MATERNAL CARE IN A HIGHLY PRECOCIAL MAMMAL, THE GUINEA PIG (*CAVIA APEREA* F. *PORCELLUS*)

Dissertation

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Table of contents

General introduction

Parental investment: the parental perspective

An individual's fitness is expressed by its contribution to the gene pool of following generations (Clutton-Brock 1991). Its life time reproductive success depends on the number and quality of young produced in each reproductive event and on the number of such events over lifetime (Sikes 1995). Parental care provided to current progeny enhances the chances of these offspring to survive to maturity and their fertility. Thus the fitness that the parent accrues from these young is increased. However, given that parental resources are limited, parental care for one brood may reduce a parent's future reproductive success as it may reduce parental survival and future fertility or a parent's ability to care for future broods (Trivers 1972; Clutton-Brock 1991). Trivers (1972) defined parental investment (PI) as anything done by the parent for the offspring that increases the offspring's chance of surviving, while decreasing the parent's ability to invest in future offspring. For example, parents that invest heavily in one brood may have less time to rear additional broods (Maynard Smith 1977); or a parent's physical condition may deteriorate as a result of rearing one brood and thus the subsequent brood may be delayed or its size may be reduced (Carlisle 1982).

Life-history theory predicts that parents should be selected to optimize PI in relation to its benefits to their current offspring and its cost to their future reproduction in such a way as to maximize the number of surviving and recruiting offspring (Roff 1992; Stearns 1992; Trivers 1972). Benefits and costs of PI can vary in relation to parental and offspring state and PI should be adjusted accordingly (Clutton-Brock 1991). For example, older parents have fewer opportunities for future reproduction. With increasing age it may be advantageous to devote remaining resources to caring for current young (Williams 1966; Clutton-Brock 1991; Isaac & Johnson 2005). In mammals maternal reproductive state may also affect allocation to the current litter: females that are simultaneously pregnant and lactating additionally have to allocate resources to the litter in utero and may therefore reduce milk yield and wean earlier than non-pregnant mothers (Worlein et al. 1988; Green et al. 1991; Duncan et al. 1984; Bruce 1958; Gomendio et al.1995;

Fortun-Lamothe et al. 1999; Norris & Adams 1981; Parkening & Collins 1991). Benefits of PI increase with offspring demand, i.e. long term need or short term hunger (Godfray & Johnstone 2000). Long term need refers to the total investment an offspring requires over the period of parental care to reach a target condition, whereas short-term need or "hunger" is dynamic and can change rapidly. Long term need may be influenced by the target condition (the larger sex has the greater long term need) or by body condition (smaller offspring have a greater long term need than larger ones) (Price et al. 1996). As the effects of parental expenditure on offspring fitness are smaller for superior offspring, parents may provide more resources and over a longer period to offspring in greater need. However, if the young are in very poor condition parents may desert their offspring (Godfray 1991; Clutton-Brock 1991).

In order to adjust parental care in relation to offspring needs parents must assess offspring demand either via offspring appearance – for example, in large precocial mammals weaning has been suggested to occur as soon as the young have reached a threshold weight (Lee et al. 1991) – or via active communication (Maynard Smith & Harper 2003; Searcy & Nowicky 2005; Dawkins 1976). Solicitation intensity may express offspring need and help parents to allocate resources when and where they are of most use (Kilner and Johnstone 1997).

Factors complicating parent-offspring interactions: the offspring perspective on parental investment

Full-siblings share on the average half of their parent's contribution to their genome. Thus an offspring's relatedness to its full siblings is only half as high (0.5) as its relatedness to itself (1.0). Therefore the offspring should be selected to value its own well being higher than that of siblings, whereas parents - being equally related to all of their offspring - should value all offspring equally. Consequently, what proves optimal for parents is less than optimal for the offspring, leading to parent-offspring conflict (Trivers 1974). Squabbling observed among family members might be an expression of this evolutionary conflict, in which the fitness gains of one party to result in fitness costs to the other party. This is, however, difficult to test (Mock & Forbes 1992).

The outcome at conflict resolution may be a parent wins outcome, an offspring win outcome or a compromise between the two parties (Parker & MacNair 1979). While Alexander (1974) suggested that the outcome of parent-offspring conflict should be a parent wins outcome, simply because of the parent's physical superiority, Trivers (1974) proposed that even though offspring may be in a physical disadvantage they will commonly win by inflating their signals of need. Parents that monitor the requirements of their offspring could then become subject to offspring manipulation (Trivers 1974).

Costs of signalling can provide a way out of the parental dilemma. Begging may increase predation risk and may incur metabolic and opportunity costs (Parker et al. 2002; Maynard Smith & Harper 2003). As offspring condition reflects the value of extra resources to young, young in greater need can afford the higher costs of more intensive begging. Empirical studies mostly on birds have provided convincing evidence that solicitation increases with need (Redondo & Castro 1992; Price & Ydenberg 1995; Smith & Montgomerie 1991; Smiseth et al. 2003) and parental food-provisioning has been found to correlate with begging rate (Quillfeldt & Peter 2000 (storm-petrels); Weary & Fraser 1995 (pigs) (Mondloch 1995 (canaries); Redondo & Castro 1992 (Magpies)).

Parker & MacNair (1979) developed the first quantitative model about the resolution of parent-offspring conflict. They concluded that if ignoring solicitation carried no cost – the outcome would be a parent wins outcome. However, under these circumstances solicitation could not have evolved. They suggested that insensitivity comes at a fitness-cost resulting from the costs of begging and an additional cost because real needs are ignored. With increasing cost of insensitivity the outcome should be shifted in favour of the offspring. Parents should provide more food if begging intensity increased and young should respond to an increase in supply by a decrease in begging intensity. They assumed that parents provided a fixed amount of care per unit solicitation and that offspring reacted to changes in the amount of care received with a fixed response. The model has therefore been referred to as "Pro-rata model". With these assumptions an ESS pair exists where parents provide more food than is optimal for them, but still less than is optimal for the offspring. The outcome of parent-offspring conflict is neither a complete parent wins nor a complete offspring win outcome but an intermediate between the two (Parker & MacNair

1979).

Godfray (1991, 1995) showed in his model that the costs of signalling can lead to honest signalling if misrepresenting needs and hence receiving more care, is balanced by the cost of signalling at a higher level. At the ESS, young signal at a level that corresponds with their state and parents use this information to allocate an appropriate (from their point of view) amount of resources. In this model the parent wins the conflict (although the victory comes at the cost of signalling costs).

While different modelling approaches agree that increased begging should positively affect parental supply, they make different assumptions about the effect of supply on solicitation intensity (Royle et al. 2002). If begging is an honest one-to-one representation of true need (Godfray 1991, 1995), the amount of supply should not affect begging intensity at all. The model by Parker & MacNair (1979) implies a negative effect of supply on demand. Experiments in altricial birds show that the slope of the function representing the effects of supply on demand can be zero or negative but also positive (reviewed by Royle et al. 2002) (See Fig.1).

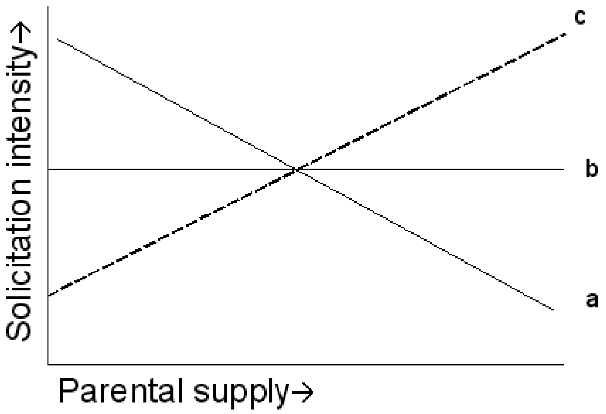


Figure 1: Effects of supply on solicitation a) negative effect of supply on solicitation (Parker & MacNair 1979; Hussel 1988), b) no effect of supply on solicitation intensity (Godfray 1991, 1995), c) positive effect of supply on solicitation intensity (after Royle et al. 2002).

Parents and offspring do not only disagree over the amount of PI at any given moment, but also over the optimal timing for the transition to independence. Parents may increase the cost of solicitation to speed up the process to independence by ignoring solicitation behaviour, by avoiding and even by rejecting their offspring (Davies 1978; Pugesek 1990; Leonard et al. 1988). Towards the end of parental care, some internal state may decrease the parent’s readiness to respond and thus end the effectiveness of the previously functioning communication system (Davies 1978; Jensen & Recén 1989; Numan & Insel 2003).

Parental care in mammals

Parental care in mammals is characterized by the production of milk that nourishes the young after birth, making them nutritionally dependent on the mother well after they are mechanically independent (Pond 1977). Gestation and lactation are both periods of high energetic demands. Gestation increases daily energy expenditure by 30% (Gittleman & Thompson 1988) and lactation is even more expensive: Energetic intake increases between 66 to 188% in lactating compared to non-lactating females (Clutton-Brock 1991; Gittleman & Thompson 1988) and the energetic ceiling may not be reached during normal lactation (Hammond et al. 1994). Optimization of milk supply with regard to maximal fitness benefits is therefore expected. Milk production and composition can be affected by diet (Sutton 1989) and food availability (Landete Castillejos 2003; Loudon 1983). Nutritional condition of mothers can affect the rate of the transfer of nutrients to young and hence offspring growth and survival. Lee et al. (1991) hypothesized that offspring should be weaned once they have reached a threshold weight. If so, mothers in good condition may wean earlier than females in moderate condition as they are able to provide their offspring with larger quantities of milk, which increases growth rates. Mothers in moderate conditions may compensate their lower milk production by nursing more frequently and over a longer period. Results from correlational studies on large precocial mammals confirm this hypothesis (Loudon & Kay 1984). However, whether early weaning is a reaction of mothers to their own body condition or to offspring demand remains unclear in these investigations as both are affected by the same environment. In rats and mice, females prolong lactation when their offspring is replaced by younger pups (Lichtman &

Cramer 1989; Nicoll & Meites 1959) indicating that mothers react to offspring demand.

Solicitation behaviour has been widely studied in birds (review Kilner & Johnstone 1997). Similar studies in mammals are rare (but see studies in pigs: Spinka & Algers 1995; Illmann et al. 2001; Weary & Fraser 1995, and in seals: Smiseth & Lorentsen 1995). While offspring of many bird species solicit food from parents by intense displays and loud vocalizations, solicitation in mammals is less obvious due to the physiological interactions between mother and offspring. Sucking intensity and sucking frequency can vary as a function of offspring state (Delgado et al. 1982; Loudon et al.1983). Via sucking young can influences milk production as the sucking stimulus prompts the release of prolactin, which in turn stimulates milk synthesis and secretion of milk in the mammary gland (Cowie 1984). Contrary to costly-begging theory however, there is little evidence that sucking itself is particularly costly (Wells 2003). The opportunity for sucking must first be established by a behavioural interaction, which is mainly initiated by the young in precocials and in altricials particularly when they have become more mobile (Bøe 1993 & Jensen 1988 (pigs), Smiseth & Lorentsen 2001 (grey seals)). A relation of condition and calls has been found in pigs (Illmann et al. 1995) and humans (reviewed by Wells 2003). However, vocal communication in relation to need may not be typical for mammals in general. For example, the calls of young meerkats do not seem to indicate their hunger state (Manser & Avery 2000) and no correlation was found between calling rate and hunger in grey seals (Smiseth & Lorentsen 2001). The control over the success of a sucking attempt probably lies with the mother as she can block access to teats, avoid pups or chase them away (Reisbick et al. 1975: rats; König 1985: guinea pigs; Houpt & Boyd 1994: Przewalski's horse; Alley et al. 1995: goats; Künkele & Hoeck 1995: *Galea musteloides*). This may make sucking attempts a costly signal of need and maternal supply may be adjusted to the offspring's persistence which should vary with demand.

In relation to parent-offspring conflict in mammals most attention has been drawn to the timing of weaning. Trivers (1974) predicted the conflict to be most pronounced at the end of lactation, as mothers should terminate nursing before the offspring ceases demanding. The conflict is expected to be most pronounced in species where continued lactation may lead to lactational anoestrus (Green et al. 1993).

Martin (1984) suggested however, that the conflict should be most intense in an intermediate phase, after peak lactation because the effect of milk on the offspring's future reproductive success may be negligible at the end of lactation. Parent-offspring conflict has been subject to correlational studies (Berger 1979; Green et al. 1993). Experimental work has been limited mainly to altricial small mammals (Reisbick et al. 1975; Pfister et al. 1986; Lichtman & Cramer 1989 – but see Babbit & Packard 1990; Laurien-Kehnen & Trillmich 2003, 2004). Studies in rats (Pfister et al. 1986) and cats (Martin 1986) showed that young suckled even far beyond normal weaning age when provided with an anesthetized dam, or when rats were continuously kept with dams and their preweanling litters. The findings indicate conflicting interests over the duration of lactation.

Many of the studies carried out in mammals have been correlational and experimental studies have been mainly done in altricial species. Hardly any similar studies have been carried out for precocial mammals (but see Laurien-Kehnen & Trillmich 2004; Babbit & Packard 1990; Dwyer et al. 1998). The situation may be very different from that in altricial mammals: young are born naked and entirely helpless. They are totally dependent on maternal milk and warmth. The milk yield increases over lactation until a peak shortly before weaning to meet the increasing demands of growing young. In contrast, precocial young are born after a relatively long gestation, are highly developed at birth and in many herbivorous precocials, young may consume solid food within a few days after birth (Derrickson 1992). Milk yield peaks early in lactation and decreases with the increasing ability of young to feed on solid food. In precocial young that can feed by themselves from early on, the fitness gains through maternal care seem to be reduced. However, like in altricials, the high costs of milk production lead to a potential for mother-offspring conflict. The mother-offspring interaction in these highly precocial mammals is particularly interesting as the ability of young to contribute to their food intake by independent foraging may influence maternal strategies to allocate resources and also the offspring decision to beg for milk. In the following, by the use of the term "precocial", I will refer to these highly precocial mammals.

It is conceivable that young that are highly developed and mobile may be better able to extract maternal care. On the other hand solicitation and the time spent sucking may incur an additional cost to the offspring in terms of missed foraging opportunities. Mothers may be less sensitive to offspring demand than mothers of altricial offspring because misjudging offspring need may be less costly than in altricials. A low sensitivity may balance the outcome of parent-offspring conflict in favour of parents (Parker & MacNair 1979).

Furthermore, the pattern of energetic expenditure of concurrent lactation and gestation differs from that in altricials due to a long gestation (Peters 1989) and an early peak of milk yield (Anderson et al. 1984) in precocials. Maternal reproductive state may therefore not affect lactation in the same way as found in many altricials.

Aims of this study

Studies similar to those carried out in altricial mammals using a precocial model organism, may allow conclusions if the findings in altricial mammals and the theory on parent-offspring interactions can be confirmed for precocials. In my thesis I investigated the influence of pup demand and of maternal reproductive state on nursing performance in the guinea pig (*Cavia aperea f. porcellus*). Guinea pigs are extremely precocial. Their short reproductive cycles (for a precocial mammal) and their easy handling make them an ideal model system to complete the experimental studies that have been carried out in small altricial mammals. Manipulation of demand and supply can be easily achieved by cross-fostering young of different ages (see Chapter 1) or by cross-fostering guinea pig pups with pups of the much smaller and lower yielding cavy (*Cavia aperea*) (see Chapter 2). Furthermore, similar to small altricial mammals – but unlike most large precocial mammals – guinea pigs have a post partum oestrus and are therefore ideal to test if the effects of concurrent pregnancy on lactation can also be found in a precocial mammal (see Chapter 3).

I addressed the question: Do mothers – similar to the findings in rats an mice (Nicoll & Meites 1959; Lichtman & Cramer 1989) – react to pup demand by adjusting nursing performance? I used milk yield, milk quality, time spent nursing, and the timing of weaning as measures for nursing performance. (Chapter 1). I further ask: Which cues do mothers use to determine how much care they should provide?

Do they react to some correlate of age like pup mass? (Chapter 1) Or do pups actively communicate their need? If so, does common theory on the effects of demand on supply and of supply on demand also apply to these precocials mammals? (Chapter 2).

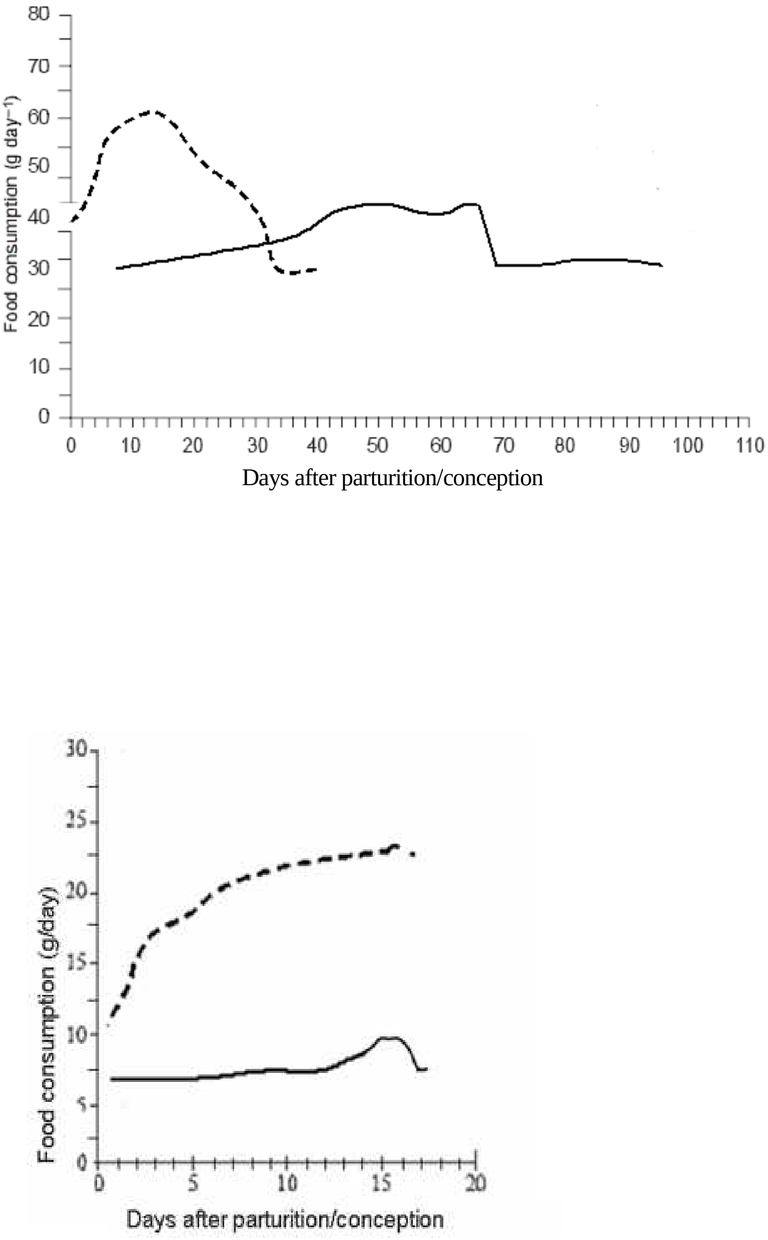
Is the outcome of parent-offspring conflict over maternal resources a parent wins outcome, an offspring win outcome or a compromise between the two? (Chapter 1 and 2).

Similar to the litter replacement experiments in rats and mice (Nicoll & Meites 1959; Lichtman & Cramer 1989) in which mothers were continuously supplied with young foster pups, cross-fostering different aged young allows to determine if mothers adjust the timing of weaning to pup age or to some correlate of age like weight (see Chapter 1). Mothers with cross-fostered younger pups should prolong lactation while mothers with cross-fostered older pups should wean early. Additionally, this experimental set up allows to find out, if mothers react to pup demand by adjustments in milk yield and time spent nursing throughout lactation. If and to what extent nursing performance varies according to the age of foster pups can furthermore allow conclusions about the resolution of parent-offspring conflict. If cross-fostered older pups suck longer relative to normal circumstances, this would provide unambiguous evidence that interests of mothers and pups over the duration of the lactation diverge and that mothers wield the power in this conflict.

I also tested if the models on the effects of supply and demand that are derived from observations in altricial birds (Godfray 1991, 1995; Parker & MacNair 1979) can be applied to a precocial mammal (see Chapter 2). Cross-fostering guinea pig pups with cavy pups, creates a low supply situation for pups raised by cavy mothers and a high supply situation for pups raised by guinea pigs. Females nursing cavy pups confront low demand, those nursing guinea pig pups high demand. Pup attempts to gain access to the teats were used as a measure for solicitation. Following the models, mothers caring for guinea pig pups should confront more sucking attempts than those raising cavy pups and should respond with an increase in supply. Pups cared for by high yielding guinea pig mothers should show less sucking attempts than pups nursed by low yielding cavy mothers.

In Chapter 3 I show the effects of reproductive state on nursing performance in guinea pigs. In altricial mammals the nursing performance has often been found to be reduced in females that were concurrently pregnant and lactating (Bruce 1958 (mice); Xiccato 2005 and Partridge et al. 1986 (rabbits)). This can be interpreted to result from an allocation problem as peak energetic expenditures of pregnancy and lactation overlap to a great extent in many altricial mammals – for example, mice (Johnson et al. 2001) and rats (Oswald and McClure, 1987) (see Fig.2). Johnson et al.(2001)found that despite the additional burden of pregnancy, lactating mice did not increase their food intake and suggested that females may be limited in their capacity to absorb energy. However, Hammond et al.(1994) exposed lactating rats to cold temperatures and thereby showed that the energetic expenditure to pups was not affected by an increase of energetic demands beyond those of normal lactation. Females could still meet the energetic demands of their pups by an increase in food intake, increases in gut mass and in intestinal absorptive capacity. Therefore it is questionable if reduced nursing performance results from their simultaneous allocation of energy to the suckled litter and to the litter in utero.

In guinea pigs energetic peaks of concurrent lactation and gestation are widely separated (Künkele 2000, Künkele & Trillmich 1997) (see Fig.2). Should under these circumstances lactational and gestational performance be reduced when mothers are concurrently pregnant? If so, this would suggest that reduced nursing performance is not exclusively a result of energetic constraints.



a)

b)

Figure 2: Energetic demands during pregnancy and lactation in a precocial and an altricial mammal: Food consumption during lactation (dotted lines) and during gestation (black lines) of a) guinea pig (after Künkele 2000), b) house mouse (after Johnson et al. 2001)

Chapter 1: Weaning in the guinea pig (*Cavia aperea* f. *porcellus*) – Who decides and by what measure?[1]

Abstract

Offspring should be selected to influence maternal effort in order to maximize their own fitness, whereas mothers are selected to limit investment in present progeny. In mammals, this leads to a conflict over the amount of milk provided and the timing of weaning. The intensity and time course of such conflict has so far mostly been investigated experimentally in altricial rodents. However, it is expected that offspring options for conflict will depend on developmental state. We therefore investigated in the highly precocial domestic guinea pig (*Cavia aperea* f. *porcellus*) who decides over nursing performance and weaning, and how pup state influences these decisions. Specifically, we tested whether a threshold mass of pups predicts weaning time. By exchanging older litters against neonates and vice versa, we produced a situation, in which females differed in lactational stage from the cross-fostered pups. Our results indicate that females decide about the timing of weaning as cross-fostered younger pups were weaned at a much younger age than controls and older pups benefited from continuing lactation of foster mothers. Growth rates did not differ in the treatment groups and different weaning ages resulted in differing weaning mass refuting the hypothesis that weaning is based on a threshold mass of offspring. This constitutes clear evidence that in a precocial rodent, the guinea pig, decisions about maternal care are primarily determined by maternal state and little influenced by pup state despite the extreme precociality of offspring. We suggest that precocial pups show little resistance to early weaning when food is abundant as they reach sufficient nutritional independence by mid lactation to enable independent survival.

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1) Submitted

Introduction

Parental investment serves to ensure offspring growth and survival to independence and thus increases parental fitness. However, parental investment is costly on a proximate and ultimate level (Clutton-Brock 1991). Life history theory predicts that parents should only invest in offspring until fitness gain through continued investment in present offspring becomes less than potential gains achievable through investment in future offspring (Roff 1992; Stearns 1992; Trivers 1972). The optimal amount of parental effort depends on the state of parents and offspring and should be adjusted dynamically and adaptively. As the effects of parental expenditure on offspring fitness become marginal for superior offspring, parents may reduce their investment early when offspring are in better state. Conversely, they may provide resources over a longer period to offspring in poor state (Bateson 1994). If the young are in very poor state, however, parents may cease investment completely (Clutton-Brock 1991; Godfray 1991). These adjustments require parents to assess varying need and state of the young by offspring appearance or by the young actively communicating their need (Godfray 1991, 1995; Parker et al. 2002).

Parents are not the only active participants in interactions concerning parental investment: Offspring are selected to influence parental effort in such a way that their own fitness is optimized through their own reproduction and via the parents' future offspring (Mock & Parker 1997; Trivers 1974). While parents are equally related to all offspring, offspring are more closely related to themselves than to future siblings, in particular when these are fathered by a different male. This creates a genetic battleground of conflict of interests between parents and offspring (Mock & Parker 1997) in which both parties value fitness gains via the parents’ future offspring differently. Given the underlying genetic conflict, the question arises how conflict resolution depends on the power held by the parties involved. As suppliers of resources to the offspring via feeding and other acts of care, parents generally wield much more power than offspring and thus may be able to decide the conflict in their favour (Trivers 1974).

Most of the research on parent-offspring conflict and its resolution has been conducted on altricial birds and the theory developed also refers to situations typical for these birds (reviewed by Mock & Parker 1997; Royle et al. 2004). Much less work has been done on mammals probably because communication between mother and offspring is less obvious in mammals than in birds, as begging is rare and lactation provides a means of direct physiological interaction and may take less conspicuous forms such as intense nudging of the udder or increased sucking (Wells 2003). Also, mammalian maternal provisioning is harder to study than feeding in birds since time at the teat often does not provide sufficient information to estimate milk transfer (Cameron 1998). On the other hand, in many mammals, the level of sucking stimulus is directly related to the generation of milk supply. Sucking prompts the release of prolactin, which stimulates the synthesis and secretion of milk in the mammary gland (Cowie 1984). The amount of teat stimulation positively relates to the amount of prolactin released (Algers et al. 1991; Hart & Linzell 1977). Through this feedback females may adjust - unknown to the observer - milk yield and quality to offspring needs (for example, yield: Mepham & Beck 1973; composition: Jacobsen et al. 2004; Mueller & Sadleir 1977). Thus, offspring stimuli are critical for eliciting maternal care and young may communicate their need by varying sucking intensity (Wells 2003).

However, this feedback regulation of milk supply may not be typical for mammals in general. For example, in marsupials sucking of young has little or no effect on prolactin levels. Although milk yield and composition change more in marsupials than in eutherians, these changes appear to be an intrinsic character of the mammary epithelium (Tynedale-Biscoe & Renfree 1987). Cross-fostering tammar wallaby young (*Macropus eugenii*) to females at differing stages in lactation resulted in abnormal growth rates indicating that young were not able to influence milk production to the extent expected from the age of the young (Findlay & Renfree 1984; Trott et al. 2003).

Perhaps due to these difficulties in measuring changes in mammalian provisioning during lactation the process of weaning has attracted most attention. Lee et al. (1991) predicted offspring to be weaned at a threshold body mass and thus earlier under optimal than under moderate environmental conditions. They argued that females in good bodily condition are able to provide their offspring with larger quantities of high quality milk, allowing higher growth rates and earlier weaning than is possible when females are in poor bodily condition. Most of the work on determinants of weaning is correlational (Dahle & Swenson 2003; Fairbanks & McGuire 1995; Gomendio 1991; Lee et al. 1991; Trillmich 1986; White & Luick

1984) and less experimental work has been done (Babbit & Packard 1990; Gomendio et al. 1995; Laurien-Kehnen & Trillmich 2003, 2004; Loudon & Kay 1984; Martin 1986). The most detailed experimental work has been done on altricial rodents – mainly rats and mice. Most of these investigated the physiological mechanisms that induce and maintain maternal care (Nicoll & Meites 1959; Cramer & Lichtman 1989; König & Markl 1983; Pfister et al. 1986, Williams et al. 1980). Rats (*Rattus norvegicus*) can be induced to lactate much longer than normal by repeatedly exchanging pups for younger foster pups (Bruce 1958; Nicoll & Meites 1959). This finding indicates that stimuli presented by the young maintain lactation and that pup state obviously influences the timing of weaning. However, they do not allow conclusions as to whether females or offspring determine the timing of weaning. Pfister et al. (1986) demonstrated that older rat pups will continue to nurse far beyond normal weaning age when fostered to a female nursing much younger offspring. This finding suggests that pups will use the mother as a food source much longer than normal if given the chance and indicates a potential for conflict between mother and offspring about the timing of weaning.

For precocial mammals hardly any comparable studies are available. Short reproductive cycles and ease of handling in the laboratory makes the guinea pig an ideal model for precocial mammals. A previous experiment with guinea pigs indicated that food-restricted females delayed weaning (Laurien-Kehnen & Trillmich 2004), but this seemed a response of the female to her own body condition rather than a reaction to pup needs. Food-restricting pups in early lactation led to a slight increase in time spent nursing by the mother and effects on milk yield were not found. The effects on weaning were not well documented (Laurien-Kehnen & Trillmich 2003). Therefore, these experiments did not answer the question of whether mother-offspring conflict over weaning is resolved by a one-sided maternal decision, by a compromise between mother and offspring, or by offspring manipulation of maternal effort. While gains in offspring fitness through maternal care are immediately obvious for altricial mammals where young depend completely on maternal resources for warmth, protection, and nutrients, in precocials these benefits seem reduced in precocials. This applies particularly to the extremely precocial guinea pig (in the wild, *Cavia aperea*, as well as the domestic form

C. aperea f. *porcellus*). Young thermoregulate from birth by efficiently using their well developed brown fat to keep warm (Brück 1970). Guinea pigs are highly mobile from birth (Trillmich et al. 2006) and this leads to a much reduced risk of predation to the extent that juveniles in the field may survive as well as adults (Kraus et al. 2005). Furthermore young begin to feed on solid food right from birth (Künkele & Trillmich 1997) and. exceptional for mammals, may survive (under ideal laboratory conditions) without ever getting any milk (Linzell 1971).

Nevertheless, lactation is costly to female guinea pigs as they produce about their own weight in milk (Mepham & Beck 1973) and milk intake has important effects on pup growth (Künkele & Trillmich 1997), early development (Weaver et al. 1988), and probably on later fecundity as female body mass negatively correlates with age at first estrous (Trillmich et al. 2006). Given that offspring look and behave like small adults and therefore the imbalance of power between parent and offspring seems reduced, they may be better able than altricial offspring to extract additional maternal care. This makes a study of the potential for weaning conflict particularly interesting in this exceptional mammal. We investigated the conflict and its resolution by cross-fostering different aged pups.

If cross-fostered younger pups were weaned too early and cross-fostered older pups sucked longer relative to normal circumstances this would provide unambiguous evidence that interests of mothers and pups over the duration of the lactation diverge and that mother wield the power in this conflict. Adjustments to pup age would demonstrate that pup age or some correlate of age like a threshold pup mass influences the timing of weaning.

If and to what extend nursing performance i.e. milk yield and quality and the time spent on nursing vary according to the age of foster pups will allow conclusions about the conflict and its resolution throughout the nursing period. Consequently, we ask the following questions: (1) Are nursing time, milk yield and milk quality adjusted in response to young of differing ages, and (2) is weaning age adjusted to pup age or some correlate of age like mass, leading to a decrease or increase in length of lactation for cross-fostered, differently-aged young? In addition, we ask (3) who decides about the timing of weaning, mother or offspring?

Methods

Animal subjects and experimental design

Multiparous (n=22) and primiparous (n=11) outbred domestic guinea pigs (*Cavia aperea* f. *porcellus*) from the breeding stock at the University of Bielefeld were used for the experiments. Pellet food (guinea pig chow, Höveler, Langenfeld, Germany), hay and water were provided *ad libitum* and a carrot was fed additionally on week days. Drinking water was supplemented with vitamin C (Ascorbic acid, Roth, Karlsruhe, approx.1g/l) once a week. Temperature was about 21°C. The photo cycle was 14 hours light and 10 hours dark. Wood chips were used for bedding. During pregnancy two females were kept together in enclosures with 0.8 m^2 floor space. From about 14 days before giving birth they were housed individually in an enclosure of 100x80x50cm (open above). After birth, all litters were adjusted to three pups. If necessary this number was achieved by fostering supernumerary pups to lactating females not used in the experiment or by fostering additional pups to the original litter. Three litters were excluded from the experiment as mothers or single pups of the litter appeared sick after parturition or died. We created the two following experimental groups:

Group 1 consisted of females (n=11) to which older pups were cross-fostered, Group 2 (n=10) consisted of females with cross-fostered younger pups. Pups of litters in the first group were exchanged on day one of life (=day of birth) against pups from litters of the latter group that were around 7 days of age (5 litters = 6 days old, 6 litters = 7 days old). A control group (n=12) was run simultaneously in which same-age litters were exchanged to control for effects of the cross-fostering procedure. Females in the treatment groups did not differ in parity, age, maternal mass, or litter size (one-way ANOVA: F2,32=0.114, p<0.892; maternal mass, one-way ANOVA: F2,32=1.2; p<0.311; litter size, one-way ANOVA: F2,32=0.609; p<0.551) (SPSS).

Observed traits

We observed behavior for one hour in the home box every other day. Maternal nursing can be recognized by a specific nursing position, where mothers crouch motionless, enabling access to the teats (see Hennessey and Jenkins 1994; Kunkel and Kunkel 1964). Nursing was recorded when females took up the nursing position for more than 30 seconds. To look at changes in nursing performance throughout the lactation period we analyzed the amount of time spent nursing. Following Laurien-Kehnen & Trillmich (2003, 2004) weaning age was defined as pup age when mothers terminated nursing. Whether or not mothers were still nursing was checked every day (during observations in the home box or during milk yield measurements) and nursing was considered terminated on the first of three subsequent days on which the female was never seen in nursing position.

On the days not used for observations, milk yield was measured. For milk yield measurements the time-nurse method described by Mepham & Beck (1973) was used. Pups were separated from mothers for one hour (not 2-4 hours as in Mepham & Beck's experiment to avoid inadvertent weaning), during which the pups were deprived of solid food and water. Pups and mothers were then weighed to the nearest 0.1 g and placed together in a macrolone box with a grid bottom for a period of 30 minutes. At the end of this period pups were weighed again. We used mass difference as an index of milk yield and mass gain of pups was taken as a minimum estimate of milk production over 1.5 hours. The actual milk yield is higher than the measured mass increase, as individual pup mass decreases by about 0.3 g over a half hour period due to water loss via the lung by exhalation and insensible water loss through the skin (Hille 1993). Feces of pups could easily be distinguished from maternal feces by size and were added to the final litter mass. Voided urine could not be assigned to mothers or pups. Consequently we excluded all measurements where urine excretion was observed (136 of 407).

Analysis of milk composition

Females were milked by hand after a one hour separation from their pups on day 3, day 8 and day 13 (±1 day). Not all females could be milked (control: n=12, females with cross-fostered older pups: n=9, females with cross-fostered younger pups: n=8) and at least 0.6 ml were needed for the analysis. Usually no more than 1 ml of milk was obtained by manual milking. This precluded duplicate measurement. Standardization was done with cow milk samples. Repeated measurements of cow milk samples produced an average intra-assay coefficiency of variation of 1.1%. Fat content was analysed using nuclear magnetic resonance spectroscopy. Samples were recorded on a Bruker FT-NMR spectrometer Avance 600 at a proton-resonance frequency of ^{1}H:600.13MHz characteristic for hydrogen atoms in fatty acids. A solution of D2O and Pyridine was used as a standard (50.1g: 3.01g,). Measures were taken over all aliphatic protons of fatty acids and all pyridine protons (Weber and Thiele 1998).

Statistical Analysis

We first analysed whether the treatment affected maternal effort throughout the lactation period by measuring changes in milk fat content, milk yield, and the time mothers spent in nursing position. To find out how changes in these traits influenced pup development we compared growth rates of pups among the treatment groups. We then analysed how the treatment affected the timing of weaning in the three groups from the mothers' as well as from the pups' perspective and finally we compared pup mass on the day of weaning to test if a mass threshold could serve to time weaning. Data was analysed with SPSS (version 12.0 for Windows). Sample size was defined by the number of litters. Effects of original litter size can not be excluded. We therefore used factorial ANOVAs with the mother's original litter size as second fixed factor to analyse the effects of cross-fostering on weaning age, length of nursing period, and mass at weaning. Mean pup mass within a litter was used to analyse pup growth and mass at weaning. Growth rates over two days were analysed until day 22 of the maternal lactation period (day 22 was the day the first mother weaned her pups and effects of milk yield should be small thereafter). A factorial repeated measures ANOVA (repeats: 11) with the mothers original litter size as second fixed factor was used for the analysis.

Normal distribution was tested by graphical methods and Levene tests were carried out to test for homogeneity of variance. We used mixed linear models with original litter size as second factor and female identity as a random factor to analyse the repeated measurements of milk yield, milk fat content and time spent nursing. Repeated measures ANOVAs were inappropriate as the duration of the nursing period varied between mothers and for milk yield and fat content individual data points were missing (due to failed milk yield measurements or insufficient material for fat analysis).

Results

No effect of original litter size was found in any of the analyses. We did not find any significant differences in milk fat content or milk yield among the three groups (Tab. 1). Fat content increased over the nursing period and milk yield started out low, peaked around days 6 to 8 and then decreased again, without any detectable difference among groups (Tab. 1). Overall growth rates were not significantly different (Tab. 1). From the milk yield curve (Fig. 1) one would expect that 7-day old young transferred from their own mother at peak lactation to a mother that had just given birth would have suffered a reduction in milk intake because maternal milk yield starts out low after parturition. Indeed, on day 6-8 of pup life growth rates were significantly lower in cross-fostered older pups (means: 5.1 ± 2.2 g per day) than in control pups (means: 7.9 ± 2.2 g) or cross-fostered newborns (means: 8.0 ± 2.3 g) (ANOVA, F2,27=5.372, p=0.011, post-hoc (LSD): control – older pups: p=0.006; control – younger pups: p=0.95; older pups – younger pups: p=0.008).

Milk yield (g/day) (days 2-26)	Older pups: 1.50 ± 1.89 Younger pups: 1.62 ± 1.95 Control: 1.41 ± 1.93	Mixed linear model: F(2,32.3)=0.274, p=0.762
Milk fat content in percent (day 3 -15)	Older pups:6.72 ± 2.93 Younger pups: 7.47 ± 2.35 Control: 6.34 ± 2.96	Mixed linear model: F(2,24.8)=1.207, p=0.316
Growth rates (g/day) (days 2-22)	Older pups: 7.78 ± 3.18 Younger pups:8.28 ± 5.43 Control: 8.09 ± 2.77	Repeated measures ANOVA: 10 repeats, F(2,29)=0.446, p=0.645

Table 1: Milk yield, milk fat content, and pup growth rates. Values are means and SD.

Mothers terminated all nursing bouts in all groups, independent of pup age. Time spent nursing decreased over the nursing period and did not differ significantly among groups (Fig. 2; means ± SD: older pups: 0.38 ± 0.217, younger pups: 0.31 ± 0.206, control: 0.34 ± 0.225; Mixed linear model: F(2,27.8)=1.903, p=0.168).

To determine the effect of pup age on a female's decision about nursing, the length of nursing periods for mothers with cross-fostered younger pups, mothers with cross-fostered older pups, and mothers of the control group were compared. Mothers with younger pups nursed for 31.3 ± 3.7 days, mothers with older pups for 28.2 ± 3.3 days and mothers of pups of the control group for 29.1 ± 2.6 days (Fig. 3; ANOVA, $F_{2,27}$=2.675, p=0.087). Thus, despite a trend in the direction expected from the age of young, groups did not differ significantly in nursing duration as seen from the mother's perspective.

Looking at the length of the nursing period from the pups' perspective, effects were marked: females ended nursing of their cross-fostered younger litters when the pups were only 26.1 ± 3.8 days old. These litters were nursed for a shorter period than litters of the control group, for which nursing ended at age 29.1 ± 2.6 days. Cross-fostered older litters continued sucking until they were 33.6 ± 3.4 days old (Fig. 4a) (ANOVA, $F_{2,27}$=11.86, p=0.0002; post hoc (LSD): older pups – control: p=0.003; younger pups – control: p=0.043; younger pups – older pups: p=0.00002).

As overall growth rates did not differ among treatment groups but age at weaning did, this must result in differences in litter mass at weaning between groups. Indeed, total mass of litters of cross-fostered older pups was significantly higher at weaning than mass of litters of the control group (Fig. 4b) (means: younger litters: 825.6 ± 95.9 g, control litters: 858.2 ± 95.9 g, older litters: 1002.4 ± 128.6 g) (ANOVA, $F_{2,27}$=6.173, p=0.006; post hoc (LSD): older pups – control: p=0.007; younger pups – control: p=0.522; younger pups – older pups: p=0.002).

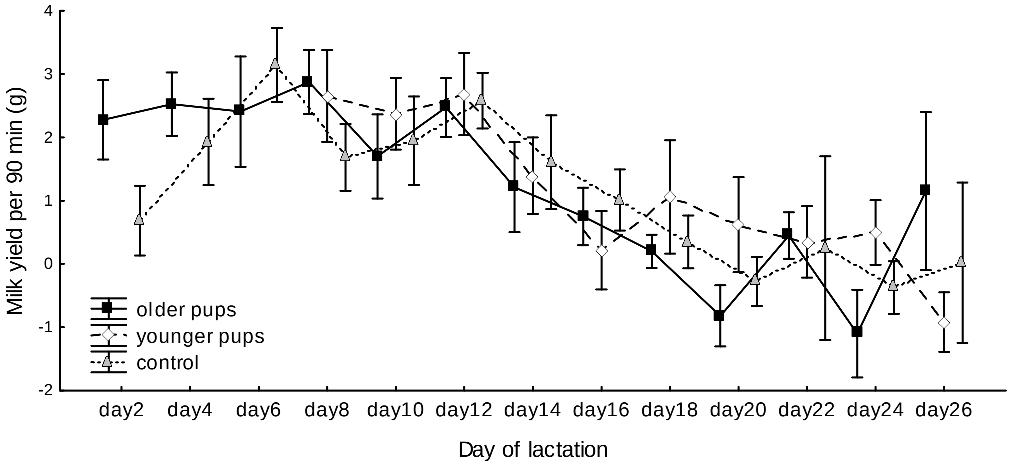

Figure 1: Milk yield per 90 minutes (=pup-mass after nursing minus pup-mass before nursing; see Methods). Groups: control pups had the same age as original pups; younger pups were 6 days younger than the original litter; older pups were 6 days older than original litter. Values given as means ± SE. (Mothers with cross-fostered younger pups are shown from day 8 – one day after the exchange took place.)

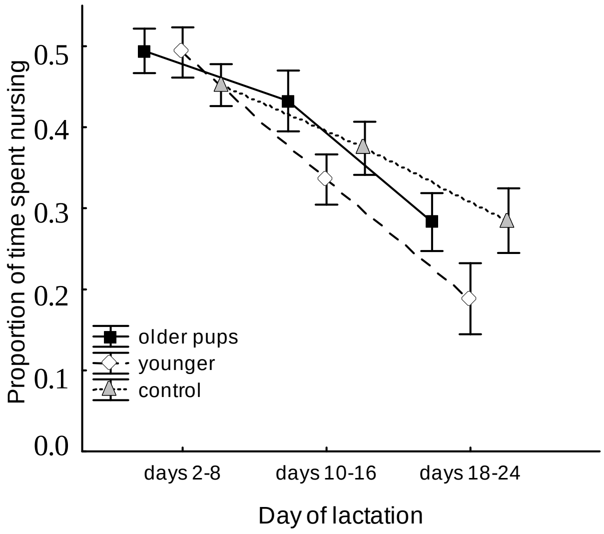

Figure 2: Mean proportion of time spent nursing per 1h observation.

Groups: control pups had the same age as original pups; younger pups were 6 days younger than the original litter; older pups were 6 days older than original litter. Values given for 3 Periods of lactation (Peak of lactation around day 8). Values given as means ± SE.

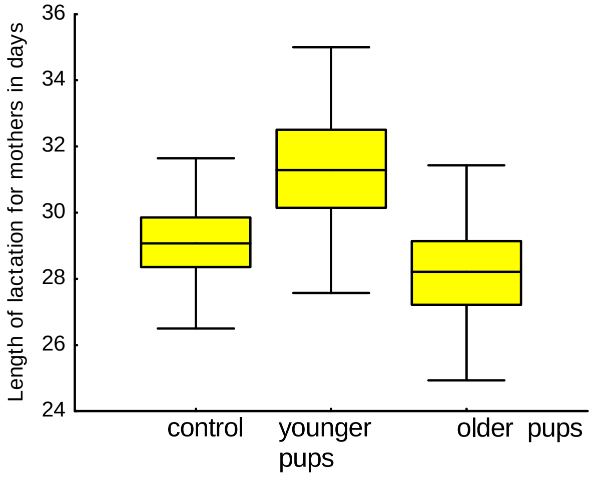

Figure 3: Length of nursing period for mothers of cross-fostered pups.

Groups: control pups had the same age as original pups; younger pups were 6 days younger than the original litter; older pups were 6 days older than original litter. Values given as means, SE (boxes) and SD (Whiskers).

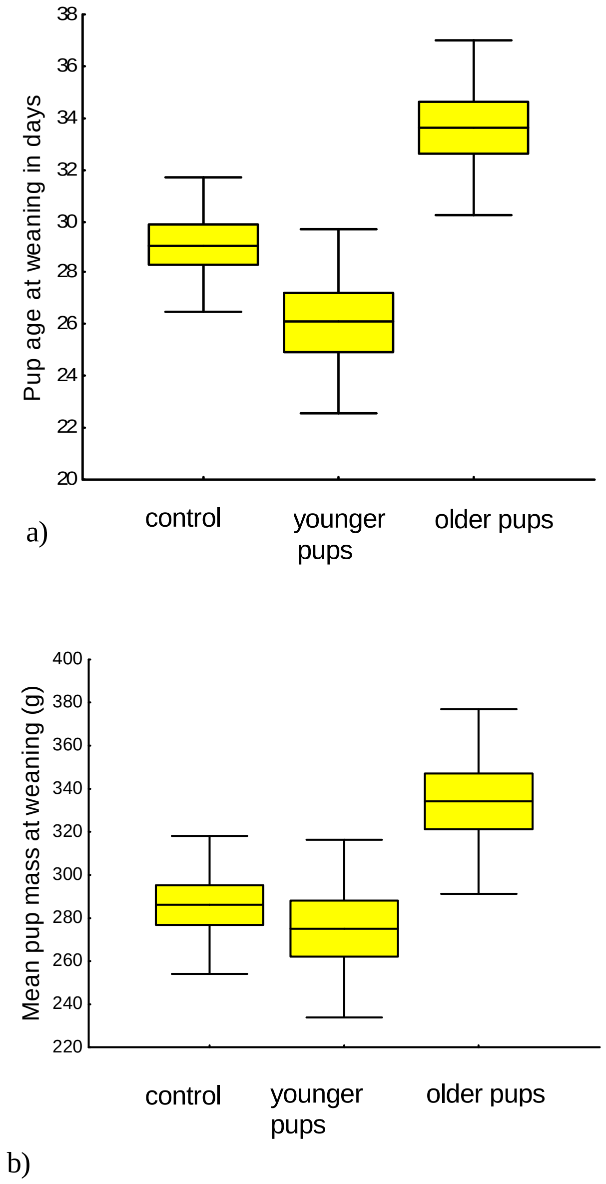

Figure 4:

a) Mean age of pups at weaning.

b) Mean mass of pups on the day of weaning.

Groups: control pups had the same age as original pups; younger pups were 6 days younger than the original litter; older pups were 6 days older than original litter. Values given as means, SE (boxes) and SD (whiskers).

Discussion

Younger foster-pups were weaned much earlier than controls, and older pups continued to suck from their foster mothers for longer than normal. If under normal conditions pups were deciding about the end of lactation, cross-fostered younger pups should have lengthened the lactation period of their foster mother and cross-fostered older pups should not have taken advantage of the surplus care provided and should have stopped sucking once they had reached normal weaning age. However, mothers showed only a non-significant tendency (p=0.087) to prolong nursing for 2 days or shorten the nursing period by 1 day, much less than the 6 days predicted from foster pup age. Thus, our results demonstrate a conflict over the timing of weaning and establish that under normal conditions pups are weaned earlier than they would of their own accord suggesting that mothers hold the power to decide the conflict.

Lee et al. (1991) predicted that the weaning decision should be based on maternal and on pup state and suggested a threshold pup mass as a proximate cue. For the guinea pig, our data clearly refute this hypothesis, as cross-fostered older pups were significantly heavier at weaning than control pups.

In contrast to findings in altricial rodents (Nicoll & Meites 1959; Pfister et al. 1986) nursing performance in guinea pigs appears to be determined by a mechanism that works in an hourglass like fashion. However, earlier findings by Laurien-Kehnen & Trillmich (2003) demonstrated that weaning was delayed when mothers were food restricted. Lactating female guinea pigs seem to decide primarily based on their own rather than on pup state. Using such a mechanism can lead to an adaptive decision under natural conditions, because maternal and pup condition will be highly correlated as both are influenced by identical food abundance in the environment. Therefore her own state will reliably inform a female about food available to her pups since pups begin to feed on solid food in the first few days after birth and contribute a substantial proportion of their total food intake during the lactation period by independent foraging (Künkele & Trillmich 1997). Much of the differences between findings in many altricial mammals and the precocial guinea pig may relate to the fact that pups can self-feed early during lactation thereby enabling pups to become largely independent of maternal milk before the end of lactation (Künkele & Trillmich 1997).

The amount of time spent nursing decreased similarly over lactation for all mothers indicating that not only the timing of weaning but also at any stage of lactation the time spent nursing is under maternal control. The observation that females terminated almost all nursing bouts further supports this conclusion (see also König 1985). Fat content and milk yield were not adjusted in response to differently aged pups (Tab. 1, Fig. 1). The yield curve was similar to those described for guinea pigs by Anderson et al. (1984), Laurien-Kehnen & Trillmich (2003), and Mepham & Beck (1973), who found the highest milk yield near day 7 of lactation. No influence of the intensity of the sucking stimulus on milk yield could be demonstrated for guinea pigs as has been reported for mice (König et al. 1988), rats (Drewett 1983), pigs (Gill & Thompson 1956), and humans (Wells 2003). Precocial guinea pigs differ strongly from altricial rodents in the regulation of maternal care. In rats milk production increases if the sucking stimulus is increased due to nervous and hormonal feedback between the nipple, the brain and the milk gland (Nelson 2000). In guinea pigs, however, the milk yield did not increase when pup demand was increased experimentally by food restriction (Laurien-Kehnen & Trillmich 2003) or by experimental increases in litter size (Davis et al. 1979). This finding makes the precocial guinea pig a rather unique case among eutherian mammals. Contrary to most theory (Hussel 1988; Godfray 1995; Parker et al. 2002), maternal supply of milk does not interact detectably with demand. This lack of responsiveness of the milk producing system closely

resembles findings in marsupial females that react little if at all to changing pup demands. Over the course of lactation, in marsupials, the amount and composition of milk change more drastically than in eutherian mammals (Tynedale-Biscoe & Renfree 1987), but these changes in milk supply were found to be an intrinsic characteristic of the marsupials' mammary epithelial cells and occur as cells age without adjustment to offspring age (Tynedale-Biscoe & Renfree 1987). Apparently here as well as in the guinea pig a mechanism is set that follows a fixed trajectory independent of feedback from the sucking of offspring.

Remarkably similar to findings by Findlay & Renfree (1984) in wallabies, cross-fostered younger guinea pig pups received more milk in their first days of life than same-aged pups of the control group. As a consequence of the mother's fixed schedule of milk yield cross-fostered younger pups received more milk in their first days of life than same-aged pups of the control group, since the foster mothers were at peak lactation. For cross-fostered older pups, milk yield was lower immediately after fostering and they found a peak in milk yield when they were about 13 days old. Our measurements of milk intake demonstrate that, if given a chance, pups take more milk than their natural mother supplies. Thus, mothers do not only hold power over the timing of weaning, but also over the amount of supply throughout lactation.

The results demonstrate a conflict about the amount of milk provided and the end of lactation. This became perhaps most obvious when cross-fostered older pups took advantage of a prolonged lactation. Nevertheless, evidence of active squabbling between mother and offspring was too rare to be analysed, even in the group of cross-fostered younger pups that were weaned too early relative to standard conditions. Much rather pups seemed to accept their mother's decision to wean without major squabbling and we could not observe any mother aggressively pushing her pups away towards the end of lactation (see also König 1985). The relative value of parental milk supply versus self-feeding is likely to shift in favor of self-feeding as young age. Davies (1978) demonstrated that parent birds can speed up offspring transition to independence by increasing resistance to begging. When feeding in response to begging was delayed, young tits started self-feeding earlier compared to chicks of 'generous' parents. Similarly in mammals by reducing milk yield and increasingly refusing sucking attempts mothers may succeed in making sucking unprofitable for their offspring which then achieve energy intake more efficiently by independent foraging. On the other hand, milk remains a welcome addition to independent foraging when it can be gained cheaply, as demonstrated by the cross-fostered older pups that sucked far beyond normal weaning age. Our findings are in accordance with studies in cats: recently weaned kittens used the opportunity to suck, when their mothers were placed in front of them anaesthetized (Koepke & Pribram 1971) in rats that were kept with mothers caring for preweanling litters (Pfister et al. 1986).

The question remains: Do our findings of the diverging interests of mothers and pups over the amount of milk and the length of lactation reflect the genetic conflict predicted by Trivers (1974)? Mock & Forbes (1992) suggested that not every phenotypic conflict, i.e. squabbling, among family members must indicate the underlying genotypic conflict, as the latter concerns fitness consequences for parent and offspring. For the case of the guinea pig we would like to stress that our results though indicative of a phenotypic conflict, do not necessarily indicate a fitness conflict in the sense of Trivers (1974). However, the timing of first estrous depends on offspring mass which is influenced by the amount of milk received throughout lactation (Trillmich et al. 2006). Thus, the conflict over the amount of milk provided in earlier phases of lactation may indicate a fitness conflict. Low energetic benefits to pups as well as low energetic cost to mothers at the end of the lactation may support Bateson’s (1994) suggestion that optimal weaning times for mothers and offspring will coincide. Guinea pigs are aseasonal breeders and can conceive post partum. Under conditions of high food abundance females are almost constantly pregnant while lactating (Rowlands 1949) and a continuation of lactation may negatively affect the maintenance of pregnancy and the development of the litter in utero. Thus, even though energetic expenditure may be low towards the end of the lactation period a longer lactation may incur maternal fitness costs indicating that a Triversian weaning conflict may exist.

Chapter 2: Changing supply and demand by cross-fostering – effects on the behaviour of pups and mothers in guinea pigs and cavies[2]

Abstract

The regulation of parental supply and offspring-demand was modelled for the situation in altricial birds. The situation might differ for precocial mammals, where offspring have the alternative to feed themselves. We investigated the effect of supply and demand on mother-offspring interaction in precocials by cross-fostering pups between the domestic guinea pig (C*avia aperea* f. *porcellus)* and its ancestor, the cavy (*Cavia aperea*). The two forms are closely related, yet guinea pigs are heavier, and produce more milk than cavies. We thus created a low supply situation for pups raised by cavy mothers and a high supply situation for pups raised by guinea pigs. Females nursing cavy pups confront low demand, those nursing guinea pig pups high demand. Pups in a high supply situation showed more sucking attempts than in a low supply situation indicating a positive effect of supply on demand. High demand pups showed more sucking attempts than low demand pups. Mothers with high demand pups nursed more frequently and spent more time nursing than mothers with low demand pups. Milk production remained unaffected and timing of weaning was only slightly adjusted to the kind of pup fostered. Weaning was marked by a decrease of sucking attempts, a decrease in maternal responsiveness and an increase in aggressive behaviour. The findings show that mothers get information about pup demand, but some inner maternal state seems to induce the weaning process largely independent of pup demands. The effects of supply differed from the predictions derived from current models of parent-offspring interaction.

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2) Submitted

Introduction

Parental investment ensures offspring growth and survival to independence and thus increases fitness, but this comes at a fitness cost in terms of potential future offspring (Trivers 1972). Parents should optimize allocation to obtain maximal fitness benefits. As the effects of additional parental expenditure on offspring fitness become marginal for superior offspring, parents may reduce or abbreviate investment, but may provide resources over a longer period to offspring in poor state. For young in very poor state, however, parents may cease investment completely (Clutton-Brock 1991; Godfray 1991). Such adjustments require parents to assess the needs of their young (Godfray 1995). Parents may use offspring appearance (Lee et al. 1991) to determine the optimal amount of care and offspring may actively communicate their demand (Mock & Parker 1997; Searcy & Nowicki 2005). Solicitation behaviour may have evolved as a result of the mutual benefit obtained, when parental investment is regulated effectively according to offspring need (Kölliker & Richner 2001). Hussel (1988) assumed higher demand expressed through more solicitation to result in increased supply and a high supply to lead to less solicitation**.** Supporting his suggestion, parental provisioning was found to be proportional to begging intensity in several bird species (Smith & Montgomerie 1991; Kilner 1995; Mondloch 1995).

However, Trivers (1974) proposed that different fitness interests of parents and offspring lead to disagreements over the optimal amount and duration of care. Consequently, offspring may try to solicit a disproportionate share of parental resources. To maintain honest communication solicitation must incur some cost to the young (Kilner & Johnston 1997; Parker et al. 2002a). Affordable costs increase with the benefit of begging, thus offspring in greater need can beg more intensely (Parker et al. 2002b). While different modelling approaches agree that supply should increase with increased demand they make different assumptions about the effect of supply on demand. Godfray (1991, 1995) predicted begging to be an honest one-to-one representation of true need and assumed that supply should not affect begging intensity. An extension of his honest signalling model by Johnstone (1996) also allows a negative effect of supply on solicitation. The scramble model by Parker & MacNair (1979) implies a negative slope of the supply on demand function. Food supplementation experiments on bird nestlings show that the slope of the function can be zero or negative, but also positive (Royle et al. 2002).

With increasing offspring condition and offspring ability to feed themselves the benefits of parental investment to offspring decline. Consequently costly solicitation becomes inefficient and should decrease accordingly If parents do not agree with offspring over the optimal timing for the transition to independence (Trivers 1974), they may speed up this process by increasing the cost of begging through ignoring solicitation, avoiding, and rejecting offspring (Davies 1978; Pugesek 1990, Leonard et al. 1988). Thus, towards the end of parental care, a decline in the parents' readiness to respond reduces the effectiveness of solicitation and makes independent foraging the better alternative (Davies 1978; Jensen and Recén 1989).

Most research on the effects of supply and demand on parent-offspring interaction has been conducted in altricial birds. In mammals, lactation provides a means of more direct physiological interaction than feeding in birds. Sucking intensity can vary as a function of offspring condition and influence the amount of milk provided and the duration of lactation (Delgado et al. 1982; Loudon & Kay 1984; Lee et al. 1991). It remains questionable, though, if sucking intensity can act as a reliable signal of need as the energetic cost of sucking seems low. In contrast to sucking intensity, gaining access to the teats to initiate a nursing bout may be seen as a costly signal of need (Wells 2003). In precocial, and also in many altricial mammals once they become mobile, young initiate all or most nursing bouts while the control over the success of such a sucking attempt probably lies with the mother as she can block access to teats, avoid pups or chase them away (Reisbick et al. 1975: rats; König 1985: guinea pigs; Houpt and Boyd 1994: Przewalski’s horse; Alley et al. 1995: goats; Künkele & Hoeck 1995: Galea musteloides). This may make sucking attempts a costly signal of need and maternal supply may be adjusted to the offspring’s persistence which should vary with demand. Mothers may initiate weaning by becoming less responsive to these attempts and thus further increasing these costs. In precocials, the situation may be quite different from that in altricials as offspring feed themselves besides soliciting parental care. In this case, solicitation may incur an opportunity cost not known to altricial offspring and the decision to beg or self-feed may follow the rules of optimal foraging.

The guinea pig is an ideal model to study the effects of supply on demand and vice versa in a precocial mammal. Demand and supply can be manipulated by cross-fostering pups of domestic guinea pigs (*Cavia aperea* f. *porcellus*, referred to as guinea pig: GP) with pups of their ancestral form, the cavy (*Cavia aperea*, referred to as cavy: C). GPs are heavier than Cs (GPs: 900g, Rowlands 1949; Cs: 515g, Rood 1972), they give birth to larger litters (GP: 3.7 pups per litter, König 1985; C: 2.2 pups per litter, Rood 1972), and mean pup birth mass is higher (GP: 99.9 g, König 1985; C: 60 g, Weir 1974). Mothers accept pups of the other kind just as well as foster pups of their own kind. Weaning occurs much earlier in Cs (day 16 of lactation, Trillmich et al. 2006) than in GPs (day 29, Rehling & Trillmich subm.). According to the smaller adult size of Cs, and to their smaller mean litter size at birth, we assume supply and demand to be lower for Cs than for GPs, as energy output in milk and milk yield are positively correlated to female body mass (Hanwell & Peaker 1977) and to litter size (Oftedal 1984). In contrast to other mammals (rats: Bruce 1958, goats: Linzell and Peaker 1971; pigs: Algers et al. 1991, humans: Cowie 1984) GPs seem to follow a milk yield pattern that varies little in response to sucking stimulus (Davis 1979; Anderson et al. 1984, Rehling & Trillmich subm.). Cross-fostering thus creates a low supply situation for GP-pups raised by C-mothers and a high supply situation for C-pups raised by GP-mothers.

We here tested the models on the effects of demand and supply in precocial GPs and Cs and made the following predictions: (1) If gaining access to the teats represents solicitation as suggested by Wells (2003) and the function of the effect of supply on demand is negative as suggested by Hussel (1988), then pups in a low supply situation (GP-pups fostered to C-mothers) should try to initiate more bouts (successfully or not), and pups in a high supply situation (C-pups fostered to GP-mothers) should try less to initiate bouts. (2) The effect of demand on supply should be positive. We therefore predicted females to adjust to high demand (C-mothers raising GP-pups) by an increase, and to low demand (GP-mothers raising C-pups) by a reduction in nursing performance. In particular, females should adjust the timing of weaning to pup demand. (3) Initially demand is high and should decrease with the offspring's increasing ability to look after themselves. The number of sucking attempts is therefore predicted to decrease over lactation. (4) If mothers and pups disagree over the timing of weaning, maternal responsiveness should decrease towards the end of lactation leading to conflict behaviour, i.e. we expect an increase in maternal aggression as well as in unsuccessful attempts by pups. (5) Conflict behaviour should be more pronounced for pups weaned early than for pups weaned late compared to normal conditions.

Methods

Animal subjects and experimental design

Twenty outbred short-hair guinea pigs and twenty cavies of the breeding stock of the University of Bielefeld (for details see Trillmich et al. 2004) were used for the experiment. Pellet food (Höveler, Langenfeld, Germany) and water were provided *ad libitum*. Drinking water was supplemented with vitamin C once weekly. Hay was fed daily and carrots were added to the diet every second day. Wood chips were used for bedding. The temperature was maintained at 21°C. The photocycle was 14 hours light and 10 hours dark. During pregnancy, two females were kept together in enclosures with 0.8 m^2 floor space. From about 14 days before parturition they were housed individually in these enclosures. The following experimental groups were created by cross-fostering pups on their first day of life: The GP control group, consisted of guinea pig females with guinea pig pups "GPgp" (n=10). The "GPc" group (n=9) consisted of guinea pig females to which cavy pups were fostered; one of initially ten females had to be excluded because of an inflamed teat). The "Cgp" group (n=10) consisted of cavy females with guinea pig pups. All litters were adjusted to three pups (if necessary supernumerary pups where fostered to lactating females not used in the experiment or same aged additional pups where fostered to the litter).

The "Cc" group (n=10), consisted of cavy females with their original litters (2 to 4 pups per litter, mean litter size: 2.8 ± 0.6). Only behavioural observations were carried out in this group. Neither GP females of the GPgp and the GPc-group nor C females of the Cgp and the Cc-group differed in parity, original litter size, and litter mass (all one-way ANOVAS): Parity (all groups compared): F(3, 35)=2.171, p=0.109. Maternal mass: among GP-mothers: F(1, 17)=1.346, p=0.262; among cavy mothers: F(1, 18)= 0.012, p=0.915. Original litter size: among GP-mothers (GPgp and GPc group): F(1, 17)=1.368, p=0.258; among cavy mothers: F(1, 18)=0.450, p=0.511.

Observed traits

Behaviour was observed on day three (day one=day of parturition) and on every third day until weaning. On every observation day four 15 minute observations with at least 30 minute intervals between sessions were carried out. Beginning on day 16 for C-mothers and on day 19 for GP-mothers additional observations (2 times 15minutes) took place on the days between these observation days to determine the exact day of weaning.

We measured the total number of sucking attempts by pups. Each nursing bout was counted as a successful attempt. Nursing can be recognized by a specific nursing position, where mothers crouch motionless, enabling the access to the teats (Kunkel & Kunkel 1964; Hennessey & Jenkins 1994). Nursing was recorded, when females took on this position for more than 30 seconds. Unsuccessful attempts were recorded when pups tried to push mothers into nursing position by squeezing underneath her ventrum and the mother did not take on that position for at least 30 seconds and also when pups ran towards mothers and were chased away or were avoided by the mother.

Milk yield and quality were measured to allow conclusions about physiological adjustments of maternal care. by Guinea pigs were milked by hand for 10 minutes after a 50 minute separation from their pups on days 4, 8, 12 and 16. For milking the female was sat on a towel and drops of milk were elicited by gently massaging her teats. The milk was taken up with a disposable micro pipette (200µ, Ø 1.4mm). While the guinea pigs in our lab are used to handling and did not seem to be stressed by the procedure, the cavies are not. We therefore did not milk cavies. Milk samples were weighed to the nearest 0.0001 gram to determine the milk yield (corresponding to expressible milk production over one hour). Samples were stored at -20°C and later analysed for fat content. 0.6 ml were needed for the analysis. Usually no more than 1 ml of milk was obtained per milking. This precluded duplicate measurements. Standardization was done with cow milk samples. Repeated measurements of cow milk samples produced an average intra-assay coefficiency of variation of 1.1%. Fat content was analysed using nuclear magnetic resonance spectroscopy. Samples were recorded on a Bruker FT-NMR spectrometer Avance 600 at a proton-resonance frequency of 1H: 600.13 MHz characteristic for hydrogen atoms in fatty acids. A solution of

D2O and Pyridine was used as a standard (50.1g : 3.01g). Measures were taken over all aliphatic protons of fatty acids and all pyridine protons (Weber & Thiele 1998).

Milk intake was measured on days 5, 9. 13 and 17. We used the time-nurse method described by Mepham & Beck (1973). Pups were separated from mothers for one hour (not 2-4 hours as in Mepham & Beck's experiment to avoid inadvertent weaning), in which the pups were deprived of food and water. Pups and mothers were then weighed to the nearest 0.1 g and placed together in a macrolone box with a grid bottom for 20 minutes and then weighed again. Mass difference was used as an index for milk production over 80 minutes. The actual milk yield is higher than the measured mass increase, as individual pup mass decreases by about 0.3 g over 30 minutes due to water loss by exhalation and insensible water loss through the skin (Hille 1993). Feces of pups could easily be distinguished from maternal feces by their size and were added to the final litter mass. Measurements in which urine was excreted were excluded (31 of 116).

As measures of nursing behaviour we recorded the number or successful nursings, the time mothers spent nursing their pups and the timing of weaning. Following Laurien-Kehnen & Trillmich (2003, 2004) weaning age was defined as pup age on the first of three subsequent days on which the female had not been seen in nursing position. Towards the end of lactation we checked daily if milk could still be expressed manually to ensure that weaning was not a consequence of lack of milk. Milk colour changed from a saturated opaque white to a transparent colour and stayed like that thereafter. This change in colour proved to be a more reliable indicator to define the end of milk yield than dry teats – as watery drops reoccurred after the teats had been dry for several days.

We recorded events of maternal aggression and the number of unsuccessful sucking attempts. As the total number of sucking attempts may be higher when pups are younger and more dependent on milk, the absolute number of unsuccessful attempts might not differ between begin and end of nursing period, yet mothers might be more responsive to their pups in the beginning of the nursing period. We therefore further compared the proportion of unsuccessful attempts in the beginning and at the end of lactation.

Statistical analysis

Data was analysed with SPSS (version 12.0 for Windows). Sample size was defined by the number of females.

Original litter size may correlate with maternal condition . However, no influence on any of the measured traits was found and we therefore did not include original litter size as a random factor in the analysis. Mixed linear models with two main factors (kind of mothers and kind of pups) were used to analyse milk yield, -intake, -fat content, and pup mass. A 2factorial ANOVA (factors: kind of mother, kind of pup) was used to compare timing of weaning. For the analysis of behavioural changes (time spent nursing, absolute number of sucking attempts, proportion of unsuccessful attempts, number of feedings) we used the data collected during the first three observation days (day 3, 6 and 9) and the three days before weaning. These periods were chosen for comparison as the duration of nursing periods varied greatly and we assumed that the weaning process would not influence behaviour in any of the groups during the early period, but would become noticeable during late lactation. We used 2factorial repeated measures ANOVAS with two repeats: early and late lactation. Normal distribution was tested by graphical methods and Levene tests were carried out to test for homogeneity of variance. For lack of homogeneity of variance a Wilcoxon test was used to determine if aggression in the end of lactation differed from aggression in the beginning of lactation.

Ethical note

Guinea pigs are highly precocial and thermoregulate entirely autonomously right after birth (Brück 1970). Thus, when separated from their mothers at room temperature they are well able to maintain body temperature. Moreover, under natural conditions, the highly mobile cavy pups may well become separated from their mothers for short periods. For separation from their mother pups were placed in a grid box (40x60cm, provided with bedding and a hut) that was placed next to the home box. Consequently, mother and pups could always keep in contact with each other. Furthermore, pups were never alone, as they were always kept together with their littermates. No freezing or other evidence for stress was noted. In contrast many young spent a lot of the separation time resting, indicating that they did not judge their situation as dangerous.

No adverse effects of food deprivation were found. Although guinea pigs feed throughout the day, main feeding time in the lab are the morning hours when the animals have been provided with fresh hay and carrots. Experiments were not done in the hour following the feeding of the animals. In the lab and probably also under natural conditions long intervals between feeding bouts occur. The experimental food-deprivation was well in the normal range of theses intervals.

Results

Milk supply and milk intake

In agreement with our expectation, GP- as well as C-pups gained significantly more mass during the first 17 days (when in none of the groups pups were weaned) when nursed by GP-mothers than when nursed by C-mothers (Fig. 1; mixed linear model: GP-pups: F(1, 249)=43.31; p=0.000; C-pups: F(1, 190)=43.21; p=0.000). Thus the bigger GP-mothers (mean 810±130 g) produced more milk than the much smaller C-mothers (mean 501± 87 g). However, our measurements did not show significant differences in milk intake in timed nursing bouts (Mixed linear model (2factors: group, day): F(2, 24.02)=1.274; p=0.305); Tab. 1). For GP-mothers, milk yield as measured by hand milking and milk fat content did not differ whether GP-mothers raised C- or GP-pups (mixed linear model (2 factors: Group, day): Milk fat content: F(1,15.41)=3.19; p=0.094); Milk yield: F (1, 17.80) =0. 032; p=0.860; Tab.1). Thus, mothers did not react demonstrably to pup demand by changing the quality or quantity of milk.

Tab. 1: Effects of cross-fostering on milk quality (% fat), milk yield (g per 10 min hand milking) and milk intake by pups (during 20 min with the mother). None of the differences between groups are significant. Values are given as means ±SD. Milk fat content: mixed linear model (2 factors: Group, day): F(1,15.41)=3.19; p=0.094); Milk yield: mixed linear model (2 factors: Group, day): F (1, 17.80) =0. 032; p=0.860); milk intake: mixed linear model (2 factors: Group [including 3 groups], day): F(2, 24.02)=1.274; p=0.305).

Milk fat content (%)	GPgp	6.08±2.88
	GPc	7.66±3.31
Milk yield (g)	GPgp	0.41±0.19
	GPc	0.42±0.18
Milk intake (g)	GPgp	1.37±1.86
	GPc	1.80±1.72
	C gp	1.09±1.56

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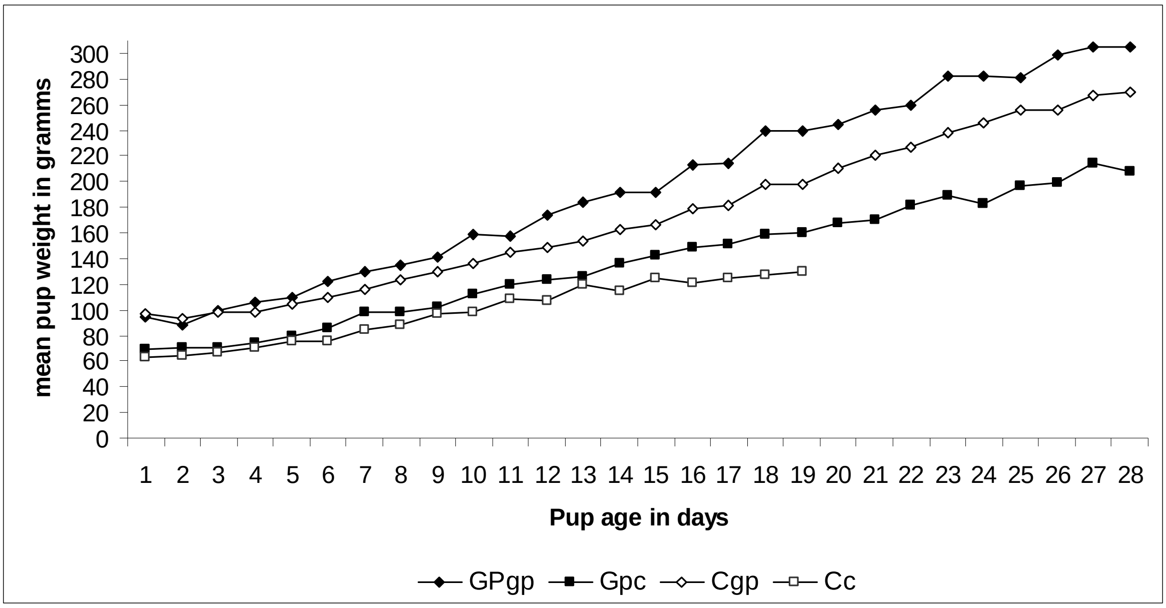


Figure 1: Pup growth over the lactation period. Values given are means of litter means.

Sucking attempts

We measured the total number of sucking attempts of pups reared by mothers of the same or the other type. The absolute number of sucking attempts was influenced by offspring demand and by maternal supply. If pups indicated their demand through sucking attempts low demand pups should show less sucking attempts than high demand pups raised by the same kind of mothers. Indeed, more attempts were found in GP-mothers caring for GP-pups than in GP-mothers caring for C-pups and similarly C-mothers raising GP-pups faced more attempts than C-mothers raising C-pups (Tab.2).

To test our first hypothesis that pups in a low supply situation should try to initiate more bouts we compared the total number of the pups' sucking attempts. Pups raised in a high supply situation, i.e. by GP-mothers, showed more attempts than those raised in a low supply situation, i.e. by C-mothers (Tab. 2). Sucking attempts were divided into those that led to successful nursing bouts and those that remained unsuccessful. The number of successful nursing bouts depended on the total number of attempts pups initiated and on maternal responsiveness to these attempts, i.e. the proportion of successful feedings resulting. Overall, the proportion of successful and unsuccessful feedings did not differ between groups (Tab. 2, Fig. 2a). An increase in absolute number of sucking attempts thus resulted in more unsuccessful attempts (Fig. 2b) but also in more successful nursing bouts for C-pups when raised by GP-mothers than for those raised by C-mothers. In early lactation, however, the proportion of unsuccessful attempts in the Cgp-group was significantly lower than in any other group (Tab. 2, Fig. 2a). Therefore, no difference in the number of successful nursings was found for GP-pups raised by different kinds of mothers, although GP-pups raised by C-mothers showed fewer sucking attempts (Tab. 2).

The time spent in nursing position was clearly affected by pups as GP- and C-females raising GP-pups spent more time in nursing position than those raising C-pups. Neither C- nor GP-pups differed in the time spent sucking, when reared by different kinds of females (Tab. 2). According to these data, mothers of both types reacted to the increased number of sucking attempts by the larger and more demanding GP-pups by increasing total nursing time.

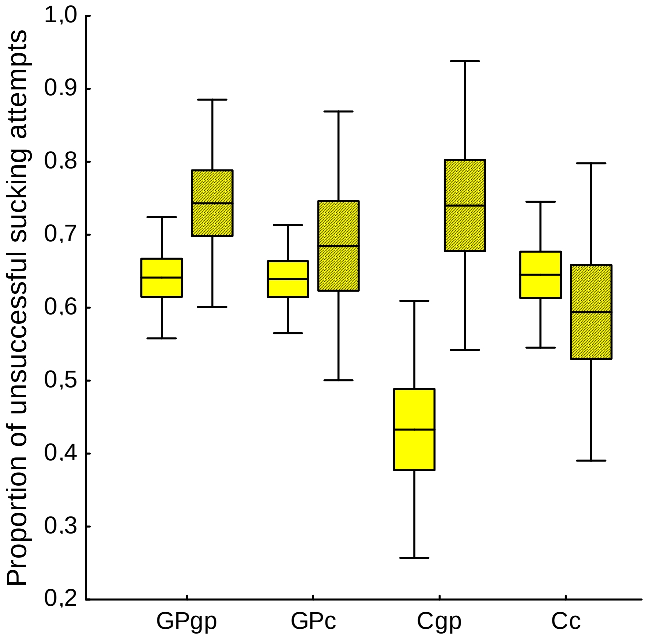
		GPc	GPgp	Cc	Cgp	maternal influence	offspring influence
Weaning		28.7±3.8	29.7±2.6	18.6±2.0	21.5±3.7	F(1,36)=82.734 p=0.000	F(1,36)=3.957 p=0.054
Absolute sucking attempts	begin: end:	2.96±0.630 1.81±0.867	3.60±1.117 2.33±1.172	1.69±0.347 1.07±0.576	2.59 ±0.899 2.29 ±1.203	F(1,36)=13.375 p=0.001	F(1,36)=15.816 p=0.000
Unsuccessful sucking attempts	begin: end:	1.96±0.53 1.31±0.88	2.31±1.09 1.86±1.23	1.11±0.34 0.69±0.55	1.25±0.90 1.70±1.137	F(1,36)=10.984 p=0.002	F(1,36)=6.366 p=0.016
Successful sucking attempts	begin: end:	1.00±0.165 0.49±0.225	1.27±0.207 0.46±0.119	0.58±0.130 0.38±0.131	1.34±0.233 0. 59±0.55	F(1,36)=1.427 p=0.240	F(1,36)=21.322 p=0.000
Successful sucking attempts (proportion)	begin: end:	0.35±0.060 0.32±0.185	0.38±0.096 0.26±0.142	0.36±0.100 0.41±0.203	0.57±0.1770.2 6±0.196	F(1,36)=3.586 p=0.066	F(1,36)=0.066 p=0.800
Time spent nursing in seconds	begin: end:	337±88.5 133± 93.8	503±89.8 165±76.7	359±83.9 114±45.5	506±94.4 134±75.9	F(1,36)=0.119 p=0.732	F(1,36)=22.710 p=0.000

Table 2: Effects of cross-fostering on nursing. Values are given as means per observation ± SD. For weaning: 2factorial ANOVA (factors: kind of mother and kind of pups), for all other traits: 2factorial repeated ANOVA (factors: kind of mother and kind of pups, repeats:2 (phase: begin and end). Interactions between maternal and pup influence were non significant except for successful sucking attempts (maternal influence*pup influence: F(1,35)=9.520, p=0.004).

Weaning behaviour

The total number of sucking attempts was lower (Tab. 2) and the proportion of unsuccessful sucking attempts was significantly higher during the last three days before weaning than during the first 9 days of lactation (Tab. 2, Fig. 2a). Interestingly, the number of unsuccessful sucking attempts declined in all groups towards the end of lactation except in the C-mothers raising GP-pups (Tab. 2, Fig. 2b). This may indicate that GP-pups indeed resisted to some extent the early weaning occurring when they were raised by C-mothers. In all groups sucking attempts were observed in the days after weaning. Maternal aggressiveness was significantly higher during late than during early lactation (Wilcoxon; N=39, T=13, p=0.00003; Fig. 3) and did not differ at weaning whether pups were weaned early or late (GPgp – GPc: Wilcoxon; N=9, T=15, p=0.374; Cc – Cgp: Wilcoxon; N=10, T=22, p=0.575).

If mothers reacted strongly to the different demand and developmental schedule of pups we would expect weaning of C-pups by GP mothers to be much advanced in comparison to the GPgp-group and, reversely, weaning in the Cgp-group to be much delayed compared to the Cc-group. The type of pup reared tended to influence the timing of weaning. Milk production always ended about 6 days after weaning. The timing of weaning resulted in GP-pups raised by C females being weaned much earlier (-8 days) compared to those raised by GP-mothers. Conversely, C-pups raised by GP-mothers experienced a major extension of the nursing period (+10 days) (Tab. 2; Fig. 4).



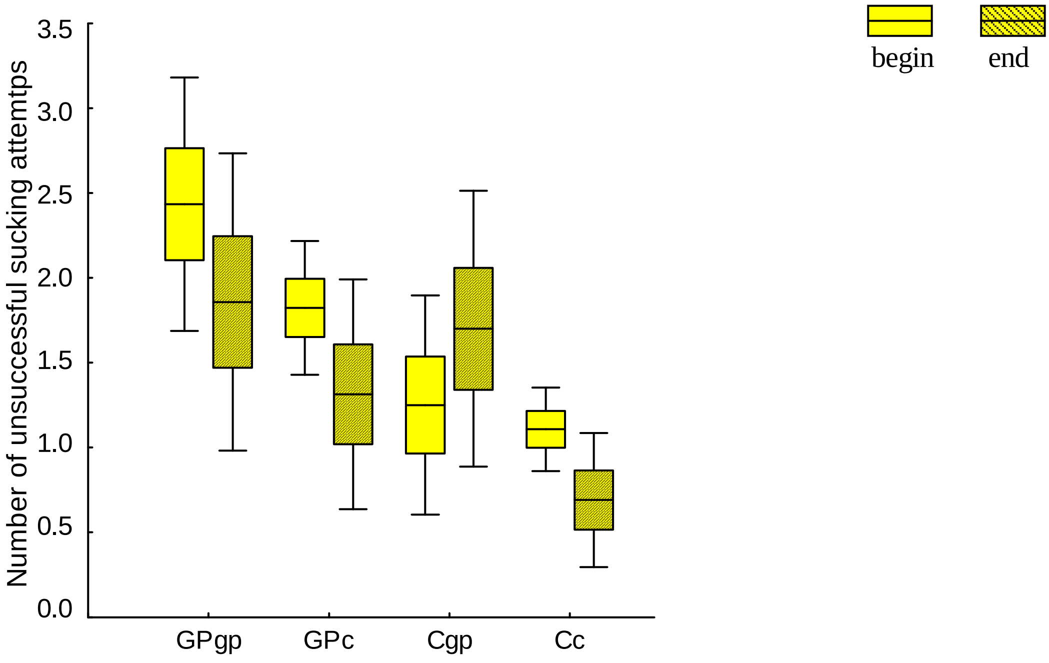
a)

Figure 2:

a) Proportion of unsuccessful sucking attempts per 15 min observation in the early lactation period (begin) and in last the three days before weaning (end).

b) Mean number of unsuccessful sucking attempts for the same times.

Values given as mean ± SD (whiskers) and SE (boxes).**)**



b)

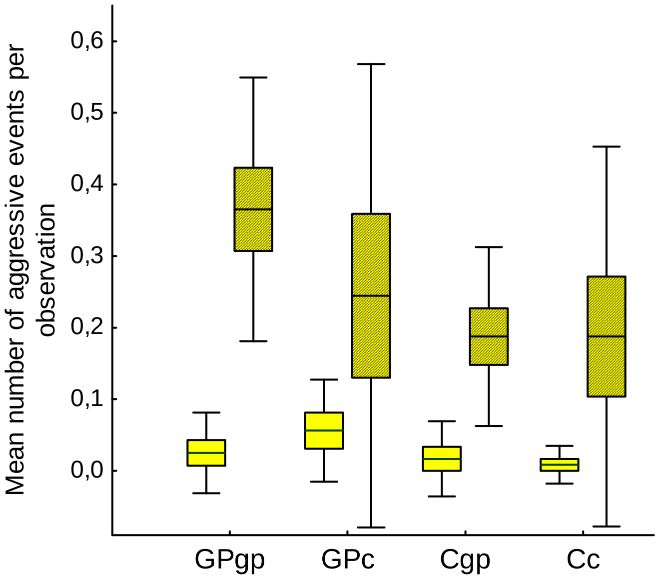


Figure 3: Number of aggressive events by the mother per 15 min observation in the early lactation period (begin) and in last the three days before weaning (end). Values given as means ± SD (whiskers) and SE (boxes).

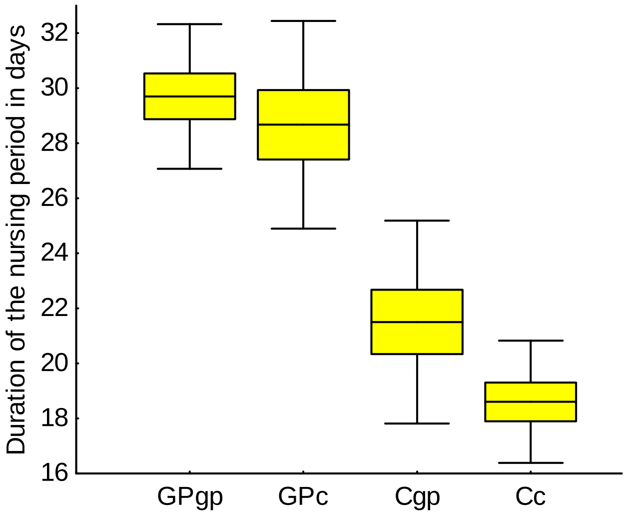


Figure 4: Age at weaning for the four combinations of mothers and offspring. Values are means ± SD (whiskers) and ± SE (boxes).

Discussion

In agreement with our assumption that GP-mothers yield more milk than C-mothers, pups nursed by GP-mothers gained significantly more mass than when nursed by C-mothers, indicating that milk supply was indeed higher in GP-mothers than in the much smaller C-mothers (Fig. 1). This result also shows that mothers limit the amount of milk supplied throughout lactation as pups take more milk if given the opportunity. The time-nurse method did not allow to detect the milk yield differences as variance of the measurement was high. In addition, the normal milk yield available to pups over one hour is likely higher than the yield measured in a single nursing bout after a one hour separation due to limited storage capacity of the milk gland. In any case, the effect clearly showed up in the higher growth rates of pups when reared by GP-females.

Following Wells (2003) we assumed that the frequency of sucking attempts may be taken as a measure for solicitation behaviour. As solicitation must increase with increasing offspring demand (Parker & MacNair 1979; Hussel 1988; Godfray 1991, 1995) high demand pups should show more sucking attempts than low demand pups. Indeed, independent of the type of mother GP-pups attempted more frequently to suck than C-pups. A high number of attempts led to a high number of successful nursing bouts and resulted in more time spent nursing. (Fig. 2a, Tab. 2). This was due to the proportion of successful attempts, which did not differ between groups (except C-mothers with GP-pups showed a higher proportion than any group, yet only during early lactation). However, the number of sucking attempts may not be the only cue mothers use to estimate pup demand. The increased proportion of success during early lactation in the Cgp-group (Fig. 2a) resulted in GP-pups raised by C-mothers enjoying a higher number of successful feedings and spending more time nursing than GP-pups raised by GP-mothers, even though they showed a lower number of sucking attempts. Thus, besides the frequency of attempts mothers must use other cues from their pups e.g. vocal cues or sucking intensity to judge their offspring’s demand.

Solicitation is predicted to be honest in that it relates to the level of demand. To maintain honesty, solicitation must be costly (Godfray 1991, 1995). Consistent with honest signalling theory C-pups did not increase the number of attempts to elicit as many successful feedings as GP-pups raised by the same kind of mother (Tab. 2). It seems unlikely, though, that the additional energetic costs incurred in only a few more sucking attempts (GP-pups nursed by C-mothers showed only 3.6 more sucking attempts per hour than C-pups) could outweigh the benefits of extra nursing bouts (GP-pups nursed by C-mothers had 3.0 more successful nursing bouts per hour (calculated from Tab. 2). We therefore conclude that other costs must be involved. Although Wells (2003) suggested that sucking itself is energetically not very expensive – thus should be a dishonest signal – sucking may incur opportunity costs as it reduces the time budget for other activities. This could play an important role in precocial mammals where sucking may reduce the opportunity to search for and eat solid food.

Effects of supply and demand

Parker & MacNair (1979) assumed a negative effect of supply on demand, while Godfray (1991, 1995) assumed the slope of the function to be zero. In contrast to both predictions pups raised by GP-mothers, providing more plentiful resource, showed more sucking attempts than those raised by cavy mothers (Tab. 2). This indicates a positive effect of supply on demand, driving solicitation by pups. Solicitation may increase when it is positively reinforced by parents as found in budgerigars (Stamps et al. 1985). As the higher milk yield in the GP-mother groups is more rewarding, pups may be encouraged to try to suck more often, while pups in the Cgp-group do not gain more milk by more frequent sucking when the milk glands have been depleted.

The alternative to forage for solid food may also explain the positive effect of supply on solicitation. In highly precocial mammals that feed on milk as well as on solid food from very early on, the decision to suck may be a case of optimal foraging: both food sources are important for pup development (Künkele 2000), however, once a sufficient intake of both has been achieved, pups may prefer the source that is easier available or supplies them with more energy per unit time. A high supply may outweigh the costs of missed foraging opportunities while pups in a low supply situation may decide to spend more time foraging on solid food. A study on foraging activities and food intake could serve to test this hypothesis. Furthermore, a positive relationship of supply and solicitation should not be found in altricials that do not have the alternative of self-feeding. Here cross-fostering two closely related altricial mammals that differ in demand and supply could provide further insights.

If the positive effect of supply on solicitation can also be found under more natural conditions where milk yield will often correlate with environmental quality needs further investigation. In contrast to our results correlational studies in large precocial mammals demonstrated that in low quality environments mothers had low milk yields and sucking frequency was raised (Loudon & Kay 1984; Fairbanks & McGuire 1995; Lycett et al. 1998; Sarno & Franklin 1999) In a poor environment milk yield may be low, but at the same time self-feeding in this environment where food is scarce or of low quality may not compensate for sucking.

Supply should be positively affected by demand according to models (Parker & MacNair 1979; Hussel 1988; Godfray 1991, 1995). In contrast to the findings in many other mammals, we did not find maternal adjustments to pup demand in milk yield and fat content of milk (Tab. 1). The proportion of success was not affected by cross-fostering, except for C-mothers with GP-pups early in lactation, when the proportion of success was higher than in all other groups (Fig. 2a). Regarding the frequency of nursing bouts and the time spent on nursing the effects were as predicted: Mothers with GP-pups confronted more sucking attempts and – due to the generally unchanged proportion of success – had a higher nursing frequency and thus spent more time in nursing position than mothers with C-pups. The pro rata model by Parker & MacNair (1979) that suggests parental investment to be paid in proportion to solicitation level fits well with the finding that parents respond to a certain proportion of attempts.

Confirming our previous experiment (Rehling & Trillmich subm.) adjustments in the duration of the nursing period were small. However, we could show that weaning was delayed by 3 days when C-mothers raised GP-pups indicating that mothers received information about pup demand and adjusted to it to a limited extent.

With increasing pup independence solicitation behaviour should become less cost effective and decrease over the nursing period (Davies 1978). In accordance with this prediction less sucking attempts were found late than early in lactation. However, cavy pups nursed by GP females sucked far beyond normal weaning age (nursing period 50% longer than normal) whereas for GP-pups the duration of lactation was decreased (by 28%). The results indicate that mothers and pups disagreed over the timing of weaning. Therefore, mothers may artificially increase the costs of solicitation to speed up the transition to independence (Davies 1978; Pugesek 1990). Indeed, the proportion of unsuccessful attempts and maternal aggression increased, indicating that maternal responsiveness to solicitation declined. This was the case even when pups were weaned early relative to normal conditions. As the affordable costs of begging increase with offspring condition the intensity of squabbling should be higher when pups are weaned early than when pups are weaned late. However, we found no differences in the number of sucking attempts, the proportion of success or maternal aggression between groups.

The timing of weaning seems to be determined mainly by females (Laurien-Kehnen & Trillmich 2004) and not by the young as found in altricials, when fostering young pups to females at later stages of lactation (rats: Nicoll & Meites 1959; Reisbick et. al 1975). In contrast to GPs, altricials are completely dependent on maternal milk. In mice a comparable reduction in duration of lactation (33%) leads to high mortality rates (27%) and low pup mass (Fuchs 1981). Thus, if maternal responsiveness decreases in rats younger foster pups may still solicit food as benefits are high, while even in the early weaned GPs in this study self-feeding may outweigh the benefits of sucking when females increased the costs of solicitation.

Our findings are mainly consistent with the predictions derived from models on the effects of demand and supply (Godfray 1991, 1995; Parker & MacNair 1979). Consistent with the predictions from these models and with findings in altricial birds, high demand pups showed a high level of solicitation and mothers reacted to this with an increase in supply. However, in contrast to the predictions from both modelling approaches, offspring in high supply situations showed more solicitation., probably as a consequence of the offspring’s alternative of self-feeding. If this relationship can be found under natural conditions where maternal supply relates to the supply of solid food remains questionable, though. Like in altricial birds (Davies 1978) the communication ends as a consequence of a reduced maternal responsiveness. In the precocial GP the influence on the timing of weaning seems to be minor compared to altricials, probably because benefits of sucking past mid lactation are low.

Chapter 3: Is maternal effort state dependent? – effects of concurrent pregnancy and lactation[3]

Abstract:

Whereas in many mammals lactation reduces the probability of conception most small altricial mammals have a post partum-estrus and are often simultaneously pregnant and lactating. This may cause resource allocation problems as pregnancy and lactation overlap to a great extent in altricial rodents and lagomorphs. Influences of concurrent pregnancy on lactation and on nursing performance are commonly observed. In particular early weaning, and reduced gestational and lactational performance have been interpreted to result from high simultaneous energetic demands of gestation and lactation. The precocial guinea pig (*Cavia aperea* f. *porcellus*), like many rodents, has a post-partum estrous, yet in contrast to altricial mammals peaks in energy expenditure of lactation and gestation are widely separated. This situation allows to investigate if factors other than simple resource allocation decisions are responsible for changes occurring when lactation and gestation overlap. Here we show that females nurse less and wean earlier but do not reduce investment in the following litter when concurrently lactating and pregnant. We conclude that regulatory constraints may be more important than problems of energy allocation.

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3) Submitted

Introduction

Reproductive success depends on the number and quality of young and on the number of broods throughout a lifetime (Clutton-Brock et al.., 1989). As parental resources are limited high investment increases offspring quality but at the same time limits the number and quality of young that can be produced within a given brood or in subsequent broods (Clutton-Brock, 1991; Trivers, 1972). To maximize the number of young interbirth intervals should be short. However, this may come at the cost of reduced quality of young if uninterrupted care reduces parental condition (Verhulst & Hut, 1995).

In mammals mothers bear the costs of gestation and lactation which are critical periods of maximal energy expenditure (Gittleman & Thompson, 1988; Loudon & Racey, 1987). During gestation energy investment involves the growth of fetal, uterine, placental and mammary tissue and an increase in maintenance costs. After birth, lactation is even more costly and is of equal importance for offspring survival (Gittleman & Thompson, 1988). In many large mammals, lactation inhibits ovulation and thus the following pregnancy is delayed until after weaning of the previous litter (Gomendio, 1989; Wells, 2003). Lactational anestrus can be considered an adaptive strategy to avoid depletion of maternal resources (Marquis et al., 2002). However, under food abundance lactating females may conceive at later stages of lactation (Cowie, 1984; Merchant et al., 1990). If so, mothers must simultaneously allocate resources to the suckled litter as well as to the litter in utero. Concurrent pregnancy may therefore negatively affect nursing performance. Indeed, pregnancy has been found to result in a decrease in milk yield (Brody et al., 1923) and a reduction in the duration of the nursing period (Duncan et al., 1984; Green et al., 1993; Worlein et al., 1988). In contrast to large mammals, the majority of small rodents and lagomorphs have a postpartum estrus and conception occurs on the day of parturition (Martínez-Gómez et al., 2004, Oswald & McClure, 1987, Roy & Wynne-Edwards, 1995, Parkening & Collins, 1991 Clark et al. 2006). In altriticials, gestation is shorter than in precocials of the same size (Peters 1989) and in non-pregnant mothers lactation may even be longer than the gestation period. Thus, the two phases largely overlap. Peak energy expenditure on lactation and gestation coincide towards the end of both phases in altricial small mammals such as mice (Johnson et al., 2001) and rats (Oswald & McClure, 1987). A delay in blastocyst implantation as documented for mice and rats (Fuchs, 1982; Mantelenakis & Ketchel, 1966, Oswald & McClure, 1990; Woodside et al., 1981) can reduce the overlap of peak energy demands. Nevertheless, lactation and pregnancy overlap to a great extent. The effects on the litter in utero are controversial: second litters are smaller in woodrats (Oswald & McClure 1990) and may be sacrificed, when the energetic squeeze is high as found in Norway rats (Woodside et al., 1987). In rabbits fetal survival and weight of second litters were also reduced and these effects were related to the size of the suckled litter (Fortun-Lamothe et al., 1999). However, Martínez-Gómez et al. (2004) found no evidence for lower birth mass or higher mortality in pups of lactating rabbit does under ad libitum conditions. Mainly negative effects on the suckled litter have been documented: pregnant mothers had lower milk yields (Bruce, 1958: mice; Xiccato, 2005: rabbits) or the milk yield declined more rapidly towards the end of lactation (Partridge et al., 1986: rabbits). Pups where weaned at an earlier age than those of non-pregnant mothers (Bruce, 1958; Fuchs, 1981: mice; Gomendio et al., 1995: rats; Fortun-Lamothe et al., 1999; Martínez-Gómez et al., 2004: rabbits; Norris & Adams, 1981: Mongolian gerbils). In mice, offspring of pregnant mothers were smaller at weaning than those of non-pregnant mothers (Bruce, 1958; König & Markl, 1987).

All of these effects can be interpreted as due to an allocation problem of energy limited mothers leading to a cost of reproduction in terms of reduced investment in a subsequent litter. However, it has not been shown directly that the effects described above are due to energetic limitations rather than being correlates of regulatory changes in the maternal physiology due to parallel processing of the differing demands of pregnancy and lactation. If regulatory constraints determined maternal options this should show up in a situation in which energetic limitations are less likely to be directly causal for the effects described. Precocial rodents that have a postpartum estrus offer a model system for studying the effects of concurrent lactation and pregnancy without the almost complete overlap observed in altricial rodents because these species wean offspring long before energetic costs of gestation become important. In the precocial domestic guinea pig (*Cavia aperea* f. *porcellus*) the gestation period is much longer (68 days, Rowlands 1949) than the lactation period (29 days for non-pregnant females, Rehling & Trillmich, subm.). The shape of the milk yield curve resembles that of precocial ungulates rather than that of altricial rodents:

peak milk yield is reached early in lactation between day 5 and 10 after parturition (Anderson et al., 1984; Mepham & Beck, 1973). An increase in energy intake of pregnant females can first be shown around day 30 of gestation (Künkele, 2000), that is after weaning previous young. Thus, peaks of energy demand do not overlap between lactation and gestation and energetic constrains are unlikely in guinea pigs kept under ad libitum conditions. Influences of gestation on nursing performance in this species would suggest that delays in pregnancy and negative effects on nursing and future reproduction found in other species may not be exclusively caused by energetic constraints.

To document such primarily non-energetic constraints we compared maternal effort in pregnant and non-pregnant guinea pigs under ad libitum conditions. From the findings for small rodents with post partum estrus we predicted (1) the quality and amount of milk to be lower for pregnant than for non-pregnant mothers and nursing behavior to be less intense, (2) weaning to occur earlier in pregnant than in non-pregnant mothers, and (3) that the reduction in nursing performance negatively affects the condition of the suckled litter, and (4) lactation to have a negative effect on the size and the development of the litter in utero.

Methods

Animal subjects and experimental design

32 outbred, short-haired, adult domestic guinea pigs (*Cavia aperea* f. *porcellus*) of the breeding stock of the University of Bielefeld were paired for the experiments. The females had not been pregnant for at least 3 months prior to the experiment. For the first mating two females and one male were kept in enclosures with 0.9 m² floor space. Pellet food (guinea pig chow, Höveler, Langenfeld, Germany) and hay were provided ad libitum and carrots were fed additionally every day except on weekends. Drinking water was provided ad libitum and supplemented with vitamin C once weekly. Temperature was maintained at 21°C. The photocycle was 14 hours light and 10 hours dark. Wood chips were used for bedding. Females were randomly assigned to two treatment groups: "non-pregnant females" that were separated from the male about two weeks before parturition and "pregnant females" that were left with the male until the second day after parturition to allow post-partum mating. Females that did not conceive, failed to give birth and those whose litter size could not be adjusted to three pups were excluded from the experiment. Also females initially assigned to the pregnant group that did not conceive post partum were excluded. This left a sample size of 8 females in the non-pregnant and of 15 females in the pregnant group. Of these initially 15 pregnant mothers 5 were not observed until weaning for time limitations. However, for analysis of maternal mass and offspring mass and litter size at the following parturition these five females were included. Females in both groups did not differ significantly in parity, age, maternal mass, and first litter size (one-way ANOVA; parity: F(1,21)=1.513, p=0.232, age: F(1,21)=0.063, p=0.804, maternal mass: F(1,21)=0.928, p=0.346, litter size: F(1,21)=0.585, p=0.453. After parturition, all litters were adjusted to three pups. If necessary this number was achieved by fostering surplus pups to lactating females not used in the experiment or by complementing litters with same-aged pups. From the second day after parturition onwards all females were housed individually with their litters, each in an enclosure of 0.9 m^2.

Observed traits

Maternal nursing behavior can be recognized by a specific nursing position, where mothers crouch motionless, enabling pups to access the teats (see Hennessey & Jenkins, 1994; Kunkel & Kunkel, 1964,). Nursing was recorded, when females took up the nursing position for more than 30 seconds. To determine influences of concurrent pregnancy on nursing performance behavioral observations where carried out on day 3 (day 1 = day of parturition) and on every third day from then on until weaning. On each observation day, 4 observations of 15 minutes each with at least 30 minutes intervals between sessions were made, during which the time spent nursing was recorded. From day 20 onwards observations of the same length were carried out daily to determine the end of the nursing period. Weaning age was defined as the first of three subsequent days without nursing. On day 4 – that is the beginning of peak milk yield (Mepham & Beck 1973) – between 10 and 12 am, mothers were separated from their pups for 40 minutes and then milked manually for twenty minutes to determine milk yield. Samples were stored at -20°C and later analyzed for fat content. 0.6 ml were needed for the analysis. Usually no more than 1 ml of milk was obtained per milking. This precluded duplicate measurements. Standardization was done with cow milk samples. Repeated measurements of cow milk samples produced an average intra-assay coefficiency of variation of 1.1%. Fat content was analyzed using nuclear magnetic resonance spectroscopy. Samples were recorded on a Bruker FT-NMR spectrometer Avance 600 at a proton-resonance frequency of 1H: 600.13 MHz characteristic for hydrogen atoms in fatty acids. A solution of D2O and Pyridine was used as a standard (50.1g : 3.01g). Measures were taken over all aliphatic protons of fatty acids and all pyridine protons (Weber & Thiele, 1998). From day 24 onwards we checked daily if milk was still available by trying to milk a drop from both teats. The day when the milk changed to a watery, less opaque consistency, was taken as the end of milk yield.

To study influences of concurrent pregnancy on pup development and on maternal condition pups were weighed daily and maternal weight was determined at parturition and at weaning. A potential influence of concurrent lactation on the litter in utero, was studied by comparing original litter size, pup mass at birth and pup mortality between first and second litters.

Statistics

Data was analyzed with SPSS (version 12.0 for Windows). Sample size was defined by the number of litters (pregnant group, n = 10, non-pregnant group n = 8). Effects of original litter size cannot be excluded. We therefore used factorial ANOVAs with a mother's original litter size as random factor to analyze the effects of pregnancy on the length of the nursing period. We compared the time spent in nursing position until day 24 or until the day before weaning, if weaning occurred before day 24, using a linear mixed model. Mean pup weights within a litter were used to analyze pup growth. Growth rates were analyzed for the period from day 3 to day 20 (as the initial presence of a male might have affected nursing behavior on the first day and the first animals were weaned at day 20). We used a repeated measures ANOVA. For the comparison between first and second litters the Wilcoxon test or a paired t-test were used for the analysis, depending on normality of the data.

Results

Effects of concurrent pregnancy on nursing performance and the development of suckled litters

No significant differences were found between pregnant and non-pregnant mothers for milk yield and fat content of milk. Pregnant mothers spent less time in nursing position (Fig. 1; Tab. 1) and weaned pups significantly earlier than non-pregnant mothers (Fig. 2; Tab. 1). Milk flow ended significantly earlier for pregnant than for non-pregnant mothers (Tab. 1) and continued for another 6 days after weaning in pregnant and non-pregnant females alike (Tab. 1). Concurrent pregnancy had no detectable influence on pup growth between day 3 and 20 when none of the pups were weaned (Tab. 1). Weaning weights did not differ significantly between groups (Tab.1).

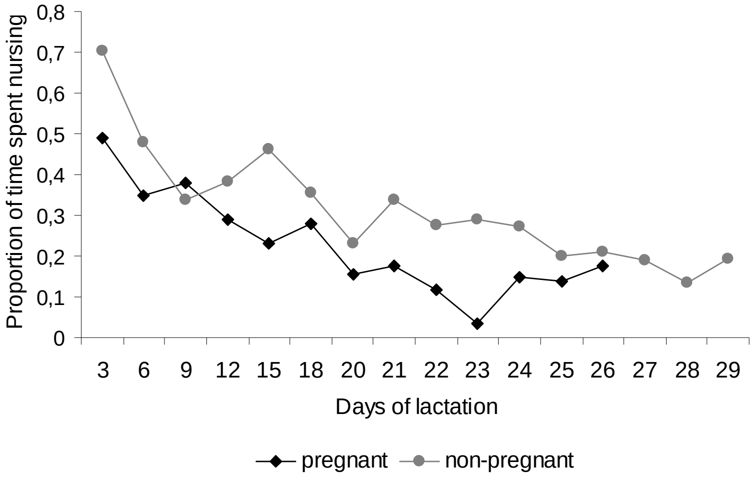

Figure 1: Mean proportion of observation time (1 h/day) spent nursing throughout the nursing period.

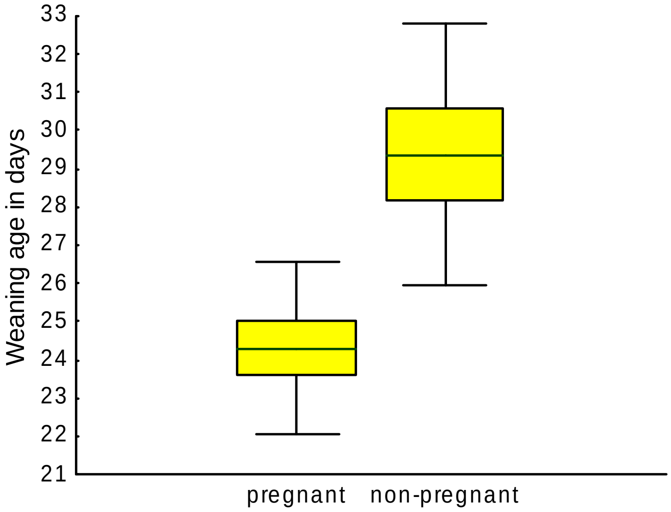

Figure 2.: Time of weaning for pregnant and non-pregnant mothers. Pregnant mothers weaned their pups significantly earlier than non-pregnant mothers. Means, SE (boxes) and SD (whiskers).

	Pregnant mothers	Non-pregnant mothers	
Duration of nursing period (in days)	24.3±2.3	29.3±3.4	Main effects ANOVA with original litter size as second factor: F(1,14)=10.947, p=0.005
Mean time spent in nursing position per 1h observation (between day 3 and day 23) in seconds	705.8±527.4	1041.3±662.5	Mixed linear model (fixed factors: group, day, litter size): F(1,14)=7.489, p=0.016
End of milk yield (day)	31.0±3.9	34.8±2.4	Main effects ANOVA with original litter size as random factor: F(1,14)=4.592; p=0.05
Milk yield on day 4 (grams)	0.75±0.15 (n=9)	0.72±0.15	Main effects ANOVA with litter size as random factor: F(1,13)=0.108; p=0.745
Milk fat content on day 4 (%)	10.8 ± 1.95 (N=9)	9.8±3.35	Main effects ANOVA with litter size as random factor: F(1,13)=0.914, p=0.337.
Pup growth (g/day) between day 3 and day 20	9.77 ± 4.25	9.02±4.08	Two factorial repeated measures ANOVA with litter size as second factor: F(2,14)=0.021, p=0.167
Weaning weight (g)	316.50 ± 32.07	329.44±44.96	F(1, 14)=0.307, p=0.589

Table 1: Effects of concurrent pregnancy on nursing performance and pup development: comparisons of pregnant (n=10; except where indicated otherwise) and non-pregnant (n=8) mothers. Milk samples were obtained after 40min separation during 20 minutes hand milking. Values given as means ± SD.

Effects of lactation on the condition of mothers and on the litter in utero

Both, pregnant and non-pregnant mothers lost weight during lactation and were significantly lighter at weaning than immediately post-partum ((N=18), mean difference: -85.6g±48.0; paired t-test t=7.563, p=0.0001). However, the weight of mothers in the pregnant-group at first parturition (i.e. without concurrent lactation during gestation) and at second parturition, (i.e. with concurrent lactation during gestation) did not differ significantly (Tab. 2). No abortions or stillbirths were observed in the second pregnancy. Perinatal mortality of pups did not differ between parities (Tab. 2). Total litter mass did not differ between first and second litters (Tab. 2), but the number of pups was significantly higher for second litters than for first litters resulting in lower pup mass of young in second litters (Tab. 2).

	First parturition	Second parturition	
Total litter mass (g)	345.2±50.0	395.4±19.6	Paired t-test: t=1.741, p=0.104
Litter size	3.47±0.64	4.67±1.23	Wilcoxon: p=0.0107
Mean pup mass (g)	102.6±9.7	91.0±12.4	Paired t-test: t=2.948, p=0.011
Maternal mass (g)	1031.9±93.8	990.6±112.7	Paired t-test: t=1.9895, p=0.0965
Mean pup mortality in %	13.3±25.6	10.0±16.4	Wilcoxon: p = 0.726

Table 2: Effects of concurrent lactation on gestation: comparisons of first and second parturition of a given female (N=15). Values are means ±SD.

Discussion

Early milk yield and fat content were not affected detectably by concurrent pregnancy. However, in accordance with our prediction derived from studies on small altricial mammals (Bruce 1958; Fuchs 1981; Gomendio et al. 1995; Fortun-Lamothe et al., 1999; Martínez-Gómez et al., 2004; Norris & Adams, 1981; Parkening & Collins, 1991; Partridge et al., 1986) pregnant mothers spent a lower proportion of time nursing and weaned significantly earlier. Milk flow continued for six days after weaning, indicating that it was not primarily lack of milk that forced weaning, but maternal behavior. As pregnant females had weaned earlier than the non-pregnant ones, consequently milk flow ended earlier in the former. This earlier termination of milk flow might be a direct consequence of hormonal changes due to gestation, but since in both groups weaning preceded the end of milk production by the same number of days it seems more likely that the lack of teat stimulation by sucking eventually ended milk production.

No influence of reduced nursing on pup growth could be detected and weaning weights did not differ. Either the amount of milk transferred to pups was not reduced in pregnant mothers and time spent nursing is not a good indicator for milk transfer (Cameron, 1998) or pups may have compensated by eating more solid food.. Maternal mass is known to correlate with litter mass and size (Kasparian et al., 2005; Laurien-Kehnen & Trillmich, 2004) and therefore female mass decrease during lactation may influence the development of the litter in utero. Indeed, maternal body mass was significantly lower at weaning than at parturition of the first litter. However, but pregnant mothers regained body mass within the remaining 6 weeks of gestation and at the time of the following parturition reached the same mass as at first parturition. Development of the litter in utero was not affected. Neither increased mortality nor reduced litter mass were detected in second litters. Surprisingly the number of pups per litter was even higher in litters of concurrently pregnant and lactating females, resulting in lower pup mass at birth. Analysis of earlier guinea pig data (Rowlands, 1949) supports this finding. Similar results have been found in mice (Fuchs, 1981; Johnson et al., 2001). However, the relationship of post-partum mating and litter size has received little attention so far. Fuchs (1981) suggested a positive effect of the hormonal environment of lactation on the ovulation and on the survival of fetuses. These findings support our assumption that energetic squeezes are unlikely in concurrently pregnant and lactating guinea pigs as increased energy demands of gestation only arise after the end of lactation. Reduced nursing performance of pregnant guinea pig females suggests that their nursing behavior is not primarily influenced by energetic constraints but by some intrinsic regulatory mechanism that depends on maternal reproductive state. We cannot exclude, however, that under less favorable – and probably more natural – conditions allocation problems may arise. A low conception rate of wild cavies (*Cavia apera*), the ancestral form of the guinea pig, during the winter months when food is scarce suggests that this may be the case (Kraus et al., 2005). Nutritional constraints may then influence lactation and pregnancy. A low protein diet in pregnant guinea pigs has severe effects on the development of fetal muscle fibers and on weight at birth (Dwyer & Stickland, 1994). The effects are reversible if nutritional rehabilitation starts at day 25 of gestation but remains permanent if the restriction continues thereafter (Dwyer et al., 1995). The critical time in gestation coincides with the timing of weaning in pregnant guinea pigs. Early weaning could therefore be adaptive. However, under ad libitum conditions as provided in the laboratory such constraints are unlikely.

Our finding therefore cautions against the frequently found interpretation (Oswald & McClure 1987; Gomendio et al., 1995; Norris & Adams, 1981; Partridge et al., 1986) that effects of concurrent lactation and gestation are caused primarily and directly by energetic constraints. The effects found may not result from the problem to allocate limiting nutrients to two concurrent litters. Trade-off studies should be expanded to include non-energetic aspects of resource allocation (Zera & Harshman, 2001). In this case, the hormonal control of resource allocation to the suckled litter and to the litter in utero may change maternal propensity to nurse and consequently the timing of weaning. In mammals the endocrinological environment of pregnancy may not be optimal for lactation and vice versa. Thus, independent or perhaps only little modulated by energetic constraints, regulatory constraints (Harshman & Zera, 2007) may be responsible for changed nursing behaviour and early weaning. Such an explanation of this particular life history trade-off may not only apply to the case of the guinea pig we investigated, but may also underlie the observed trade-offs in other rodents, even though in these cases observations are consistent with the explanation as a problem of energy allocation. Whether regulatory constraints or energetic constraints better explain this particular trade-off remains to be investigated.

Synthesis

Summary of the results: Differences and similarities in maternal allocation strategies and offspring begging in precocial and altricial mammals

Some features of parent-offspring interactions in the precocial guinea pig were similar to those found in altricial mammals and confirm the predictions derived from common theory on parent-offspring interactions. Like altricial mammals (rats (Pfister et al 1989) and cats (Martin 1986)) young guinea pigs did not wean themselves and disagreed with mothers about the amount of milk provided throughout lactation even though young are able to forage independently and milk contributes only little if at all to pup growth from mid-lactation onwards (Chapter 1 and 2).

Other features differed from those found in altricials, indicating that the high development at birth along with the ability to forage independently may indeed have led to the evolution of different maternal strategies for the optimization of parental care. Unlike findings in altricials where mothers reacted to stimuli correlating with pup demand, female guinea pigs show only very little adjustment to offspring demand in their milk yield, their responsiveness, and the timing of weaning. but seem to follow a fixed program that is set at parturition and only little influenced by pups (Chapter 1 and 2).

The findings on solicitation behaviour suggest that independent foraging of precocial young may affect the offspring decision to initiate nursing bouts. Although the results showed that in confirmation with common signalling models, solicitation intensity correlates with demand and positively influences maternal nursing frequency, the assumptions for the effects of supply on solicitation were not met. In precocial young that have the alternative to feed themselves, a high milk yield may reinforce sucking while a low milk yield may make the alternative to forage on solid food more beneficial (Chapter 2).

As noted in the introduction, energetic constraints of concurrent pregnancy and lactation are unlikely or at least very limited in guinea pigs as, in contrast to altricials, the peak energetic expenditures of the two phases do not coincide. However, the findings closely resembled those in altricials as nursing performance was reduced in pregnant mothers (Chapter 3).

Speculation: Are precocial mothers pre- programmed?

The results of both cross-fostering experiments (Chapter 1 and 2) on parent-offspring conflict closely resembled the findings in rats and cats (Martin 1986, Lichtman & Cramer 1989) in that mothers and pups disagreed over the duration of care. However, when deciding over the allocation of resources to their young, guinea pig mothers seem to follow different cues. In contrast to young altricials, young guinea pigs seem to have only little potential to influence maternal nursing performance as milk production, maternal responsiveness and timing of weaning are largely dependent on a female's lactational stage. In confirmation with Davis (1979) – who concluded that milk yield in guinea pigs must be a function of pre-partum factors – it seems that females follow a program that is set at parturition and to which pups can only make fine adjustments by influencing the frequency of feedings and the time spent nursing. Such a pre-programming could be an adaptive maternal strategy in a precocial mammal. Parker & MacNair (1979) suggested that the more sensitive the parent, the higher the potential for offspring manipulation and the further will the outcome of parent-offspring conflict be shifted in favour of the offspring, i.e. parents will provide more care than is optimal from their perspective. Parental in-sensitivity on the other hand, carries costs: the cost of solicitation and an additional cost of misjudging offspring need (Parker & MacNair 1979). The latter will presumably be higher in altricials, that are nutritionally completely dependent on maternal milk, than in precocials, that may be able to compensate a low maternal supply by an increase in independent foraging. Furthermore, as mentioned in Chapter 1, precocial mothers may be able to use their own condition to adjust their time spent nursing and the timing of weaning to pup demand. Mothers of altricial young may therefore be more sensitive and adjust their care in a more flexible way, whilst mothers with precocial young can afford to be less sensitive and thus reduce the potential for offspring manipulation. However, not all findings may be typical for precocials. For example, milk yield is completely under maternal control in guinea pigs, whereas young of large precocial mammals can influence milk yield by the sucking intensity or frequency (Loudon & Kay 1984). The question arises why such a communication mechanism should have evolved in large precocial mammals but not in guinea pigs.

Speculation: To beg or to forage?

The alternative to forage independently may indeed influence begging behaviour in precocials. Although milk and solid food differ in quality and may provide the offspring with different nutrients, the decision to solicit food from parents or to forage independently may follow the rules of optimal foraging: As suggested in Chapter 2 young may prefer the source that supplies them with more energy per unit feeding time. If so, self-feeding may be more beneficial than soliciting milk when solid food is abundant and the milk yield is low. However, the finding may be restricted to the experimental conditions as in a more natural environment, milk yield may be positively correlated with pasture quality. Solicitation intensity may also be affected by other factors such as the effectiveness of begging and learning mechanisms. Learning was suggested as an explanation for the intensified begging in budgerigar nestlings that where fed additionally by responsive fathers (Stamps et al. 1989) and in house sparrows for taking on begging postures that had been most rewarding in the past (Kedar et al. 2000). Small nestlings in great need may reduce begging if they are pushed aside by larger brood mates and thus receive very little reward for their begging efforts (Lotem, 1998). When testing the effect of ewe breed on lamb bleating rate, Dwyer et al. (1998) found the frequency of bleating negatively correlated with ewe responsiveness. The effect was not found in the first 90 minutes after birth indicating that offspring begging was reinforced by maternal responsiveness. However, it was not tested if ewes of the higher responding breed terminated nursing bouts earlier then the low responding Blackface ewes and thus caused lambs to initiate more teat contacts. Similarly in guinea pigs, high milk yields may reinforce further sucking attempts when nursed by high yielding mothers.

Speculation: The influence of maternal reproductive state on nursing performance – a physiological trade off?

I suggested in Chapter 3 that regulatory constraints may be the proximate cause of reduced nursing behaviour in lactating-pregnant females. Furthermore, I think that early weaning could be the result of a physiological trade-off due to the different hormonal requirements of gestation and lactation. If the hormonal requirements of lactation have a negative effect on gestational performance, mothers may decide to wean early if the potential fitness costs of early weaning are compensated by fitness gains accrued from the litter in utero. Unfortunately –not much information about the hormonal environment of lactation in guinea pigs is currently available. As I was very interested in the nature of such a potential trade-off, I have made some speculations about how hormones related to lactation could affect gestational success on the basis of findings in other mammalian species. In all non-ruminant animals so far studied, prolactin plays the major role in lactation (Cowie 1984). In rabbits, the concentration of progesterone – a key hormone of gestation – is known to be lowered by an increased serum concentration of prolactin (Fuchs 1984). Treating pregnant rabbits with prolactin resulted in a high foetal mortality (Fortun-Lamothe et al. 1994). The milk ejection reflex depends on the release of oxytocin which is stimulated during sucking (Cowie 1984). Studies in all mammalian species so far studied, show that oxytocin also plays a role in the initiation of labour and the expulsive phase (Blanks & Thornton 2003). From this it is conceivable that oxytocin released by sucking could potentially influence the timing of parturition. Evidence suggests that oxytocin released by daytime nursing in rabbits may negatively influence parturition in rabbits (Hudson et al. 1995).

Thus, there is evidence that high concentrations of lactational hormones may have harmful effects on gestational performance in other species. Lactational hormones could be more harmful to the litter in utero in one phase of gestation than in another and then weaning shortly before this critical time could be an adaptive maternal strategy. A correlation of the size of the suckled litter and the detrimental effect on the litter in utero (as found in rabbits (Fortun-Lamothe et al. 1999) could be explained by an increased sucking stimulus that leads to an increased release of lactational hormones (Algers et al. 1991).

During pregnancy, progesterone and oestrogen levels rise markedly and evidence suggests that raised oestrogen levels can inhibit milk production (Cowie 1984). In guinea pigs oestrogen concentration rises slightly from day 15 of gestation and increases steeply from around day 20 onwards (Challis et al. 1971). If the milk yield is reduced in pregnant guinea pigs needs further investigation. Growth rates of suckled young in my study do not support the idea that milk yield is reduced in pregnant mothers. However, at later stages of lactation a reduction in milk yield may not be reflected by offspring- growth as it contributes only little if at all to pup growth (Laurien-Kehnen 2003, Künkele 2000). Measuring the milk yield at later stages of lactation could provide further insight to this question.

Mothers seem well adapted to avoid or reduce potential negative effects of concurrent lactation and pregnancy. Rabbits for example, nurse at night when the release of oxytocin seems to be least harmful to the litter in utero (Hudson et al 1995). Similarly a reduction in time spent nursing and early weaning could be adaptive to reduce harmful effects of lactational hormones on gestation. The hormonal environment may be a compromise between the endocrinological requirements of lactation and gestation. If the young in utero partly influence the hormone levels of pregnancy and the suckled litter the hormonal environment for lactation by sucking stimuli, then the results may also be interpreted as the outcome of a sibling conflict.

Outlook

This study allowed to compare aspects of mother-offspring interactions in altricial mammals with the situation in a precocial mammal where young can feed independently. To my knowledge it is unique in that it applies the theory of the mutual effects of offspring solicitation and maternal supply to a highly precocial mammal. Dwyer et al. (1998), analysed the effect of ewe responsiveness on lamb bleating and found a positive effect of maternal responsiveness on lamb bleating rate. However, in their study if maternal milk yield may have been lower in the high responding breed or the high responding ewes may have terminated nursing bouts earlier . Thus a higher responsiveness to lamb bleating did not necessarily reflect a higher milk supply.

With regard to the results on the solicitation behaviour of pups, interesting new questions and tasks arise. For example, in Chapter 2 I argued that maternal supply is positively influenced by young because an increase in solicitation results in an increased frequency of nursing bouts and time spent nursing. However, it still needs to be shown that by influencing the time spent nursing young can indeed influence the total amount of milk supplied. Another task is to show if the decision to forage or to solicit care from the mother does indeed follow the rules of optimal foraging – for example, by measuring and comparing the intake of solid food and the time spent foraging by young that confront different milk yields. To minimize the effects of learning mechanisms and possible non-nutritive rewards of sucking, manipulating the cost of foraging may be an even better alternative to study if such a “solicitation versus self-feeding strategy” exists. This could be accomplished for example, by supplying young with food of different energetic value.

Exciting are as well the effects of concurrent pregnancy on nursing behaviour – and also the effects of lactation on the size of the following litter. Although little is known about the hormonal environment during lactation in the guinea pig, the hormonal environment during pregnancy is well studied (Challis et al. 1971). An application of gestational hormones during lactation combined with milk yield measurements could provide further insights into the question about the proximate cause for reduced nursing behaviour in pregnant females. Comparisons of hormone levels in pregnant-lactating and pregnant-non lactating females could provide information if hormone levels connected with gestation are affected by lactation.

Summary

Females should adjust costly nursing to their own and to offspring condition in order to allow optimal offspring development at minimal fitness costs to themselves. They may assess offspring condition by monitoring offspring appearance and solicitation intensity. Young should be selected to influence maternal effort to maximize their own fitness, leading to parent-offspring conflict. Experimental studies on parent-offspring interactions in mammals have mainly been carried out in altricial species and models on the effects of offspring demand on parental supply and of supply on demand refer to altricial birds. However, in highly precocial mammals where young can forage independently from early on while still being nursed the situation may be different from that in altricials.

I investigated the influence of pup demand on nursing performance and the timing of weaning in the precocial guinea pig (*Cavia aperea* f. *porcellus*) by cross-fostering different aged pups. As cross-fostered younger pups were weaned early and older pups late compared to normal conditions, the results clearly indicate a conflict in which mothers wield the power. In contrast to findings in altricials, females hardly adjusted the timing of weaning to pup age. I tested if the models on the effects of supply and demand could be applied to precocial mammals by cross-fostering guinea pig pups with same aged cavy pups (*Cavia aperea*). Females nursing the much larger guinea pig pups confronted higher demand than females nursing cavy pups. Pups raised by guinea pigs found a higher milk supply than pups raised by cavies. In confirmation with current models, high demand pups showed more solicitation and mothers reacted to this by nursing more frequently. However, the assumptions for the effects of supply were not met: when confronted with a high supply, pups increased solicitation. The results of these two experiments support the idea that the early ability to forage independently may have lead to different maternal strategies for the optimization of supply and the offspring decision to initiate nursing bouts.

Maternal state may also affect nursing performance. In altricials lactation has been found to be reduced in concurrently pregnant and lactating mothers, maybe because energetic peaks of the phases overlap. In pregnant-lactating precocials these peaks are well separated. However, I found that pregnant guinea pigs also shortened lactation, indicating that reductions in nursing performance may not exclusively be caused by energetic constraints.

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