Back in Winter 2012 !!

We always try new things in NUT 115 to offer you the best educational experience possible. Some things work while others do not, but we want to experiment.

This quarter we will conduct a study to evaluate the impact of a nutrient (lipid) on the sex ratio of offspring. The animal model that will be used is the mouse.

Mouse Study

Hypothesis: The hypothesis is that the fatty acid composition of the lipid in the diet of mice prior to and during breeding and gestation will impact the sex ratio of the pups produced.

Objective: The objective is to determine if the lipid source impacts the sex ratio of offspring. There will be two diets containing either corn oil (unsaturated fatty acids - UNSAT) or Energy Booster 100 (saturated fatty acids - SAT). The fatty acid composition of the diet will differ while all other nutrients will be unchanged.

Background: The amount of energy in the diet of mice and lizards affected the sex ratio of offspring. There are 2 papers that follow for your reading. There is no perfect study. We just try to do the best job possible. In the mouse study, the authors increased energy intake (note: energy is not a nutrient). However, all nutrients were increasing so it is difficult to say that it was an energy effect.

Corn oil is high in unsaturated fatty acids (Table 1). Energy Booster is high in saturated fatty acids. Our goal is to keep the diets identical in nutrient composition except for the fatty acid composition. There is a difference in the lipid sources. Corn oil provides fatty acids in triglyceride form. Energy Booster provides fatty acids in free fatty acid form. It would be best to provide the fatty acids in the free form for both diets if we were doing this for a research publication.

Experimental design: There will be 12 female and 4 male CD1 mice used. There will be 6 females and 2 males per treatment – UNSAT & SAT. The
diets will be fed 2 weeks prior to breeding, during gestation, and during lactation.

**Data collection:** The number of offspring born will be counted and recorded for each mouse. Mice pups will be weighed. At approximately 2-3 weeks postpartum, the sex of each pup will be determined.

To detect a difference in sex ratio, the data will be tested using a t-test.

**Summary:** Everyone will be involved. Hopefully you will find it a fun way to link a nutrient with animal metabolism and function.
Table 1. Fatty acid composition (g/100 g fatty acid) of the lipid in the lipid supplements and the diets. Corn oil diet is UNSAT and the Energy Booster diet is SAT.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Corn oil</th>
<th>Energy Booster</th>
<th>UNSAT</th>
<th>SAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4-C13</td>
<td>0.00</td>
<td>0.18</td>
<td>0.19</td>
<td>0.32</td>
</tr>
<tr>
<td>C14:0</td>
<td>0.04</td>
<td>2.67</td>
<td>0.08</td>
<td>2.00</td>
</tr>
<tr>
<td>C16:0</td>
<td>11.39</td>
<td>33.01</td>
<td>16.00</td>
<td>26.13</td>
</tr>
<tr>
<td>C16:1 cis</td>
<td>0.11</td>
<td>0.68</td>
<td>1.09</td>
<td>1.37</td>
</tr>
<tr>
<td>C18:0</td>
<td>1.97</td>
<td>49.77</td>
<td>5.26</td>
<td>26.99</td>
</tr>
<tr>
<td>C18:1 trans 11</td>
<td>0.00</td>
<td>0.35</td>
<td>0.12</td>
<td>0.26</td>
</tr>
<tr>
<td>C18:1 cis 9&amp;10</td>
<td>26.73</td>
<td>5.61</td>
<td>26.52</td>
<td>16.95</td>
</tr>
<tr>
<td>C18:2n6</td>
<td>57.01</td>
<td>0.50</td>
<td>42.06</td>
<td>16.28</td>
</tr>
<tr>
<td>C18:3n3</td>
<td>1.19</td>
<td>0.07</td>
<td>1.91</td>
<td>1.42</td>
</tr>
<tr>
<td>C20:4n6</td>
<td>0.00</td>
<td>0.01</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>C20:5n3</td>
<td>0.00</td>
<td>0.00</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>C22:6n3</td>
<td>0.00</td>
<td>0.00</td>
<td>0.44</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Just to jog your memory from O-Chem, C18:0 is stearic acid and it is 18 carbons with no double bonds. Stearic acid is a saturated fatty acid. Linolenic acid is C18:3 with 18 carbons and 3 double bonds. By the omega nomenclature linolenic is a n3 fatty acid.
Maternal Diet and Other Factors Affecting Offspring Sex Ratio: A Review

Cheryl S. Rosenfeld1,3 and R. Michael Roberts2,4

Departments of Animal Sciences,1 Biomedical Sciences,2 and Biochemistry,3 University of Missouri-Columbia, Columbia, Missouri 65211

ABSTRACT

Mammals usually produce approximately equal numbers of sons and daughters, but there are exceptions to this general rule, as has been observed in ruminant ungulate species, where the sex-allocation hypothesis of Trivers and Willard has provided a rational evolutionary underpinning to adaptive changes in sex ratio. Here, we review circumstances whereby ruminants and other mammalian species, especially rodents and primates, appear able to skew the sex ratio of their offspring. We also discuss some of the factors, both nutritional and non-nutritional, that potentially promote such skewing. Work from our laboratory, performed on mice, suggests that age of the mother and maternal diet, rather than the maternal body condition per se, play directive roles in controlling sex ratio. In particular, a diet high in saturated fats but low in carbohydrate leads to the birth of significantly more male than female offspring in mature laboratory mice, whereas when calories are supplied mainly in the form of carbohydrate rather than fat, daughters predominate. As the diet fed to the mice in these experiments were nutritionally complete and because litter sizes did not differ between treatments, dietary inadequacy seems not to be the cause for sex ratio distortion. A number of mechanisms, all of which are testable, are discussed to provide an explanation for the phenomenon. We conclude the review by discussing potential implications of these observations to human medicine and agriculture.

early development, embryo, embryonic development, fertilization, nutrition and pregnancy, sex allocation, sex ratio, trophoblast, uterus

INTRODUCTION

Darwin surmised that some animal species can exhibit statistically significant shifts in the proportion of sons and daughters that are born, although the conditions and underlying mechanisms that prompt these changes were and still are, for the most part, unclear. In insects, reptiles, and birds, sex-ratio adjustments in response to food availability and other environmental factors, e.g., extreme sex-ratio skewing due to male-selective killing by Wolbachia infection in the Samoan butterfly Hypolimnas pollina have long been characterized [1-5]. This work has contributed greatly to evolutionary theory, even to the extent that the experimental data can be fitted accurately to mathematical predictions [6-9]. It is now clear that the male-to-female sex ratio at the time of conception (primary sex ratio) and the secondary sex ratio at birth can be strikingly skewed from the theoretical 1:1 expected ratio [reviewed in 10, 11]. In the sections that follow, we first review the evidence that adaptive adjustments in sex ratio of offspring occurs in mammals in response to diet and report on some of our own experimental findings in the mouse. We conclude by discussing some of the mechanisms that might be responsible for skewing sex ratios.

Significance of Gender Differences at Birth and the Trivers and Willard Sex-Allocation Hypothesis

Trivers and Willard [12] pointed out that, in polygynous species, a small proportion of males, usually ones that are larger and more aggressive, share most of the lifetime reproductive success, while lower ranking males often father no offspring at all. By contrast, the majority of females, irrespective of their social rank and body condition, become pregnant through mating to this selective group of males. In such species, fathers often play little part in rearing the young. The sex-allocation hypothesis of Trivers and Willard predicted that females in the best body condition would tend to produce offspring the gender of which favors the sex of greater variance, namely males. Their sons would benefit from greater parental investment and most likely, as adults, join the elite group of breeding males. As a consequence, such females are likely to pass on their genes to more of their grandchildren. Conversely, females lower in the social structure or in poorer body condition would be anticipated to invest more in female progeny because their daughters, rather than their sons, are likely to have greater lifetime reproductive success. The greater variance of males in polygynous species, both in terms of early mortality and reproductive success, is well established in wild populations. Males born to high ranking/better fed females may, in turn, have greater reproductive success than their contemporaries [13, 14]. Such a correlation seems also to hold true for mice, where larger males are more attractive to females than small males [15], and males born to food-deprived mothers are generally smaller as adults than males born to females fed ad libitum, even if such variance is not evident when they are born [16]. Moreover, males born to food-deprived female mice are more likely to lose agonistic encounters than sons born to control-fed females [17]. De-
TABLE 1. Relative energy content (% Kcal) of major nutrients in mice diets.

<table>
<thead>
<tr>
<th>Diet</th>
<th>D12450B* (LF)</th>
<th>D12492* (VIF)</th>
<th>Purina 5015* (CLC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>20</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>31</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>Maltodextrin</td>
<td>4</td>
<td>13</td>
<td>NS</td>
</tr>
<tr>
<td>Sucrose</td>
<td>33</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>20</td>
<td>56</td>
</tr>
<tr>
<td>Fats</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean oil</td>
<td>6</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Lard</td>
<td>4</td>
<td>54</td>
<td>NS</td>
</tr>
<tr>
<td>Total fat</td>
<td>10</td>
<td>60</td>
<td>26</td>
</tr>
</tbody>
</table>

* Defined Research Diet (Research Diets, Inc., New Brunswick, NJ) with equivalent amounts of casein, cellulose, minerals, vitamin mixes [31]. D12450B diet had a caloric density of 3.8 kcal/g, D12492, 5.2 kcal/g. Purina Complete Life Cycle (CLC) 5015 diet, 4.4 kcal/g. (Purina Inc., St. Louis, MO) NS, Not specified.

Despite the fact that the Trivers and Willard hypothesis [12] has been often cited and sometimes overinterpreted, that some sex-ratio skewing may be nonadaptive, and that there is much literature that is conflicting [13, 18-21], the hypothesis has provided a useful theoretical framework to begin to study sex-ratio deviation.

Maternal Nutrition and Sex Ratio of Offspring in Various Species

This section will discuss what is known about how nutrition of the mother can affect the sex ratio of her progeny, first in the large artiodactyl species, second in rodents, especially mice, and finally in other animals.

Large ruminants. The prediction that females in better body condition would produce more male than female progeny has been observed in red deer [14, 22, 23], roe deer [24], mature ewes [25], reindeer [26]. Barbary sheep [27], domestic pigs [28], and a number of other species, although there are exceptions [13, 29]. Dairy cows, but not heifers, on a high plane of nutrition give birth to proportionately more bull than female calves than cows on a poorer diet [30]. Repeat breeder cows, i.e., ones that have problems becoming pregnant by artificial insemination, also tend to produce more males [31]. The data on roe deer [24] were obtained with farmed animals on a diet controlled for low- and high-energy intake by varying the oil content. In that study, 75% of the calves born to the high-energy does were male, while the low-energy group produced only 46% males. Most other studies have been performed on wild populations, which are less well-controlled.

Rodents. There have been surprisingly few studies aimed directly at testing the Trivers and Willard hypothesis [12] in mice, although there are several reports that are consistent with its applicability in this species. Numerous studies have shown that maternal nutrition, particularly a diet that is inadequate, can affect litter size and viability [16, 32-34]. Rivers and Crawford [32] fed mice either a low-fat or control diet. Females on the low-fat diet had litters with a significant sex-ratio distortion (~1:3 males:females) relative to controls, where the sex ratio was 1:1. Females on the low-fat diet also had smaller litters, suggesting that there had been selective loss of male embryos or fociuses. Drickamer [33] noted that dominant female mice could appropriate more food than lower ranking females and produced a greater proportion of male-biased litters. Mekle and Drickamer [34] found that both wild and laboratory mice deprived of food for 1 wk before mating produced fewer males than control wild and laboratory females. In a follow-up study, Mekle and Thornton [16] showed that intermittent feeding of wild mice both prior to and during gestation gave female-biased litters relative to controls. Food restriction of female rats results in a skewing of offspring sex ratio, which has been attributed to a decrease in uterine glycercylphosphorylcholine diesterase activity [35]. In rats, a maternal diet high in sodium and potassium but low in calcium affects the sex ratio of offspring [36, 37]. Interestingly, hamsters dosed with caffeine have significant skewing of the sex ratio toward females [38], speculatively attributed to inhibition of cAMP phosphodiesterase activity.

In our experiments, we chose to examine the effects of two defined, nutritionally complete diets [39; Table 1], which differ primarily in their sources of energy, on the sex of offspring born to female NIH Swiss mice [39]. Diet 1 was low in saturated fat (LF), with the majority of calories provided as sugars and complex carbohydrate. The second was very high in saturated fat (VHF), with 54% of its energy provided as lard (Table 1). The goal was to determine whether these diets could influence the sex ratio of pups born.

NIH Swiss mice maintained on the two diets from 30 days of age delivered four successive litters of pups after being bred at approximately 10, 20, 28, and 40 wk of age, resulting in 1048 young born over 108 pregnancies (Table 2). The effects of diet on litter size, maternal weight, gestation length, and sex ratio were tested by using a mixed model procedures with a repeated measures design [40, 41]. Because each female had multiple correlated records within...
TABLE 3. Effect of diet on sex ratio of first litter born to mature mice, aged 20-27 wk before breeding.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Conception weight (g)</th>
<th>Litter size</th>
<th>Gestation length (days)</th>
<th>Sex ratio</th>
<th>No. of male-biased litters</th>
</tr>
</thead>
<tbody>
<tr>
<td>LF (n=14)</td>
<td>31.0 ± 4.9b</td>
<td>9.2 ± 3.6</td>
<td>20.4 ± 1.5</td>
<td>0.38b</td>
<td>2</td>
</tr>
<tr>
<td>VHF (n=11)</td>
<td>41.4 ± 7.4b</td>
<td>9.4 ± 3.4</td>
<td>20.4 ± 1.7</td>
<td>0.64c</td>
<td>10</td>
</tr>
</tbody>
</table>

* Values for maternal weights at conception, litter size, and gestation length are means ± SD.
* Values for VHF diet were significantly heavier (P < 0.001) than ones on LF diet.
* Sex ratios deviated significantly from 0.5 (P < 0.05).

In the two treatments was used to determine the effect of the diets. Parity and treatment by parity interactions were tested with residual error.

Sex ratio (fraction of male pups) for the VHF and LF groups was tested against the expected value of 0.5 by using a t-test statistic [42]. Gestation length (~20 days) and litter size (~9 pups) did not differ between the VHF and LF groups and did not change as the mice aged, although the mice did become progressively harder to breed. The sex ratio of pups (fraction of males) born to mothers on the VHF diet was unusually high (0.67) and to the mothers on the LF diet very low (0.39), spanning litters 2.4. Importantly, this skewing of the sex ratio was related to the diets fed and not to the individual weights of the mothers.

Mice that were first bred at 10 wk of age delivered similar numbers of sons and daughters, whereas virgin mice bred later than 20 wk of age produced pups for which the sex ratio was skewed according to diet (Table 3). The experiments showed that the source and possibly amount of calories provided to mature female mice on a nutritionally complete diet can influence sex of offspring born and are consistent with the Trivers and Willard sex-allocation theory [12]. The second set of experiments, which employed older females, clearly showed that age of the mother rather than parity order affected offspring sex ratio. Only mature females showed a significant response to the diets in terms of the sex of the offspring they produced.

Marsupials. Audi and Sunquist [43] performed an experimental field study with the American opossum (Didelphis marsupialis), in which randomly selected females had their diet supplemented with sardines. The provisioned group produced a male-biased sex ratio of pouch young, while the controls produced males to females in about the same number. This study is of particular interest because, in opossums, the young make their way to the pouch within 14 days after conception so that sex selection must occur early in development. Also, the supplement was high in lipid and rich in n-3 essential fatty acids, which has been suggested to influence sex ratio toward males in humans [44].

Primates. Although in most societies, humans are not generally considered to be polygynous, retrospective census studies have indicated significant, although somewhat inconsistent, changes in sex ratio associated with particular socioeconomic conditions, geographic areas, and social groupings [45-48]. Crawford et al. [44] speculated that a high content of essential fatty acids in the diets of pregnant females favors boys and suggested that male fetuses are more susceptible to fatty-acid deficiencies than females. Williams and Glosker [49] concluded that there is a positive correlation of male births and food availability, and that if caloric availability declines, so does the male to female ratio, although the changes are generally quite small and certainly not of the magnitude noted by us for mice.

A large group of African women, most of whom were malnourished as determined by their height and weight, for example, produced more daughters than sons [50]. A study examining birth rates of women from rural Ethiopia also demonstrated that a positive correlation existed between women who were in better nutritional state, as determined by body mass and muscle indices, and percent of male births [51]. Analysis of over 10,000 children born in Modena, Italy, revealed that thinner mothers were less likely to give birth to sons [52]. In humans, males appear to have higher in utero caloric demands than females [53]. Vulnerability of male offspring to in utero malnutrition and other environmental stressors might, therefore, have arisen through natural selection, by maximizing the mother's reproductive success, so that she tends to give birth to the more energy-demanding male offspring during auspicious environmental cycles [53].

Male births have also been loosely correlated with a masculine phenotype of the mother, high estrogen and androgen levels at the time of conception, and android patterns of fat distribution in women [55, 56]. Ash in rodents, electrolytes within the pregnant mother's diet might also affect sex ratio in humans [57].

Studies on sex-ratio biases in nonhuman primates, many of which are polygynous, have been as controversial as those with humans [14, 58], and many of the outcomes noted have been obtained on small sample sizes where stochastic variation can easily lead to erroneous conclusions [58]. High-ranking females of some species tend to produce mainly males, but in others, e.g., baboons, the opposite occurs, and thus, another hypothesis, that of the advantaged daughter has been proposed because daughters of high-ranking females tend to inherit the elite social status of their mothers [14, 59]. Indeed, the complex social structure and intensity of competition for local resources in primate populations means that the predictions of Trivers and Willard [12] would likely not apply in many instances. Nevertheless, it still seems likely that nutritional status of the mother, and the cost of reproduction, play a significant role in adjusting sex ratios among primates [58].

Possible Nonnutritional Causes of Sex-Ratio Skewing

Distortions in the sex ratio have been attributed to factors other than nutrition of the mother [see 14]. In rodents, females stressed in some manner tend to produce fewer sons than nonstressed females [60-65]. Housing pregnant females under crowded conditions reduces sex ratio (fraction of male offspring) [63], while mating at first postpartum estrus tended to produce more males [66]. When subordinate female hamsters are paired with dominant females, they produce smaller litters and fewer sons than their dominant counterparts [64]. Treating such subordinate females with either dexamethasone [64] or progesterone [67] abrogates this stress-induced selective loss of male pups, sug-
suggesting an endocrine basis for the phenomenon. Parity has been observed to influence sex ratio of pups born to golden hamsters [68]. Litter sizes and sex ratio increased until the third litter and then declined in subsequent litters. For females bred only once in their lifetimes, male-biased litters occurred in hamsters aged between 100 and 455 days but was female-biased in younger and older animals. Body condition and amount of food consumed, which could have been important variables, were not considered in the statistical models used to analyze the data in any of these studies.

The timing of insemination has long been held to affect sex ratio in species that ovulate spontaneously, particularly in livestock [14, 69]. Early studies with rabbits suggested that fewer male offspring are produced from early matings [70]. In hamsters, more male births occur if fertilization occurs late in estrus, possibly as the result of low vaginal pH [71]. Data from cattle have been mixed, with many older studies indicating that breeding early in the estrous period favored females [72]. More contemporary experiments have generally shown little effect of early insemination [73, 74], but the method used for estrus synchronization may have influenced the outcome of recent work [74]. Gutiérrez-Adán et al. [75] presented evidence that, in cattle, the timing of insemination relative to maturation stage of the dominant follicle influences sex ratio. Moreover, there is little doubt that, in deer [69, 76] and sheep [69], early insemination skewed the ratio toward females, while late insemination favors males.

Timing of mating in mice may affect sex ratio in some strains of mice, but not others [77]. B6/CBA F1 hybrid mice produce more females when they are mated early and more males when mating is delayed by a few hours [78]. In addition, the timing of embryo transfer to such mice was found to skew the sex ratio. If embryos were allowed to develop in vitro to the two-cell stage and then transferred to females the morning after they had copulated, i.e., the embryos were 24 h advanced of the recipients, more female fetuses resulted, whereas early or synchronous transfers tended to favor males. The experiments of Jimenez et al. [78] revealed an additional interesting fact, namely that late-stage absorptions were essentially randomized between males and females in the asynchronously bred mice, indicating that selective late-stage abortion could not provide the basis for the sex skewing.

Not unexpectedly, the effect of timing of insemination on sex of offspring in humans is unclear. Some studies indicate that more males are born with natural insemination 3 or more days before or 1 or more days after ovulation [79–83]. However, other results dispute whether timing of intercourse, artificial induction of ovulation, and artificial insemination has any affect on sex ratio in humans [84].

Studies of birth rates from preindustrial Finland (1775–1850) indicate that more sons were born during periods when adult males declined in the population [85]. After the industrial revolution, female births began to outnumber male births in more developed countries. In humans, one reason for the recent upsurge in female relative to male births in Western societies may be age of the mother. Gutiérrez-Adán et al. [86] analyzed birth records in Spain from 1945 to 1997 and showed that only two variables—mean age at marriage and the older age at which women give birth—correlated (P < 0.01) with the reduction in the ratio of male to female births. Similar findings, especially for nonwhites, have been found in a U.S. study [87]. Analysis of baboon births in Gombe National Park reveals subordi-
cervical mucus, nutrient/energy status of tract secretions, vaginal pH relative to the precise time at which copulation occurred in relation to estrus [Fig. 1A] [71, 94]. One class of sperm might have intrinsic physiological differences in viability, capacitation, or the dynamics of the acrosome reaction [75, 95].

2. Sperm of one sex might be more capable of effecting fertilization once the egg has been reached, depending on factors such as the condition of the female reproductive tract and the penetrability of the zona pellucida, which likely vary according to the time of ovulation relative to time of insemination. Depending on the maturation state at the time of fertilization, the oocyte might preferentially bind X- or Y-bearing sperm (Fig. 1B) [96].

3. Differences in the rate of development or in the sensitivity to conditions of XX versus XY embryos within the female reproductive tract cause a selective loss of embryos of one sex prior to placentation (Fig. 1, C and D). Such selection might be favored by particular nutritional components or developmental asynchrony between the embryos and uterus. For example, faster growth of embryos of one sex in a litter-bearing species, where the space available limits the number of fetuses that survive, could provide a competitive advantage to that gender.

4. Selective fetal resorption/abortion is the final possible means of skewing offspring sex ratio. It would appear to provide a relatively costly means for adapting sex ratio to maternal and environmental selective pressures and was not found to be the cause of sex-ratio skewing in the recent studies of Jimenez et al. in mice [78]. Nevertheless, as a result of fetal resorption following implantation, the Norwegian rat produces litters biased toward females if mating occurs at first postpartum estrus following removal of the first litter [97]. Induced uterine crowding also leads to a female bias in these rat litters. In each case, the bias arose from absorption of male fetuses after they had implanted but within the first half of pregnancy. Examination of implantation sites in subordinate female hamsters reveals a preferential fetal loss of male pups between Days 5 and 10 of pregnancy [65]. Analysis of perinatal mortality records from the Medical Birth Registry in Norway revealed that human male embryos appear to be more sensitive to uterine stress and thus likely to be aborted than females [98].

In some species of birds, changes in sex ratio have been observed relative to abundance of food [99–101]. Because the female is the heterogametic sex in birds, prototypical gamete selection must provide the means whereby avian sex bias within the resulting clutch is adjusted [101].

Sexual Dimorphism in Development of Preimplantation Embryos

Male and female preimplantational embryos differ in their mRNA expression patterns. For instance, some genes located on the X chromosome are expressed more robustly in bovine and human female versus male embryos [102–105]. Several autosomal genes expressed in trophoblast, such as IFN-γ [106] and hCG [107], and a variety of imprinted genes [108–110] are also not expressed or methylated identically across the sexes.

The most frequently reported manner in which early male and female embryos differ is in their rates of cleavage in the first few days after fertilization. Embryos produced in vitro in a number of species seem to fall into fast-cleaving and slow-cleaving groups, which are predominantly male and female, respectively. This phenomenon has been observed for bovine [111–115], murine [116], and ovine embryos [117, 118]. Male in vivo-produced porcine embryos, both prior to and subsequent to blastocyst hatching, have also been reported to be larger and to have more cells than female embryos [119, 120]. That male embryos develop faster is by no means universally accepted, however, as some studies have reported no differences in human [121], bovine [122], and cultured mouse embryos in the time to reach the blastocyst stage [122 and unpublished work from this laboratory on bovine and mouse]. Similarly, male and female porcine embryos have been reported to grow at similar rates in vivo [123, 124]. Nonetheless, male bovine blastocysts have significantly more cells than females immediately posthatching [125].

There could be several explanations for these contrasting observations. One is species and breed/strain differences. Another is that the culture conditions employed for the in vitro studies influenced the results. For example, the presence of glucose in the medium may preferentially favor either the growth or the development of male over female bovine embryos [106, 126–128]. A third explanation may relate to the manner in which growth rates are measured. In many cases, the end-point employed for in vitro studies has been the time taken to reach a readily observable stage in development, most usually the formation of the blastocyst. By such a standard, all embryos could have equivalent growth rates during the early cleavage stages, but the female embryos might be less capable than male embryos in making a particular developmental transition, e.g., to form a blastocoele or to advance from early to late blastocyst (see Fig. 1D). Thus, a failure to develop or to grow at the same rate as the other sex is probably due to inadequacies of the culture medium or to other environmental stresses. There are several studies indicating that IVP male bovine embryos predominate among blastocysts and that this skew in sex ratios becomes more exaggerated at the expanded and hatched stages [129–132]. Meanwhile, embryos arrested in development prior to the blastocyst stages have been shown to be predominantly female [128, 133, 134]. Our laboratory has shown that the block to female bovine embryo development in a glucose-containing medium occurs at about the time the blastocoel cavity begins to form [106]. Moreover, the data show no differences in growth rate between male and female embryos up to Day 6 of development and that the females that advance to expanded blastocyst do so at the same rate as the male embryos. The cohort of females that fail to advance to expanded blastocyst appear to be less tolerant of the high glucose concentrations in the medium than the successful females. In mouse embryos, a high concentration of glucose (5.56 mM) in the media does not detrimentally effect female or male embryonic development (unpublished observations), which is consistent with the finding that glucose does not always inhibit preimplantational murine embryo development [135].

Implications of Sex-Ratio Skewing to Agricultural and Human Medicine

If there is a difference in the relative numbers of male and female IVP embryos at the blastocyst stage, a skew toward males born after embryo transfer might be anticipated unless, of course, female blastocysts have some advantage over males posttransfer. A preponderance of bull
calves has been noted in at least one such study with cattle [136]. Usually, however, transfer of embryos in cattle is carried out with a mixture of compact morulae and early, rather than expanded, blastocysts. Under such a regimen, it is unlikely that a marked difference in sex ratios would be noted.

Importantly, many successful human IVF programs now utilize blastocyst-stage embryos because it ensures that the embryos are developmentally competent through the cleavage stages. In earlier days, IVF embryos were cultured only through the very early cleavage stages before they were transferred [137]. While such early studies showed no skew in the sex ratio [e.g., 138], several recent reports show a distinct male bias after selection of the most advanced embryos for transfer [139–141]. In other words, inadvertent sex selection may be occurring in human IVF programs. These data suggest that, in the human as well as in the bovine, male embryos make the transition to blastocysts better than females. It also seems possible that, if embryos are selected at the expanded- to-hatched blastocyst stage in either species, the bias toward males will be exaggerated.

Maternal skewing of offspring sex ratio might have important agricultural implications. Offspring of one gender may be preferred over the other. For instance, females are preferred in the dairy industry, whereas males are favored in the beef industry. Altering the diet content prior to breeding might provide a means of manipulating the sex ratio, e.g., a lower plane of nutrition might result in more female offspring.

In summary, sex-ratio skewing occurs in some mammalian species under both field and laboratory conditions, and these alterations might be adaptive, particularly to the mother who bears most of the lifetime burden of caring for the young. The underlying mechanisms are likely to be complex and are not well understood. However, by combining field and laboratory results, reasonable inferences may be drawn. Our studies in the mouse indicate that maternal diet, possibly its caloric content, can play a directive role in skewing offspring sex ratio. As Sheldon and West [29] discuss, past studies testing the sex-allocation theory of Trivers and Willard [12] in various animal populations have employed the nebulus term maternal condition. Maternal condition in wild populations has been assessed either by the animal’s dominance behavior or has been based on morphological/physiological characteristics. A unifying definition of maternal condition needs to be established before proper inferences can be drawn across populations and among various species. Importantly, the diet of the mother, both before and after conception, needs to be considered as causative factors in skewing offspring sex ratio in animals.

ACKNOWLEDGMENTS

We thank Kristie M. Grinnan, Kimberly A. Livingston, Angela M. Brokman, and Angela M. Davis, who performed the majority of the mouse studies reviewed here and originally published elsewhere; and Dr. William R. Lamberson for his assistance with the statistics [39]. We are grateful to Karla Carter and Jim Bixby for their invaluable assistance.

REFERENCES

15. Meikle D, Kreper J, Browning C. Adult male house mice born to undernourished mothers are unattractive to oestrus females. Anim Behav 1995; 50:753–758.
33. Dickman L. Delay of sexual maturation of female mice by a urinary
Maternal nutrition affects reproductive output and sex allocation in a lizard with environmental sex determination

Daniel A. Warner¹,* , Matthew B. Lovern² and Richard Shine¹

¹School of Biological Sciences, University of Sydney, New South Wales 2006, Australia
²Department of Zoology, Oklahoma State University, Stillwater, OK 74078, USA

Life-history traits such as offspring size, number and sex ratio are affected by maternal feeding rates in many kinds of animals, but the consequences of variation in maternal diet quality (rather than quantity) are poorly understood. We manipulated dietary quality of reproducing female lizards (Amphibolurus muricatus; Agamidae), a species with temperature-dependent sex determination, to examine strategies of reproductive allocation. Females maintained on a poor-quality diet produced fewer clutches but massively (twofold) larger eggs with lower concentrations of yolk testosterone than did conspecific females given a high-quality diet. Although all eggs were incubated at the same temperature, and yolk steroid hormone levels were not correlated with offspring sex, the nutrient-deprived females produced highly male-biased sex ratios among their offspring. These responses to maternal nutrition generate a link between sex and offspring size, in a direction likely to enhance maternal fitness if large body size enhances reproductive success more in sons than in daughters (as seems plausible, given the mating system of this species). Overall, our results show that sex determination in these animals is more complex, and responsive to a wider range of environmental cues, than that suggested by the classification of ‘environmental sex determination’.

Keywords: Amphibolurus muricatus; dietary quality; maternal effects; sex ratio; temperature-dependent sex determination; yolk steroid hormones

1. INTRODUCTION

Life-history traits such as offspring size and number vary enormously both within and among species (Roff 2002). At an intraspecific level that variation is tightly linked to individual survival and reproductive success (and hence, paternal and especially maternal fitness) and is thus expected to be under strong selection (Sincero et al. 1992; Kaplan 1998). However, such traits also are very flexible even within the lifetime of a single female; for example, shifts in maternal nutrition can massively alter the frequency of reproduction, total reproductive output and the ways in which that output is partitioned (e.g. into many small versus a few large offspring and relative numbers and sizes of sons versus daughters; Price 1998; Kudo & Nakahira 2005). Hence, rather than producing highly canalized life histories, selection generally fashions norms of reaction that link environmental variation to reproductive variation (Kaplan & Cooper 1984). Quantifying the form of such links is a major challenge for evolutionary ecologists (Dougherty & Reznick 2004). Although correlational studies can be useful in this respect (e.g. Madsen & Shine 1996), experimental manipulation of resource levels provides the strongest evidence for causal links between maternal nutrition and reproductive output (Meijer & Langer 1995; Kudo & Nakahira 2005; Du 2006).

Most previous work on this topic has focused on the amounts of food available for reproducing females and documented strong maternal-allocation responses to experimental manipulation of resource quantity in ways likely to influence both maternal and offspring fitness (Meijer & Langer 1995; Selman & Houston 1996; Williams 1996; Ruizin et al. 2005). As well as the more obvious traits, such as offspring size and number, food availability also can influence patterns of sex allocation (relative investment into sons versus daughters; Rosenfeld & Roberts 2004; Robertson et al. 2006). Theoretical models predict that mothers will differentially allocate resources to the sexes when the fitness returns from producing sons differ from those gained by producing daughters (Trivers & Willard 1973). These relative fitness returns will often depend upon environmental situations, and accordingly, mothers in such situations tend to allocate more resources to the sex that benefits most from the present conditions (Komdeur 1996; Nager et al. 1999; Kalmbach et al. 2001). Such differential allocation of resources can be manifested in two different ways: mothers can either (i) produce biased offspring sex ratios (Clutton-Brock et al. 1984; Ditius 1998; Kalmbach et al. 2001) or (ii) they can produce balanced sex ratios, but invest more energy into individual offspring of one sex than the other (Velando 2002).

Although flexible resource-driven sex allocation strategies appear to be widespread (Appleby et al. 1997; Rosenfeld & Roberts 2004; Robertson et al. 2006), one important aspect of dietary variation largely has been neglected in previous studies. Natural environments vary through space and time not only in the quantity of food available to a reproducing female, but also in the quality of that food (Seigel & Fitch 1985). Does variation in nutritional quality, not simply in total food intake, drive...
corresponding variation in maternal reproductive tactics? This question is particularly interesting for a species in which offspring sex is labile, depending upon incubation conditions, rather than fixed by genetic inheritance; such labile systems might be more likely to exhibit adaptive sex-allocation shifts. Accordingly, we conducted experiments to explore the effects of dietary quality on maternal reproductive allocation in a lizard species with environmental sex determination. More specifically, we set out to (i) determine the effect of dietary quality (during the reproductive season) on maternal reproductive output, (ii) explore the ability of females to differentially allocate resources to males versus female offspring in response to variation in diet quality, (iii) evaluate the influence of maternal dietary quality on other phenotypic traits of offspring, and (iv) evaluate whether any differential sex allocation was mediated via adjustment of yolk hormone levels.

The jacky dragon (Amphibolurus muricatus) is a common agamid lizard found in coastal heathland habitat of southeastern Australia. This species provides an excellent model for addressing the above issues because (i) jacky dragons have a long reproductive season (October–February) and females produce 3–4 clutches of eggs within this period (Harlow & Taylor 2000). Hence, female jacky dragons likely are exposed to fluctuations in resource quality within as well as among seasons. (ii) Jacky dragons fuel reproduction with recently ingested food rather than stored energy reserves (Warner et al. unpublished data). Hence, manipulations to dietary quality during the reproductive season should have immediate effects. (iii) Jacky dragons have temperature-dependent sex determination (TSD), whereby the sex of the offspring is determined by egg incubation temperatures (Harlow & Taylor 2000; Warner & Shine 2005). Thus, if females modify sex allocation in response to diet, they could do so either by selecting nest sites with specific thermal regimes (St. Juliana et al. 2004) or by differentially allocating steroid hormones into egg yolks (Bowden et al. 2000; Lovern & Wade 2003) in order to overproduce the sex that provides the greatest fitness returns.

2. MATERIAL AND METHODS
(a) Lizard housing and husbandry
We captured adult male and female jacky dragons in the Sydney region during the austral spring and summer of 2003/2004. These lizards were maintained in large (2 m long × 2 m wide × 1 m tall) outdoor enclosures for 1 year prior to our experimental manipulations. Enclosures contained sand substrate and several branches for perching and basking. Natural vegetation and cover boards provided the animals with shelter. Three lizards were housed per enclosure (one male with two females). Animals were fed roaches and crickets (dusted with vitamin/calcium mix) thrice a week.

Immediately after the overwinter period (Spring 2004), lizards were randomly assigned to either a low- or high-quality diet treatment group. The low-quality diet group (n = 15 females) was maintained on a diet consisting of crickets that had been fed only on corn for one month prior to being offered to the jacky dragons. Lizards in the high-quality diet group (n = 28) were maintained on a diet consisting of crickets and roaches that were primarily raised on cat food, but also were given apples, carrots and several types of leafy greens. Lizards were fed thrice a week and the overall quantity of food (in terms of number of insects) given to the lizards in the two treatments did not differ. Females were weighed and measured (snout–vent length, SVL and tail length, TL) at the beginning and end of the reproductive season (September 2004 and February 2005, respectively).

(b) Egg incubation and hatching phenotypes
We monitored captive dragons regularly for signs of nesting, and collected and weighed all eggs within 24 h after oviposition. We then extracted a small sample of yolk from half of the eggs from each clutch using a sterile syringe with a 24 gauge needle. Yolk removal reduced egg mass by an average of 12.8% (s.d. = 8.7). Yolk samples were then freeze-dried overnight and stored at −80°C until hormone analysis. All the eggs were placed individually in glass jars (125 ml) filled with vermiculite and incubated at standardized hydric (∼200 kPa water potential) and thermal conditions (constant 28°C, a temperature that produces a 30 : 50 sex ratio; Harlow & Taylor 2000). Eggs were placed in one of three incubators, and rotated within and among the incubators thrice a week to minimize the potential effects of thermal variation among and within incubators. Yolk removal had no effect on hatching success (χ² = 1.1, p = 0.29; overall hatching success = 91%, N = 418 eggs from 82 clutches).

After eggs hatched, all hatchlings were measured (SVL and TL), weighed and sexed by hemipenis eversion. This method for sexing hatching lizards has been verified by gonadal histology, laparoscopy and dissection (Harlow 1996). All hatchlings were individually marked by toe-clipping and then housed in large outdoor enclosures (1.3 m long × 0.75 m wide × 0.55 m deep) containing sand substrate with branches for perching and basking, and small tiles for shelter. Water was always available, and hatchlings were fed crickets and roaches (dusted in vitamin and mineral mix) thrice a week. No more than 15 hatchlings were housed together at a single time. Hatchlings were kept under these conditions for two weeks prior to being released in the field; at this point, the hatchlings were remeasured (SVL and TL) and weighed in order to calculate individual growth rates.

(c) Yolk hormone analyses
Concentrations of testosterone (T), 17β-oestradiol (E2) and corticosterone (CORT) in freeze-dried yolk samples were measured by radioimmunoassay (RIA) following extraction and chromatographic separation (Wingfield & Farner 1975; Schwabl 1993). Samples were mixed thoroughly prior to removing a 3–12 mg subsample (recorded to the nearest 0.5 mg for each sample) and reconstituted in 0.5 ml of ddH₂O in 1.5 ml microcentrifuge tubes. We equilibrated samples overnight at 4°C with 1000 c.p.m. of 'H-T (NET-370, 70 Ci mmol⁻¹), 'H-E2 (NET-317, 72 Ci mmol⁻¹) and 1000 c.p.m. of 'H-CORT (NET-390, 71 Ci mmol⁻¹) from Perkin Elmer Life Sciences, Inc., for individual recovery determinations.

For extraction, samples were transferred to 16 × 100 mm glass culture tubes; each microcentrifuge tube was rinsed with 0.5 ml of ddH₂O and this was added to the culture tubes as well. We extracted the samples twice with 4 ml petroleum ether : diethyl ether (30 : 70 v/v), dried the extracts under nitrogen gas in a 37°C water bath and reconstituted them in 1 ml 90% ethanol. The samples were stored at −20°C overnight and centrifuged at 2000 r.p.m. at 0°C for 5 min.
The supernatant was transferred to clean test tubes and dried under nitrogen gas in a 37°C water bath and reconstituted in 500 µl of 10% ethyl acetate in isooctane.

To remove neutral lipids and to isolate T, E2 and CORT, all samples were transferred to diatomaceous Earth (celite, Sigma) columns for chromatographic separation. Columns consisted of a celite : ethylene glycol : propylene glycol upper phase (8:1:1 m/m) and a celite : dH2O (8:1 m/v) lower phase. Neutral lipids and dihydrotestosterone were eluted with isooctane and 10% ethyl acetate in isooctane, respectively, and discarded. T, E2 and CORT were eluted with 20, 40 and 52% ethyl acetate in isooctane, respectively, and saved. Samples were dried under nitrogen gas in a 37°C water bath, resuspended in phosphate buffer and placed overnight at 4°C.

Competitive binding RIAs were performed using the appropriate tritiated steroid tracer (see above) and antiserum from Wien Laboratories for T (T-3003), Biogenesis for E2 (7010-2650) and Sigma-Addrich for CORT (C87894). The standard curves ranged from 1.95 to 500 pg and were run in triplicate. Samples were run in duplicate, averaged and adjusted for individual recovery and initial sample mass. Average recoveries were 63, 60 and 46% for T, E2 and CORT, respectively. We randomized the samples across six assays. We stopped assays E2 after three assays, as the majority of samples contained non-detectable amounts of this steroid. The average intra-assay CV was 10.0% for T and 9.7% for CORT, and the inter-assay CV was 7.4% for T and 13.6% for CORT.

3. RESULTS
(a) Effect of maternal diet on reproductive output
Dietary quality had a substantial effect on maternal body condition and reproductive output. The body condition of females fed the low-quality diet declined dramatically throughout the reproductive season (mean±s.e. change in maternal body condition = -2.35±0.82), whereas the condition of females on the high-quality diet increased (mean±s.e. change in maternal body condition = +0.74±0.64; F1,35=7.5, p=0.009). Females fed the high-quality diet produced more clutches over the reproductive season than did females on the low-quality diet (figure 1a), but the number of eggs per clutch (i.e. clutch size) did not differ between diet treatments (F1,33=3.6, p=0.067). Consequently, females given the high-quality diet produced 55% more eggs over the entire season than those on the low-quality diet (figure 1b). Although the poor-quality diet reduced reproductive output, females on this diet invested more energy per clutch. In other words, females on the low-quality diet produced heavier eggs (figure 1c) and an overall greater clutch mass (F1,33=3.3, p=0.005) than those fed the high-quality diet.

Maternal diet also had a substantial effect on the timing of oviposition and hormone allocation into egg yolks. On average, females on the low-quality diet produced their first clutch much later than females on the high-quality diet (Kruskal-Wallis test: χ²=7.2, p=0.007). Consequently, the poor-quality diet reduced the reproductive season by an average of 25 days. Females fed the high-quality diet allocated more testosterone into their eggs than did females from the low-quality diet treatment (figure 1d), but the concentration of corticosterone in egg yolks did not differ between treatments (mean±s.e. for low- and high-quality

(d) Data analyses
Statistical analyses were carried out with SAS software (6.9.1, SAS Institute 1997). All variables were checked for normality and homogeneity of variances. When necessary, data were log- or square-root transformed to meet the assumptions of parametric analyses. If variables could not be normalized by transformation, we used non-parametric analyses.

To evaluate the effect of maternal diet on changes in maternal body condition and reproductive output, we used analysis of variance (ANOVA) and covariance (ANCOVA). Change in maternal body condition was calculated as the difference between body conditions (residual scores of regression of ln mass vs. ln SVL) measured at the end versus beginning of the reproductive season. Reproductive output was defined in three ways: (i) the number of eggs produced per clutch, (ii) the number of clutches produced during the reproductive season, and (iii) the total number of eggs produced over the entire season. Maternal mass was used as a covariate when evaluating reproductive output. The effect of diet on egg mass was evaluated using clutch size as a covariate.

We used two factor mixed model ANOVAs and ANCOVAs to evaluate the effect of maternal diet, offspring sex and their interaction (fixed effects) on hatching characteristics (dependent variables). For analyses of hatching size (i.e. SVL and mass), egg mass was used as a covariate. For analyses of TL, SVL was a covariate. For analyses of hatching condition, body mass was the dependent variable and SVL was the covariate. Hatching growth rate was calculated as the change in body size (mass and SVL) over a two-week period divided by the number of days between measurements. For all analyses, maternal identity was defined as a random effect. These analyses were based upon mean trait values for each sex within each clutch to avoid pseudoreplication.

diets = 7.8 ± 1.1 and 6.1 ± 0.7 pg mg⁻¹, respectively; Kruskal-Wallis test: χ² = 1.6, p = 0.20). Yolk hormone concentrations were not related to oviposition date (testosterone: r² = 0.002, p = 0.70; corticosterone r² = −0.005, p = 0.53). Maternal diet did not affect egg survival (overall egg survival = 91%; chi-square test: χ² = 1.1, p = 0.30).

(b) Effect of maternal diet on sex ratios
Offspring sex ratios were strongly affected by maternal diet, the date of oviposition and their interaction (table 1). In general, females fed the high-quality diet produced female-biased sex ratios and those on the low-quality diet produced male-biased sex ratios (figure 2a). Moreover, sex ratios shifted seasonally for females on the low-quality diet, with female-biased clutches early in the season, but male-biased clutches later in the season (figure 2b); clutches produced by females on the high-quality diet were slightly female-biased across the entire reproductive season (figure 2c). In support of these findings, the slopes of the relationship between sex ratio and oviposition date differed between maternal diet treatments (ANCOVA: F₁,₁₅₀ = 10.8, p = 0.002). Egg mass and yolk hormone concentrations were not associated with clutch sex ratios, although the significance of corticosterone levels was marginal (p = 0.056; table 1); clutches with relatively high concentrations of corticosterone produced slightly more females.

(c) Effect of maternal diet and sex on offspring phenotypes
The only phenotypic traits significantly affected by maternal diet were hatching size (SVL and mass) and body condition. These effects were statistically significant even when the data were corrected for egg size (table 2). Hatchlings produced by females on the low-quality diet were larger and in better body condition than those produced from females on the high-quality diet (figure 3). We found no significant sex differences in hatching phenotypes, and maternal diet did not affect phenotypes of sons differently from those of daughters (as indicated by non-significant interaction terms; table 2). In addition, the levels of steroid hormones within egg yolks were not significantly associated with any phenotypic trait for either male or female offspring (all p > 0.10). Only four hatchlings died during this study, thus offspring survival was not affected by maternal diet treatment (χ² = 0.13, p = 0.723) nor did survival differ between the sexes (χ² = 0.69, p = 0.406).

4. DISCUSSION
Several studies have demonstrated that diet quantity (i.e. maternal feeding rate) influences maternal reproductive
The negative impacts of the low-quality diet on maternal condition and the number of clutches produced suggest that females in this treatment had little energy available for reproduction. Moreover, females on the low-quality diet did not begin reproduction until nearly one month after those on the high-quality diet had already produced their first clutch, probably because they needed more time to gather enough energy for clutch production in the face of low nutrient availability. However, although females on the poor diet produced fewer eggs overall, they produced much larger eggs than those maintained on the high-quality diet. Interestingly, this pattern remained significant even when analyses were adjusted for clutch size, indicating that investment per egg increased without a reduction in egg number. This pattern suggests that diet quality affects the way reproductive females allocate energy towards reproduction. In this respect, our results mirror those recently reported in insects (Fischer et al. 2006).

This lability suggests that strategies of maternal reproductive allocation can shift spatially or temporally depending upon the quality as well as quantity of available energy. For example, in years or habitats when diet quality is poor, females may invest more energy into individual clutches rather than producing multiple clutches. This strategy results in larger eggs and offspring, as indicated by our study. On the other hand, in years or habitats with an abundance of high-quality food, females will produce more clutches per season, but relatively small eggs. Thus, a female exposed to poor resources may make the best out of a suboptimal situation by allocating her energy towards producing fewer, better-quality offspring. Presumably, larger offspring size enhances survival prospects under poor resource conditions (Semlitsch & Gibbens 1990).

Maternal diet quality also influenced clutch sex ratios. Females maintained on the low-quality diet produced male-biased clutches, whereas those on the high-quality diet produced female-biased clutches. At first glance, these results appear to run counter to adaptive predictions of sex allocation theory, where 'better quality' females overproduce male offspring (i.e. the sex with the higher reproductive variance; Trivers & Willard 1973). However, if large hatching body size (as produced by females on the poor diet) is more beneficial to sons than to daughters, then the patterns found in our study fit well with predictions from sex-allocation theory (Trivers & Willard 1973). Indeed, paternity analyses in jacky dragons show that large male size is associated with high reproductive success (D. A. Warner & R. Shine, unpublished work).

One fascinating complexity in the maternal responses involved seasonal shifts in offspring sex ratios: females on the low-quality diet produced female-biased clutches early in the season and male-biased clutches later in the season; no such pattern was evident in clutches produced by females maintained on the high-quality diet. The seasonal shift in sex ratio seen in offspring of our 'low-diet-quality' females may enhance maternal fitness, because previous work on this species suggests that early hatching benefits fitness of daughters more than that of sons (Warner & Shine 2005). Thus, the low reproductive output of 'low-diet-quality' females may be balanced by the relatively high fitness returns provided by their early hatched daughters. Why, then, did females on the high-quality diet not use the same strategy? We suggest that in nature,
females in this latter situation can take advantage of their prolonged egg-laying period to manipulate offspring sex ratios via selection of thermally suitable nest-sites. This option is not available to the 'low-diet-quality' females because their relatively brief nesting period reduces the thermal diversity of nests available, potentially favouring some intrinsic (non-thermally driven) sex-ratio adjustment linked to season. Long-term field studies are needed to evaluate these ideas and especially to clarify how different patterns of sex-allocation affect maternal fitness.

Recent studies have shown that maternally derived steroid hormones in egg yolks can play a significant role in sex determination (Bowden et al. 2000; Lovern & Wade 2003; Love et al. 2005). However, sex allocation in response to maternal diet was not mediated by maternally derived steroid hormones in our jacky dragons. Although dietary quality affected the levels of testosterone that females allocated to their eggs, we found no direct association between testosterone concentrations in the egg yolks and clutch sex ratios. Similar effects of diet on yolk testosterone allocation have been described in the zebra finch (Taeniopygia guttata; Rustein et al. 2005), but in contrast to our study, testosterone levels were associated with offspring sex in their study. However, corticosterone levels in the egg yolk of jacky dragons were marginally associated with offspring sex ratios (p = 0.059)—a pattern shown in other reptiles (Sinervo & DeNardo 1996; D. Allsop, personal communication) and birds (Love et al. 2005; Pike & Petrie 2005). Clutches with relatively high concentrations of corticosterone produced slightly more females. In contrast to this pattern, however, previous studies on mammals suggest that stressed mothers overproduce sons, perhaps as a result of increased levels of circulating glucose, which has also been shown to affect sex ratios (Camero 2004). Indeed, females in our poor diet treatment appeared nutritionally stressed and overproduced sons.

Our results have important implications for the mechanisms involved in sex determination. Since jacky dragons have TSD, previous research predicts that clutch sex ratios shift over the season as a response to seasonal increases in ambient (and nest) temperatures (Harlow & Taylor 2000). The seasonal shifts in sex ratios predicted by these studies resemble the pattern produced by females fed the low-quality diet in our experiment. Interestingly, clutch sex ratios of females on the low-quality diet shifted seasonally despite all eggs being incubated at a constant temperature (28°C). In addition, we found no seasonal changes in the quantity of maternally derived hormones in the egg yolks.

![Figure 3](image-url)
(Shine et al. 2002; Sarre et al. 2004). Indeed, multiple modes of sex determination have been discovered within single species of reptiles (Shine et al. 2002), fish (Conover & Heins 1987; Baroiller et al. 1995) and insects (Kozlowski et al. 2006). The sex ratio pattern exhibited by the jacky dragons in our experiment fit well with this emerging paradigm shift, providing the first evidence that maternal diet can influence offspring sex in a reptile with TSD. The pathways by which diet affects sex determination remain obscure, but we predict that future research will reveal far greater complexity than embodied in current paradigms of sex determination in reptiles.

We thank D. Allsop, M. Elphick, H. Giragossyan, M. Hagman, T. Langkilde, P. Peters, B. Phillips, R. Radder, S. Ruggeri, T. Schwartz, J. Thomas, M. Thompson, D. Van Dyk and M. Wall for their assistance in the field and/or in the laboratory. D.A.W. was supported by an International Postgraduate Research Scholarship and by an International Postgraduate Award. Funding was provided by the Ecological Society of Australia, a James Kentley Memorial Scholarship (to D.A.W.) and the Australian Research Council (to R.S.). Lizards were collected under permit SU0638 of the New South Wales National Parks Service. All protocols for this research were approved by the Animal Care and Ethics Committee of the University of Sydney (L04/12-2004/1/ 4018) and Macquarie University (2004/014).

REFERENCES


