested whether mothers bias their investment toward daughters in some circumstances and toward sons in others.

Studies in highly dimorphic species have found that dominant mothers produce more sons or invest more in sons (eg. Clutton-Brock *et al.* 1981, 1984, 1986, Cassinello 1996, Bérubé *et al.* 1996), but did not find that subordinate mothers favoured daughters, possibly because all sons required more investment due to their larger body size and growth rates. Furthermore, such differences in investment may be equalised by more long-term investment in philopatric daughters in terms of, for example, shared resources, protection from predators or inheritance of rank (Clutton-Brock *et al.* 1981, Hiraiwa-Hasegawa 1993), or future competition for mates or resources (Hamilton 1967, Clark 1978, Silk 1983). Consequently, Clutton-Brock (1991) suggested "... reliable estimates of total costs of sons and daughters are only possible where neither male or female offspring are philopatric". In addition, the TWH was formulated using mothers with a single offspring. Where the litter size is greater than one, measuring variation in maternal investment may be even more complicated (Gosling 1986, Williams 1979).

Therefore, an ideal test of the TWH would be on a species with the following characteristics:

a) Highly polygynous such that the potential reproductive success of males varies more than for females and is more responsive to greater maternal investment.

b) Minimally sexually dimorphic such that males do not have obligate faster growth.

c) Both sexes disperse from their natal social group.

d) A litter size of one.

Horses meet all of these criteria and are therefore an ideal model species for testing the TWH. They are highly polygynous, living in stable breeding groups called bands with from one to twenty six mares and usually one adult stallion (Linklater 1998). Large numbers of bachelor males are present because of a 50:50 adult sex ratio. Consequently, some males mate with many females, while others never mate (Berger 1986, Bowling & Touchberry 1990, Feh 1990). Males are only slightly larger than females (Willoughby 1974) and male reproductive success is not correlated with measures of body size (Feh 1990). However, males do appear to benefit from increased maternal investment, as sons of high-ranking mothers have higher reproductive success than sons of subordinate mothers (Feh 1990). A maximum of one offspring is born per year, and the foal is dependent on the mother for at least 4 months, with most foals being weaned after 8 months (Berger 1986). In addition, both sons and daughters disperse from their natal group (Monard *et al.* 1996).

Previous studies on sex differential maternal investment in domestic and feral horses have found conflicting results. Some studies found that sons suckled more (Duncan *et al.* 1984, Berger 1986), whereas others have found no difference between sons and daughters (Crowell-Davis 1985, Crowell-Davis 1986, Smith-Funk & Crowell-Davis 1992). However, Cameron et al. (in press [chapter 2]) showed that suckling behaviour does not index milk intake and is therefore not a useful index of investment. Furthermore, none of the studies have tested whether mares favour sons in some circumstances and daughters in others, nor have they controlled for variation in the maternal style of individual mothers.

We investigated sex differential maternal investment in feral horses, firstly on a population level. We then looked for differences in mare behaviour toward sons and daughters in relation to mare body condition. Finally, we compared mares that had a son and a daughter but whose body condition remained constant to test the hypothesis that a mare in relatively better condition would bias her investment toward her son, and that a poorer condition mare would bias her investment toward her daughter.

MATERIAL AND METHODS

(a) Study site, subjects and sampling regime

Feral horses (*Equus caballus*), known locally as Kaimanawa wild horses live in approximately 64000 hectares in central North Island, New Zealand where they have been feral since the late 19th century (Rogers 1991). Kaimanawa horses live in year round

stable breeding groups called bands composed of usually one stallion (but up to four) and one to eleven mares and their pre-dispersal offspring. The population and study area are described in Linklater (1998).

We studied 55 mares from 18 focal bands and their foals born in the September to April breeding season in 1994-95 (13 males, 12 females), 1995-96 (18 males, 16 females) and 1996-97 (18 males, 21 females). The sex of foals born in the 1997-98 season was also recorded. The birth date of all focal foals was known to within ± 5 days. Two foals born to focal mares in 1996-97 were not sampled and so are not included in the behavioural analyses, but are included elsewhere.

Mares were aged by tooth eruption and wear patterns (Tutt 1968), and others were known to be yearlings at the start of the study. As aging by tooth wear becomes less accurate with increasing age, particularly after nine years of age (Richardson et al. 1995), we classified these mares into broad age categories a priori. Young mares were three to five, mid-aged mares were six to eight and old mares were at least nine years old. We recorded whether mares were raising their first foal (primiparae), or whether they had raised a foal before (multiparae).

Foals were divided into six age categories (Table 1). Bands were located and mare-foal dyads sampled in focal animal samples (Altmann 1974) either until at least three suckle bouts (and therefore two inter-suck periods) had been recorded or until both individuals moved out of view. Dyads were sampled from birth until foal death or dispersal, or until April 1998. Samples were evenly distributed during daylight hours in all seasons.

Table 1. Foal age categories used for behavioural sampling and analysis

Period	Name	Description
0-20 days	Early lactation	Maternal behaviour varies most 50% of foal mortality to 1 year occurs
21-50 days	Peak lactation	30 day period around peak lactation (39 days)
51-110 days	Essential lactation	Foals orphaned after this period survive 85% foal mortality to 1 year occurs
110-200 days	Usual lactation	All foals not orphaned are weaned after 196 days
201 days - wean	Weaning	Extra investment to weaning
wean - disperse	Dispersal	Investment between weaning and dispersal a) before birth of new sibling b) after the birth of a new sibling
0-110 days	Combined essential investment	Essential maternal investment (first three periods combined)

Maternal behaviour varies in relation to maternal age (e.g. Green 1986, Festa-Bianchet 1988b,c), parity (e.g. Gomendio 1989), social environment (e.g. Fairbanks 1996) and time of season born (Festa-Bianchet 1988a, c) so we checked to ensure that there was no significant difference in the number of sons and daughters born to mares of differing age or parity, whether the band had a single or multiple stallions, or the time of season.

(b) Estimating mare ability to invest and maternal costs

Mare ability to invest in her foal was evaluated as her modal of body condition score in the month before foal birth. Body condition scores were estimated by visual assessment of body fat distribution on an 11-point scale from 0 (very poor) to 5 (obese) with 0.5 gradations (Carroll & Huntingdon 1988, Rudman & Keiper 1991) with the aid of 10x50 binoculars or a 15-60x telescope whenever horses were sighted, providing visibility was good. Inter-observer reliability was high (Cameron 1998 [chapter 6]) and visual body condition scores correlate with body fat percentage in horses (r=0.81, Henneke *et al.* 1983). To estimate proximal pre-natal costs to mares we recorded the modal condition of mares during the mid-gestation period of 158-178 days before birth (gestation length: 336 days, Kiltie 1982) and subtracted her modal body condition score during the 30 days before foal birth. Similarly, to estimate post-natal costs during the period around peak lactation (39 days, Oftedal 1985) we subtracted the modal condition during the third month after birth from the modal condition during the month before birth. We calculated the condition of yearlings as the mode of scores between 350 and 380 days old.

(c) Behavioural measures

Time spent suckling is not a reliable measure of milk intake or milk energy intake in horses (Cameron in press [chapter 1], Cameron et al. in press [chapter 2]). However, whether the mare or foal end suckling bouts or the proportion of suck attempts that are unsuccessful can be used to determine the degree of conflict between the mother and offspring over allocation of resources (Byers & Bekoff 1990).

Within a suckle bout the periods of continous sucking separated by breaks in nipple contact were called sucking episodes. We recorded the number of episodes per bout, the number of episodes during which the foal bunted and whether the mare or the foal ended each sucking episode. Unsuccessful suck attempts were those that lasted less than 5 seconds, during which time milk is probably not released (Whittemore 1980).

The spatial relationship between mares and their foals is particularly variable and may reflect mare investment (Green 1992). During a focal sample, instantaneous samples (Altmann 1974) every four minutes recorded the behaviour of the mare and foal and the distance between them. A four minute interval accurately represents behaviour (Rollinson *et al.* 1956), and other studies have used a similar time interval (e.g. Becker & Ginsberg

1990, Crowell-Davis1986, Duncan *et al.* 1984). The distance between individuals was estimated in adult body lengths. For analysis of distance between mare and foal we used only samples where at least one member of the dyad was active (that is not lying down or standing resting).

All approaches across a two body length boundary around the mare and the foal were recorded. In analyses we used the percentage of approaches that were by the foal. Therefore, lower scores indicate more mare effort into maintaining contact with her foal. In 1995 and 1996 leaves across the two body-length boundary were also recorded and used to calculate a contact maintenance index (Hinde & Atkinson 1970). In addition, social interactions between the mare and foal were recorded on an all-occurrence basis.

Late maternal investment may be important for one or both sexes (Festa-Bianchet *et al.* 1994). Weaning dates were calculated as the mid point between the last sample during which the foal had a successfully suckled and the first sample during which no successful suckles. Dispersal age was calculated as the mid-point between the last sighting of the foal with its mother and the next sighting of the foal or mother without the other. We recorded whether foals dispersed in their first, second or third and later year of life.

Twenty-three mares had both a son and a daughter in different years and had the same body condition (± 0.5) prior to the birth of each foal. We took measures of maternal behaviour for 0-20 days and for 0-110 days and subtracted the daughter score from the son score to determine the difference between behavioural input into sons and daughters. Costs to each mother's condition pre- and post-natally were evaluated as was her reproductive success in the subsequent year. We also calculated the time between each mare giving birth to her son and daughter and the conception of her subsequent foal. Conception was estimated by backdating average gestation from the foals date of birth. Where mares did not foal in the year after her son and daughter she was assigned a score of 300 days, which represented an average time to conception for mares that miss a year's foaling.

(d) Statistical analysis

In population analyses of mare behaviour, each mare is used only once, usually her 1995 foal, but her 1994 or 1996 foal if she did not foal in 1995.

Data were checked for normality and were log transformed to correct for negative skew or squared to correct for positive skew where necessary. For multivariate analysis of variance and analysis of covariance we used the general linear model procedure in SAS (SAS Institute 1990). Population differences between sons and daughters were analysed using chi-squared or t-tests. Tests between sons and daughters with the same mother were analysed by paired sample t-tests.

Averages are presented ± one standard error. The results of all statistical tests presented are 2-tailed. We considered $P<0.05$ as statistically significant and results where $0.05 \leq P <0.1$ were considered a tendency.

RESULTS

There was no difference in the number of sons and daughters born to mares in relation to her age or parity, nor the band type in which she lived. However, there was a tendency for more daughters in multi-stallion bands and more sons in single stallion bands (Table 2).

Table 2. Distribution of focal foals in relation to mare age and parity and the social group the foal was raised in.

		% males	n	χ^2	P
Mare age	3-5 years	47	30		
	6-8 years	60	25		
	≥ 9 years	50	26	1.03	NS
	unaged	35	17		
Mare parity	primiparous	45	20		
	multiparous	49	57	0.10	NS
	unknown	52	21		
Band type	single stallion	58	55		
	multi-stallion	31	28		
	band changer	47	15	5.07	NS*

* $0.05<P<0.1$

(a) Population measures of sex differential maternal investment

Offspring sex was not a significant predictor of variation in maternal behaviour at any age (n=55, behavioural measures: time close, mean distance, approaches by foal, sucks ended by dam, unsuccessful suck attempts, episodes/bout, duration of sucks, time between sucks, rate of mare-foal interaction; MANOVA range in Wilks' Lambda, 0.59-0.91, all NS). If all foals born to the 55 mares were used (n=98), foal sex was still not a significant predictor of these behavioural measures (MANOVA range in Wilks' Lambda, 0.74-0.96, all NS). The average change in condition of mares both pre- or post-natally was not different for mares with sons or daughters (prenatally: males -0.03 ± 0.08, females -0.05 ± 0.06 $t_{71}=0.21$, NS; postnatally: males -0.31 ± 0.24, females -0.05 ± 0.06 $t_{94}=-1.07$, NS). Having a foal significantly inhibited mares from foaling in the subsequent year (d.f.=1, $\chi^2=5.34$, $P <0.05$) but was not influenced by foal sex (d.f.=1, $\chi^2=0.34$, NS). There was no difference between sons and daughters in weaning age (sons = 316 ±21 days, daughters 308 ± 24, $t_{47}= 0.23$, NS), or year of dispersal (d.f.=2, $\chi^2=3.68$, NS). Foal

deaths (excluding illegal shooting) to one year old were not sex biased (d.f.=1, χ^2=0.55, NS).

(b) Mare condition and sex differential maternal investment

Mare condition prior to foal birth was a significant predictor of the foal's condition as a yearling for both daughters (regression: r^2=0.17, n=26, P <0.05) and sons (regression: r^2=0.16, n=24, P <0.05).

Mare condition before birth did not have a marked effect on her behaviour toward either sons or daughters at any age (Table 3).

Table 3. Analysis of Covariance of maternal input variables with foal sex and mare condition prior to foal birth as covariates.

Behaviour	0-20 days		21-50 days		51-110 days		111-200 days	
	d.f.	F	d.f.	F	d.f.	F	d.f.	F
sucks ended by mare	49	1.03	53	0.38	51	2.05	44	2.82*
episodes per bout	49	0.56	53	4.78**	51	5.07***	45	0.04
prop sucks unsuccessful	47	1.40	53	2.93*	51	1.12	45	1.83
prop episodes foal bunted	37	0.51	40	0.07	38	0.82	34	0.77
distance btwn mare & foal	49	0.80	53	0.00	52	0.06	43	2.26
prop approaches by foal	50	0.07	50	0.30	52	1.17	43	1.08
prop time mare & foal close	46	2.20	47	0.15	51	0.47	44	0.31

* 0.05<P<0.1, **P<0.05. ***P<0.01

Weaning dates tended to vary in relation to mare condition. Mares in good condition tended to wean sons later than daughters, while poor condition mares tended to wean sons early and daughters later. (Fig 1, ANOVA, $F_{2,43}$=2.5, P <0.1).

Mare condition had a significant effect on her change in condition both pre- and post-natally. Mares in good condition lost more condition with a son both pre- and post-natally whereas mares in poorer condition lost more condition with daughters (ANOVA: prenatally sons $F_{3,33}$=11.36, P <0.0001, daughters $F_{3,32}$=1.06 NS; postnatally sons $F_{3,44}$=7.19, P <0.0001, daughters $F_{3,45}$=2.94 P <0.05 Fig 2a&b). Furthermore, mares in poor condition were less likely to foal after a daughter than mares in good condition (d.f.=3, χ^2=9.14, P <0.05) and the opposite pattern was observed for sons, but was not significant (d.f.=3, χ^2=5.05, NS, Fig 3a). Note that all mares that scored 3.5 were able to
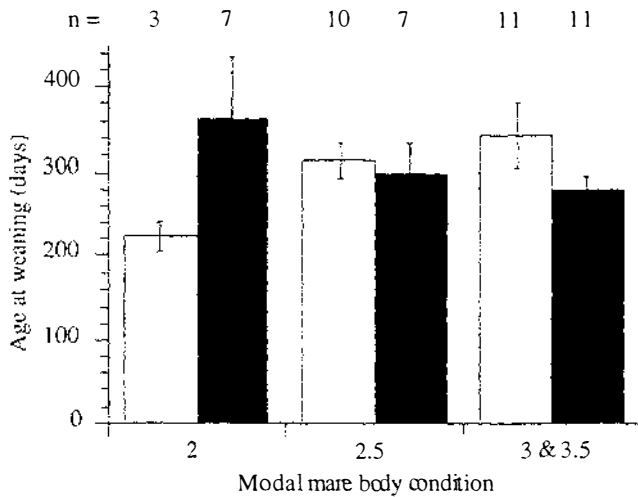
Figure 1. Age of sons (open bars) and daughters (filled bars) at weaning in relation to maternal body condition before foal birth.
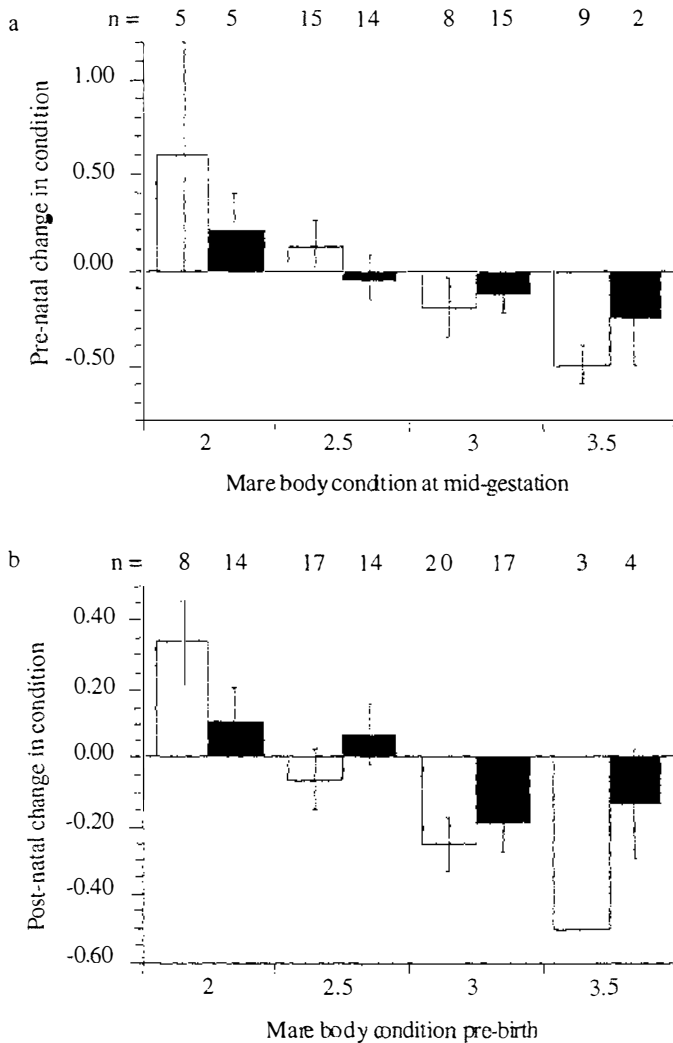

Figure 2. Proximal costs of sons (open) and daughters (filled) to mares of different condition. a) pre-natal change in mare body condition (mid-gestation minus pre-birth), b) post-natal change in mare body condition (pre-birth minus post-peak lactation).
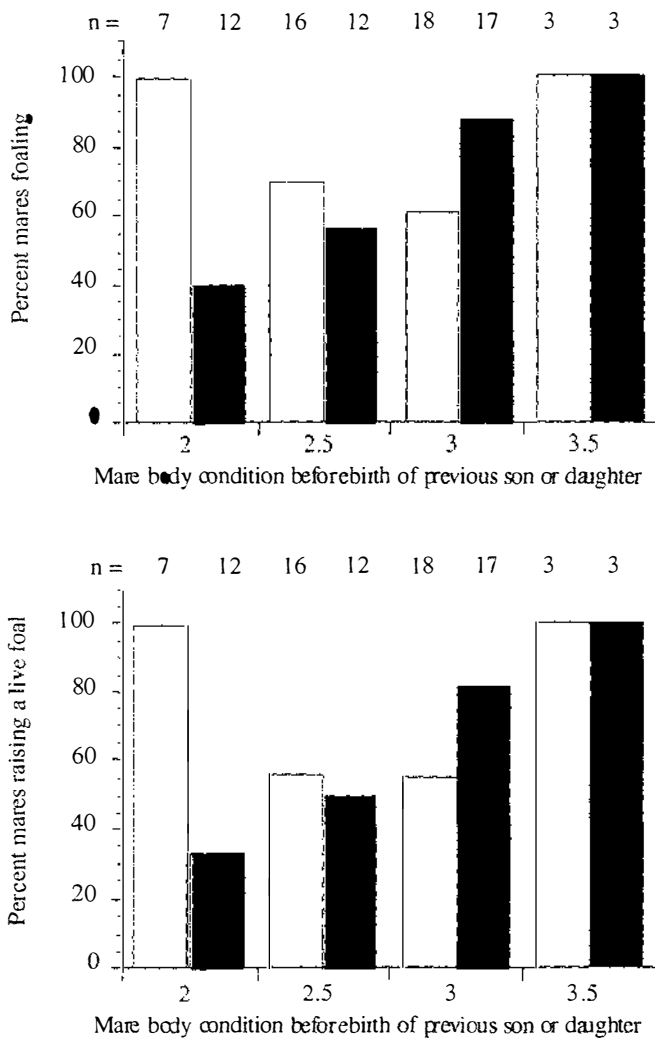
Figure 3. Reproductive costs of sons (open bars) and daughters (filled bars) to mares of different condition. a) the proportion of mares that foal in the subsequent year in relation to her body condition before the birth of her previous son or daughter, and b) the proportion of mares that successfully raised a foal in the year after having a son or daughter in relation to her body condition before the birth of that son or daughter.

foal in the subsequent year. Survival rates of foals also varied in relation to the sex of the previous year's foal and the mother's condition when she had the previous foal. Poor condition mares that had raised a daughter were less likely to successfully rear a foal in the subsequent year than were mares in better condition (d.f.=3, χ^2=9.98, P <0.05, Fig 3b). Again the opposite trend was observed for sons (d.f.=3, χ^2=6.67, P <0.1, Fig 3b), although all mares that scored 3.5 raised live foals. Therefore, sons and daughters have different reproductive costs for mares with different body conditions.

(c) Differential investment by individual mares

When mares of the same condition but with different sexed foals were compared to themselves, mothers in better condition favoured their son, whereas mares in worse condition favoured their daughter. Using a combination of eight behavioural variables we found that mare condition was a significant predictor of the difference between a mare's behaviour towards her son and her daughter for the period of essential investment (birth to 110 days, MANOVA, Wilks' Lambda = 0.36, $F_{8,14}$ = 3.05, P <0.05), and during the period when most foal mortality occurs (0-20 days, MANOVA, Wilks' Lambda = 0.34, $F_{8,12}$ = 2.89, P <0.05). In addition, several variables were singly significant or approaching significance from birth to 110 days (Fig 4a) and from birth to 20 days of age (Fig 4b). Mothers lost more condition pre-natally with a son if they were in good condition, but more with a daughter when they were in poor condition (ANOVA, $F_{2,11}$=3.81, P <0.05, Fig 5a). There were no significant difference in post-natal condition change with a son and a daughter in relation to the mare's condition at foal birth (ANOVA $F_{2,20}$=0.90, NS). There was no difference in time between foaling and subsequent conception after sons and daughters, although the pattern was for a longer time after a daughter for poor condition mares and longer after sons for good condition mares (ANOVA, $F_{2,17}$=1.08, NS, Fig 5b).

DISCUSSION

We found no difference in maternal behaviour, when we looked for sex differences on a population level, supporting some previous studies (e.g. Crowell-Davis 1985, Crowell-Davis 1986, Smith-Funk & Crowell-Davis 1992), but contrary to others (Duncan *et al.* 1984, Berger 1986). However, both these latter studies base their conclusions on differences in time spent suckling which does not measure milk intake in horses and so inferences about milk intake based on time suckling are likely to be wrong (Cameron in press [chapter 1], Cameron *et al.* in press [chapter 2]).

It is not surprising that there is no difference between males and females on a population level as greater investment in all sons is predicted only for highly dimorphic species in which body size influences reproductive success (Maynard Smith 1980). Convincing studies of male biased maternal investment on a population level have all been on highly dimorphic species (e.g. red deer *Cervus elaphus* Clutton-Brock *et al.* 1984, 1986, bighorn sheep *Ovis canadensis* Bérubé *et al.* 1996). Horses are not sexually dimorphic so population-based predictions do not apply. Indeed, if mothers in good condition favour sons, but that mothers in poor condition favour daughters, as the TWH predicts (Trivers & Willard 1973) than population differences will be obscured if mare condition is not controlled for.

When mare body condition was taken into account we found that although
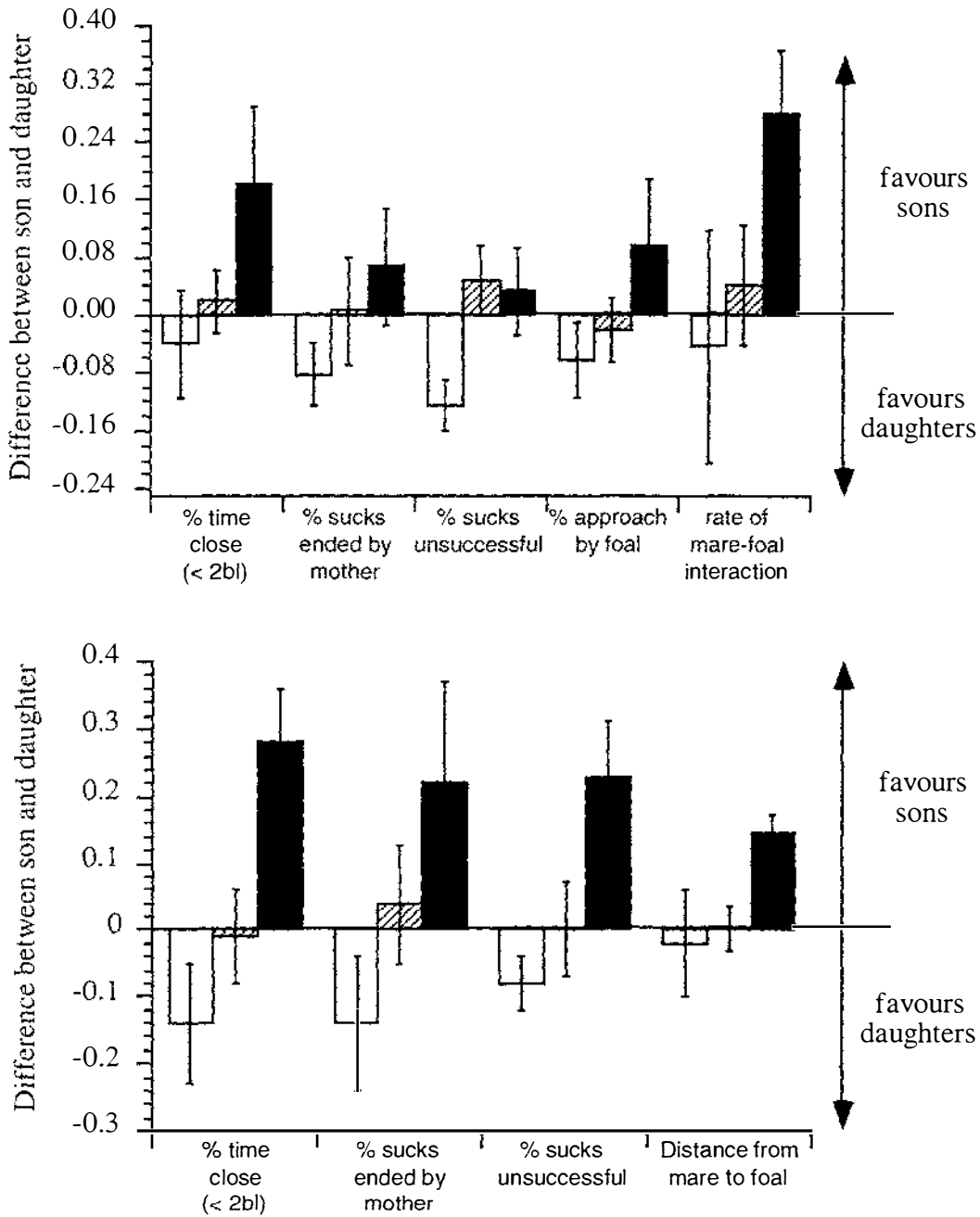
Figure 4. The difference between a mare's behaviour with her son and daughter in relation to her condition, a) from birth to 110 days, the period of essential maternal investment, and b) from birth to 20 days, the period during which most foal deaths occur. Poor condition mares n=7 (open bars), mid condition mares n=10 (hatched bars), good condition mares n=6 (filled bars).

0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8

Pre-natal change in condition

Sons cost more

Daughters cost more

poor   average   good

Mare body condition score

100
50
0
-50
-100
-150

Days between foaling and conception

Sons cost more

Daughters cost more

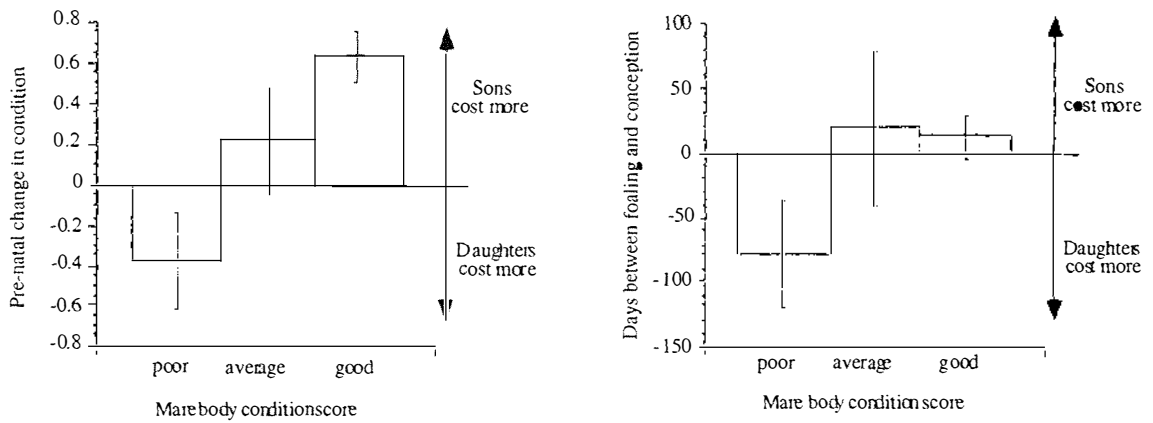poor   average   good

Mare body condition score

Figure 5. The difference between costs of sons and costs of daughters to individual mares. a) pre-natal condition change in individual mares, and b) time between foal birth and conception of next foal. Poor condition mares n=7, mid condition mares n=10, good condition mares n=6.

maternal behaviour towards sons and daughters was similar, good condition mares that raised a son suffered more costs in terms of condition loss and costs to future reproduction than if they had a daughter. Conversely, mares in poor condition suffered greater costs with daughters than with sons. Similar patterns have been found in studies looking at costs of sons and daughters to different mothers in relation to possible fitness benefits (e.g. Bereczkei & Dunbar 1997, Meikle et al. 1996). For example, Boesch (1997) found that sons cost more to dominant mothers whereas daughters cost more to sub-dominant mothers in a population of chimpanzees (Pan troglodytes) in which most daughters dispersed from their natal group. These results suggest that mares were investing more or less in sons and daughters in relation to their condition prior to, and during, investment. However, any differences in behaviour toward sons and daughters may still be obscured by variation in maternal style by individual mothers that are a feature of many species (e.g. Fairbanks 1996), including horses (Crowell-Davis 1986, Cameron 1998 [chapter 3]).

Analyses of individual mothers, in which maternal style was controlled for by paired tests over two reproductive efforts we found that the same mare in good condition favoured her son above her daughter, whereas an individual mare in poor condition favoured her daughter over her son. This was particularly marked in conflict behaviours over suckling, with mothers ending more suckle bouts, and with more suck attempts being unsuccessful in the less favoured sex. Therefore, it appears that mothers are actively discriminating between sons and daughters, and differences are not due to differential demand or extraction by offspring (Byers & Bekoff 1990). In addition, the

costs of sons are greater for good condition mares but the costs of daughters are greater for poor condition mares.

Therefore our results support the prediction from the Trivers-Willard hypothesis that mothers in good condition will favour sons, whereas mothers in poor condition will favour daughters (Trivers & Willard 1973) in terms of maternal input and both proximal and reproductive costs. Recent studies have also shown that birth sex ratios conform to the prediction that mothers in good condition at conception, or in better condition during the previous breeding season (ie. around conception time) give birth to more sons and mothers in poor condition give birth to more daughters (Monard *at al.* 1997, Cameron 1998 [chapter 6]).

Many previous studies have erred when purportedly testing the TWH by only considering bias toward sons, measuring population averages rather than individuals, not controlling for variation in maternal style and testing the TWH on species in which either its assumptions do not apply or where the relationship may be obscured by confounding variables. This is the first study showing that mothers discriminate between male and female offspring in relation to their ability to invest by favouring sons in good condition and daughters in poor condition, in a species which shows no population differences in maternal investment between sons and daughters and which conform to the assumptions of the TWH with a minimum of confounding factors.

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Discussion

*"**HOW CAN THE FOLE AMBLE WHEN THE HORSE
AND MARE TROT?
OF A RAGGED COLT COMETH A GOOD HORSE**"*
W. Camden, 1605, 'Remains concerning Britaine'.

Photo caption:

Copper (107) with her son, Crust (95107).

Photo by Elissa Cameron

Horses are a model species for testing some theories of maternal investment. Confounding factors are at a minimum and assumptions can be tested on domestic populations. When testing theories, it is appropriate that we test them initially on a model species, and secondarily on other species. In this way we can determine the general applicability of an hypothesis, and determine the influence of confounding variables.

Maternal investment consists of maternal input and the cost of such input. It is defined, however, in terms of costs to future reproduction (Trivers 1972). Nonetheless, it is frequently measured as input only (Evans 1990, Birgersson & Ekvall 1994; eg. Duncan et al. 1984, Alley et al. 1995). In mammals, the primary input is the energy that offspring receive from their mothers in the form of milk (Lee 1987, Kretzmann et al. 1993). Direct measurements of milk intake are difficult on wild animals, so time spent suckling has been used to estimate milk intake based on the assumption that the more time an offspring spends sucking the more milk is transferred (Berger 1979, Fletcher 1971). The results of several studies suggest that this may not always be the case (Festa-Bianchet 1988, Mendl & Paul 1989, Babbitt & Packard 1990a, Birgersson & Ekvall 1994). Therefore, before basing measures of maternal input on time spent suckling, I examined the evidence that suckling is proportional to milk intake. A meta-analysis of currently available data suggested that suckling behaviour was not an appropriate or accurate predictor of milk intake. The analysis showed the need for a test of the assumption that the more time spent suckling the more milk transferred. Researchers appear to have avoided such a test, which is long overdue.

I therefore tested the assumption in thoroughbred horses by labelling milk with isotopes and measuring transfer from mother to offspring, a method frequently used to measure milk intake (eg. Arnould 1997, Lydersen et al. 1997, Martin et al. 1992). The test showed that time suckling was not predictive of milk intake in horses, and therefore we can not use time spent suckling as a measure of milk intake in the study. If suckling behaviour had been used to index milk intake, our results would have been wrong; daughters sucked longer than sons, but received no more milk. Other studies using time suckling to measure milk intake (eg. Duncan et al. 1984, Berger 1986) may be wrong. Throughout the rest of my study, I use suckling behaviour only to indicate the degree of conflict between mare and foal, not as a measure of energy intake. Previous studies investigating differential maternal investment using suckling time as an index of milk intake may have made incorrect conclusions. Therefore, the conclusions of such studies should be regarded tentatively. In future, time spent suckling should not be assumed to index milk intake in a reliable way without an empirical test of the relationship in the test species. Instead, other methods of evaluating maternal investment should be used, or milk intake could be measured directly using isotope labelling techniques.

In many species, changes in maternal behaviour have been reported with differences in the social and physical environment, particularly the risk of injury to an

infant (eg. Berger 1979, Hauser 1988, Lycett et al. 1998). Equids are the only ungulates in which infanticide has been reported (Duncan 1982, Ryder & Massena 1988), and their social system is the same as those of species in which infanticide is a regular occurrence (eg. Packer & Pusey 1984, van Schaik & Kappeler 1997). I show that an infanticidal male would gain a reproductive advantage. Infanticide is generally associated with a lack of certainty of paternity, and accidental infanticide is associated with high rates of aggression within social groups (Duncan 1992). Consequently, I looked at the maternal behaviour of mares in single stallion bands, where paternity is relatively certain and rates of aggression are low. I compared them with mares in multi-stallion bands where paternity was less certain and rates of aggression, particularly between the stallions, were higher (Linklater 1998). Mares were more protective of foals in multi-stallion bands, particularly when the foal was near one of the stallions. The rate of negative interaction between the stallion and foal was a significant predictor of mare protectiveness. Mares that had their band experimentally reduced to a single stallion were less protective after the removal of the subordinate stallion, and mares that changed band types with a foal at foot were more protective in multi-stallion bands. Moreover, within one single-stallion band, those mothers whose foal had not been fathered by the band stallion were more protective of their foals than those mothers with foals fathered by the band stallion. The mares in multi-stallion bands also incurred reproductive costs. These observations and previously reported incidences of infanticide in equids suggest that mothers are more protective of foals that are at risk of infanticide. These observations are also consistent with results for other species (eg. Wright 1995, Maestripieri 1998) but add experimental evidence that male aggression alters maternal behaviour.

There is a growing body of work looking at the role of male aggression in shaping female behaviour and even social systems (eg. Nefdt 1995, Kappeler 1997, Sterck et al. 1997). Infanticide by males represents an extreme form of conflict between males and females and may be a particularly important in the evolution of social systems (eg van Schaik & Kappeler 1997). I show that rates of aggression shape maternal behaviour proximally. Infanticide may occur in horses, but it was not documented in this study. Free-living, unmanaged horses need to be studied further to determine whether male aggression causes foal death, either directly or indirectly. The role of male aggression in shaping horse social structures may be a fruitful future direction in equid sociobiology (eg. Linklater 1998).

In addition, it would be instructive to take a closer look at aspects of paternal behaviour, firstly of paternal and non-paternal stallions in single-stallion bands, and secondly at the paternal behaviour of both paternal and non-paternal multi-stallion band stallions. It is claimed that paternal behaviour in many species is related to the degree of certainty of paternity (Møller & Birkhead 1993), and stallions are reported to be

aggressive to non-paternal offspring (Berger 1986, Duncan 1982). These results, however, remain controversial (eg. Whittingham et al. 1992,Wagner et al. 1996).

In the unusual situation where a mother and daughter continued to live in the same social group, co-operative suckling and foal rearing was observed. Co-operative offspring care is rare amongst monotocous species (Packer et al. 1992) and this is the first such record for horses, which are usually intolerant of other mares in their foal's proximity (Tyler 1972, Feist & McCullough 1976, Berger 1986). Mares did not adopt orphans from their own or other bands, even when they were lactating themselves, and where the orphan was starving to death. Kin selection is implicated as an important factor in the evolution of communal suckling. The occurrence of behaviours such as non-offspring nursing in circumstances in which it does not usually occur may be particularly valuable in understanding the evolution of such behaviours (Packer et al. 1992). I suggest that the collection and collation of instances where allo-suckling occurs in unusual circumstances should be continued in an attempt to understand its evolutionary origin.

In many species, females become more successful at rearing offspring as they age (Festa-Bianchet 1988b, Meikle et al. 1996). There are two likely explanations for this. The first recognises that as a mother ages her residual reproductive value (RRV) declines. Thus, the conflict between investing in current and future offspring will decline with age. The result will be that mothers will increase investment in their current offspring as they age (Pianka & Parker 1975, Ozoga & Verme 1986, Clutton-Brock 1984, Clutton-Brock 1991). The strongest evidence in support of this theory comes from mothers that are on their last reproductive effort (ie. they die before reproducing again, eg. Clutton-Brock 1984, Green 1990). However, although the hypothesis suggests an increase in investment as females age throughout their lifetime, the evidence in support is less compelling. An alternative hypothesis suggests that mothers become more successful, not because of increased investment but because greater experience enables them to target their investment more successfully (Fairbanks 1996). This is called the Targeted Reproductive Effort hypothesis (TRE). The two hypotheses are difficult to distinguish as several of their predictions are similar (Clutton-Brock 1991). However, the RRV hypothesis predicts increased investment with age, whereas the TRE hypothesis predicts no increase in investment, or a decrease in investment (Fairbanks 1996).

Investment is ultimately defined as costs to future reproduction (Trivers 1972). Therefore, I looked at reproduction in the year after foaling for young, mid-aged and old mares. The results show that old mares are more likely to foal after successfully rearing a foal than younger mares, and that their yearly foaling rate is higher. I then looked at the pattern of input and found that old mares put in more effort in the first 20 days, during which most foal mortality occurs, and less thereafter. Similar results found in other studies (eg. Green 1990), have been used to support the RRV hypothesis. I argue that our results suggest that mares are not increasing their investment, but are targeting their

investment more effectively. Further research is required, particularly following the reproductive efforts and success of individual mares throughout their lifetimes (Clutton-Brock 1991).

Horses are a particularly suitable species for investigating theories of sex differential maternal investment and I used them to test the Trivers-Willard hypothesis (TWH) of sex-differential maternal investment (Trivers & Willard 1973). The TWH is one of the most tested hypotheses in maternal investment studies (eg Duncan et al. 1984, Lee & Moss 1986, Festa-Bianchet 1988a, Bérubé et al. 1996, Lunn & Arnould 1997). However, I argue that most of these studies are inconclusive for several reasons. Firstly, many do not test the TWH; rather they test Maynard-Smith's (1980) hypothesis of increased investment in sons in polygynous and sexually dimorphic species. The TWH relies on different strategies in different mothers in relation to their ability to invest, with sons favoured in some circumstances, daughters in others. Secondly, it is not always possible to check the applicability of the TWH assumptions. Finally, there are several confounding factors that can obscure the TWH effect (eg. Hamilton 1967, Clark 1978, Silk 1983, Hiraiwa-Hasegawa 1993). I argue that horses are an ideal test of the TWH, because all the assumptions of the TWH apply and because there are a minimum of confounding variables. I also look at individual mares and at input and costs from conception to dispersal.

I found that mares in better condition at conception had more sons than daughters but that mares in poorer condition had more daughters than sons. Such differences were not explained by differential foetal loss, or by condition later in gestation, suggesting differential conception or adjustment close to conception. In addition, mares that had both a son and a daughter in different years of the study were in poorer condition when they conceived their daughter. This supports work by Monard et al. (1997) which found that fewer sons were born in seasons following years of poor food availability.

I found no population trends in differential maternal investment. However, sons cost more to good condition mares, whereas daughters cost more to poor condition mares. When I looked at individual mares that had a son and a daughter, I found that poor condition mares favoured their daughter, and their daughter was more costly, whereas better condition mares favoured their son, and their son was more costly. Therefore the predictions of the TWH for both biased sex ratios and sex-biased maternal investment were supported in this study.

It has been hypothesised that high levels of maternal expenditure preclude extra investment in sons in species that have failed to show expected sex-biases in maternal investment (eg. Byers & Moodie 1990, Byers & Hogg 1995, Pélabon et al. 1995, Birgersson 1998). This may explain why population level studies do not conform to the Maynard Smith (1980) model. However, I argue that individual mothers may still vary

their maternal investment into sons and daughters, but that on a population level differences are obscured.

In conclusion, I conducted a study of maternal investment with an emphasis on individual mothers. In so doing, I also gathered data on population differences. I tested hypotheses for which horses provide an ideal model. I found that horse mothers have individual styles that change as they age apparently due to their increasing experience. Mothers become more protective of offspring in relation to their social environment. Furthermore, mothers change their maternal behaviour and consequently their maternal investment, in relation to the potential reproductive success of their sons and daughters.

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