

Litter sex ratios in the golden hamster vary with time of mating and litter size and are not binomially distributed

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Summary. Pregnancy rates, litter sizes, and litter sex ratios vary strongly with the time in the estrous cycle at which female golden hamsters (*Mesocricetus auratus*) are mated. Early matings tend to produce relatively high pregnancy rates, large litters, and female-biased sex ratios, while late matings tend to produce low pregnancy rates, small litters, and male-biased sex ratios. Time of mating and litter size are therefore correlated, but each appears to have an independent effect on litter sex ratio: time of mating and sex ratio are positively correlated, holding litter size constant, while litter size and sex ratio are negatively correlated, holding time of mating constant. At each litter size greater than two, the variance of litter sex ratios is less than the binomial variance expected on the hypotheses of independent sampling with a constant probability of producing a male. The main features of the distribution of litter sex ratios can be generated from a causal model in which *different* probabilities of producing a male apply to “early” and “late” conceptions within each litter. The relationship between litter size and mean litter sex ratio is potentially consistent with several different models for the evolution of adaptive sex-ratio variation.

Introduction

Williams (1979) has argued that there is no compelling evidence of adaptive sex-ratio control in vertebrates. His reasoning centers in part on the theoretical expectation that adaptive control of the sex ratio should often involve control of its *variance* as well as its *mean*. Under many different models of sex allocation, each parent in the population

has a clearly defined optimum sex ratio, in the sense that producing any other sex ratio leads to lowered fitness. To rely on the biological equivalent of coin-tossing as a means to determine the sex of each offspring seems inefficient under such circumstances. If each sex-determination event is independent, at a constant probability, then progeny sex ratios will follow the binomial sampling distribution. Even if a parent's *expected* sex ratio were exactly equal to the optimum, the *actual* sex ratio is produced would usually be higher or lower. If there really are adaptive sex ratios, argues Williams, then we should sometimes see progeny sex-ratio distributions with reduced variances (when most parents have the same optimum) or increased variances (when some parents have a strongly male-biased optimum and others have a strongly female-biased optimum). He finds no case, in vertebrates, of a sex-ratio variance that is convincingly different from the expected binomial variance.

In the years since William's review was written, several new studies have appeared, showing clearly that in some species of mammals, females of different social statuses produce different mean sex ratios. (For entries to this literature see Trivers 1985; Clutton-Brock and Iason 1986; Blaffer Hrdy 1987; McFarland Symington 1987; Altmann et al. 1988.) Most of these cases of apparent sex-ratio manipulation have been interpreted in the light of Trivers and Willard's (1973) model of adaptive condition, dependent modification of the sex ratio. (For reviews of this and many other models of adaptive sex allocation, see Charnov 1982; Trivers 1985.) Although these studies show that some mammals can adjust the probability of producing a son or a daughter, they do not provide good evidence that *variances* are being reduced, because the species involved have small brood sizes and long inter-birth intervals.

Here we describe a large set of sex ratios for laboratory-reared golden hamsters (*Mesocricetus auratus*). Average litter sizes and sex ratios vary strongly with the time in the estrous cycle when a female was mated. Although litter size and time of mating are correlated, each has an independent effect on the sex ratio. The variance of litter sex ratios is substantially less than binomial. In outline, these patterns are consistent with a formal model in which the probability that an embryo is male or female changes systematically, within litters, with the time of conception.

Methods

Golden hamsters have a 4-day estrous cycle that is closely tied to the daily light cycle. Individuals maintained under a 14L:10D photoperiod are sexually receptive from the afternoon of the day of proestrus through the morning of the day of estrus (Ciaccio and Lisk 1971; Huck et al. 1986b), and ovulation occurs between 0100 hours and 0400 hours on the day of estrus (Alleva et al. 1968; Reuter et al. 1970). Thus the female is receptive for about 10 h before and about 9 h after ovulation.

Females used in this study were in natural estrus, as determined by extravaginal examination (Orsini 1961). Different amounts of mating are required to initiate pregnancy during the early, middle, and late phases of the receptive period (Huck et al. 1986b). To maximize the probability that each mating would result in pregnancy, females were allowed to mate *ad libitum* with a well-rested male.

Average litter sizes and sex ratios may vary with parity and age in golden hamsters (Huck and Lisk 1985; Huck et al. 1986b, 1988), so we used equal numbers of nulliparous and older multiparous females (820 nulliparous and 823 multiparous females). Each female was used only once. All individuals used in the study were born in our laboratory. They are descendants of the outbred LVG strain obtained from Charles River Breeding Labs, Inc. At testing, the nulliparous females were, on average, 128 days of age (range: 91–163 d) and weighed an average of 130 g (range: 104–145 g). The multiparous females were, on

average, 207 days of age (range: 189–255 d) and weighed an average of 154 g (range: 123–170 g). Each multiparous female had reared two litters prior to this study.

The 195 males used in the study were, on average, 198 days of age (range: 168–265 d) and weighed an average of 136 g (range: 121–172 g). Each had sired two or more previous litters but had not had any sexual contact for at least 2 weeks prior to any mating that was part of the present study.

Animals were individually housed in 35 × 20 × 17.5 cm cages in windowless, air-conditioned rooms on a reversed 14L:10D photoperiod (lights off at 1900 hours). They were given water and commercial laboratory chow *ad libitum*.

Beginning at 1500 hours on the day of proestrus and continuing at hourly intervals until 1200 hours on the day of estrus, females were placed singly in transparent plastic arenas containing a male that had been habituated to the arena. Between 12 and 85 females of each parity type were used at each hourly interval. Each female was allowed to mate until a satiety criterion of 15 min without copulation was reached, or until she attacked the male. All females received 12 to 18 ejaculatory series and 6 to 28 long intromissions. Females were then placed in plastic breeding boxes and observed for signs of parturition at 8-h intervals, beginning 15 days later. At parturition, the sex of each pup was determined by examination of the anogenital distance.

Results

Pregnancy rate and litter size vary with time of mating

Almost all females that were mated before 0100 hours on the day of estrus subsequently produced litters, but females mated at later times were progressively less likely to do so. No mating at 1100 or 1200 hours on the day of estrus resulted in a pregnancy. Between 0200 and 1000 hours, pregnancy rates are somewhat higher for nulliparous than for multiparous females (see Fig. 1).

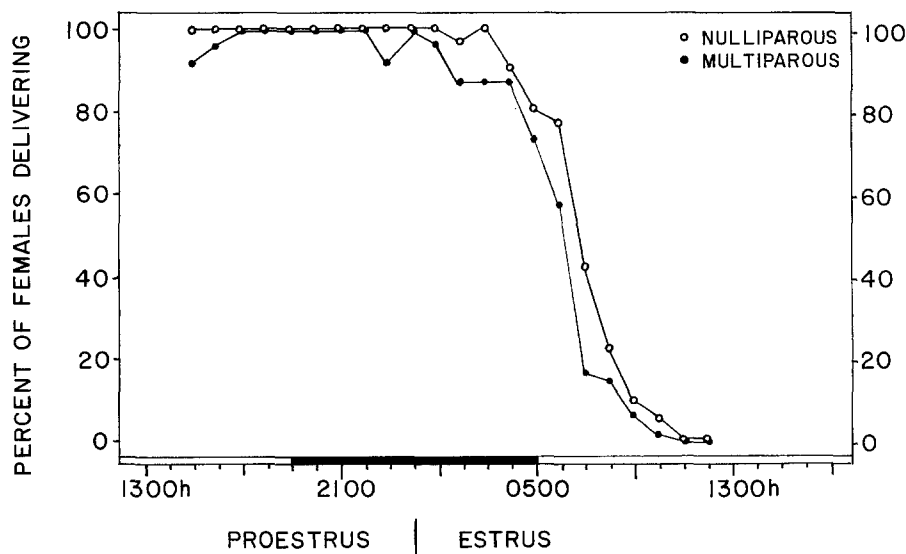


Fig. 1. Pregnancy rate as a function of time of mating. Each point shows at a given time the percentage of all females mated that subsequently gave birth. Data for nulliparous (open circles) and multiparous (closed circles) females are shown separately

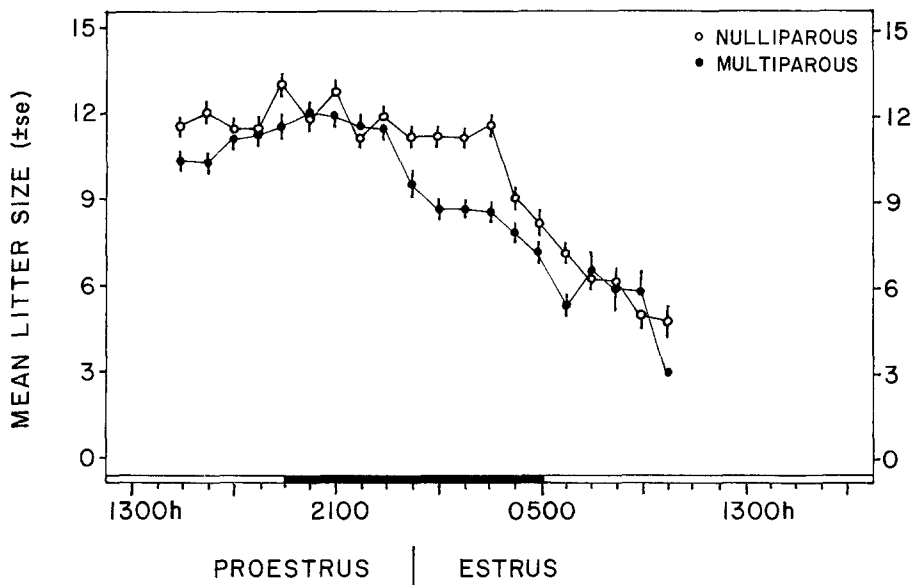


Fig. 2. Average litter size as a function of time of mating. Each point shows the mean number of pups (\pm one standard error) in litters produced by females mated at a given time. Data for nulliparous (open circles) and multiparous (closed circles) females are shown separately

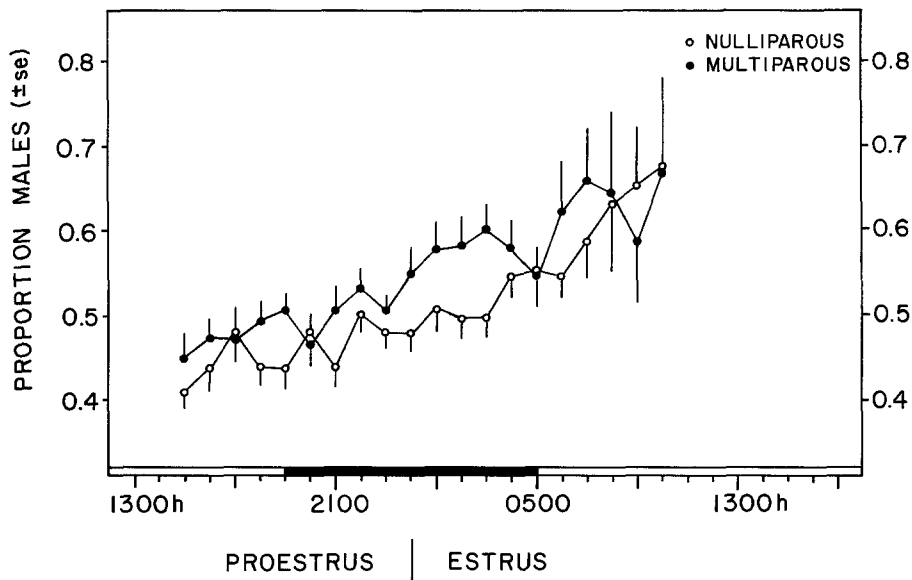


Fig. 3. Average litter sex ratio as a function of time of mating. Each point shows the mean proportion of males (\pm one standard error) for litters produced by females mated at a given time. Data for nulliparous (open circles) and multiparous (closed circles) females are shown separately

Litter size also declines with time of mating, from a mean of around 11 pups for females mated on the day of proestrus, to a mean of around 4 pups for females mated at 1000 hours on the day of estrus, the last time of mating that resulted in pregnancies. The litter sizes of nulliparous females exceed those of multiparous females at most times of mating, and are significantly larger overall (see Fig. 2).

Sex ratio varies with time of mating and litter size

The average proportion of males in a litter increases steadily from around 0.45 to around 0.65

over the 20 h during which matings give rise to successful pregnancies (see Fig. 3). Because litters tend to become smaller (Fig. 2) and more male-biased (Fig. 3) with time of mating, we should find a negative relationship between litter size and average litter sex ratio, ignoring time of mating. As expected, sex ratios decline rapidly with increasing litter sizes, from around 0.70 for litters of 4 and fewer, to around 0.40 for litters of 17 and above (Table 1).

There are at least two alternative causal hypotheses that could explain these patterns. First, sex ratio and litter size could be independent effects of a single common cause, the time of mating. In

this case the correlation between them should disappear when time of mating is controlled statistically. Second, sex ratio could depend causally on litter size, with litter size depending causally on time of mating, as in the first hypothesis. In this case the correlation between sex ratio and time

of mating should disappear when litter size is controlled.

Partial regression and correlation coefficients are presented in Table 2. Care should be used in interpreting these coefficients and their apparent significance levels, because the data violate some of the assumptions of parametric linear regression and correlation and because it is clear from Table 1 that the relationship between litter size and sex ratio is somewhat curvilinear. But the sample sizes are large, and the independent nulliparous and multiparous samples show very similar patterns, which suggests that the results are at least qualitatively valid.

Neither of the two hypotheses described above is supported in its pure form. Mean sex ratio declines with increasing litter size, holding time of mating constant, and it increases with increasing time of mating, holding litter size constant. The standard partial regression coefficients are $\beta_{S,L} = -0.205$ and $\beta_{S,T} = 0.218$, implying that litter size and time of mating have equally strong linear effects on the sex ratio. The effect of adding either variable to the regression is very highly significant ($P \ll 0.001$ in each case).

The variance of litter sex ratios is less than binomial

If each offspring in a litter of size L has an independent probability m of being male (with probability $1-m$ of being female), then the average number of males per litter is Lm , and the variance of number of males per litter is $Lm(1-m)$. The average proportion of males per litter is $Lm/L = m$

Table 1. Average litter sex ratios, by litter size and mother's parity

L	Nulliparous females		Multiparous females		All females	
	N	m	N	m	N	m
1			1	1.00	1	1.00
2			2	1.00	2	1.00
3	8	0.75	2	0.50	10	0.70
4	4	0.56	10	0.73	14	0.68
5	10	0.60	10	0.54	20	0.57
6	17	0.60	21	0.61	38	0.61
7	22	0.56	49	0.57	71	0.57
8	23	0.48	38	0.58	61	0.54
9	52	0.52	51	0.54	103	0.53
10	62	0.45	47	0.50	109	0.47
11	97	0.49	56	0.50	155	0.49
12	65	0.48	42	0.48	107	0.48
13	41	0.46	47	0.51	88	0.49
14	26	0.47	8	0.46	34	0.47
15	15	0.45	6	0.54	21	0.48
16	9	0.42	4	0.52	13	0.45
17	5	0.39	1	0.53	6	0.41
18	3	0.37			3	0.37
	459	0.49	395	0.54	854	0.51

Symbols: L =litter size; N =the number of litters of size L in the sample; m =the observed proportion of males in litters of size L

Table 2. Averages and regression and correlation coefficients for litter sex ratio, time of mating, and litter size

	Nulliparous females	Multiparous females	All females
N	459	395	854
m	0.493	0.536	0.513
T	9.3 ($s=5.2$)	9.1 ($s=4.7$)	9.2 ($s=5.0$)
L	10.6 ($s=2.8$)	9.7 ($s=2.8$)	10.2 ($s=2.8$)
a	0.52	0.56	0.56
$b(T)$	0.0070	0.0070	0.0064
$b(L)$	-0.0086	-0.0093	-0.0107
$r(T, L)$	-0.56	-0.62	-0.58
$r(T, L; m)$	-0.51	-0.58	-0.52
$r(T, m)$	0.36	0.33	0.34
$r(T, m; L)$	0.23 ($t=5.0$)	0.18 ($t=3.7$)	0.19 ($t=5.6$)
$r(L, m)$	-0.32	-0.31	-0.33
$r(L, m; T)$	-0.15 ($t=3.3$)	-0.14 ($t=2.8$)	-0.18 ($t=5.3$)

Symbols: N =number of litters; m =litter sex ratio; T =time of mating, expressed as the number of hours after 1400 on the day of proestrus (s =standard deviation); L =litter size; a , $b(T)$, and $b(L)$ =coefficients of the bivariate linear regression of litter sex ratio on time of mating and litter size; $r(X, Y)$ =the simple parametric correlation of X and Y ; $r(X, Y; Z)$ =the partial correlation, controlling for Z . (The values of t associated with these correlations are all significant at $P < 0.01$.)

Table 3. Expected and observed variances of the number of males per litter, by litter size and mother's parity

L	Nulliparous females				Multiparous females				All females			
	N	M	variance		N	M	variance		N	M	variance	
			exp	obs			exp	obs			exp	obs
1					1	1.00			1	1.00		
2					2	2.00			2	2.00		
3	8	2.25	0.56	0.50	2	1.50	0.75	0.50	10	2.10	0.63	0.55
4	4	2.25	0.98	0.25	10	2.90	0.80	0.32	14	2.71	0.87	0.37
5	10	3.00	1.20	0.88	10	2.70	1.24	1.35*	20	2.85	1.23	1.08
6	17	3.59	1.44	0.88	21	3.67	1.43	1.44*	38	3.63	1.43	1.17
7	22	3.91	1.73	0.76	49	3.98	1.72	1.19	71	3.96	1.72	1.04
8	23	3.87	2.00	1.12	38	4.63	1.95	1.21	61	4.34	1.99	1.30
9	52	4.71	2.24	1.51	51	4.84	2.24	1.37	103	4.78	2.24	1.44
10	62	4.50	2.48	2.22	47	4.96	2.50	1.90	109	4.70	2.49	2.13
11	97	5.39	2.75	1.80	56	5.52	2.75	2.04	153	5.44	2.75	1.88
12	65	5.75	2.99	1.66	42	5.74	2.99	2.16	107	5.75	2.99	1.82
13	41	6.00	3.23	1.80	47	6.66	3.25	1.93	88	6.35	3.25	1.96
14	26	6.58	3.49	3.53*	8	6.38	3.47	2.28	34	6.53	3.48	3.17
15	15	6.80	3.72	1.02	6	8.17	3.72	4.16*	21	7.19	3.74	2.16
16	9	6.67	3.89	2.50	4	8.25	4.00	2.25	13	7.15	3.96	2.82
17	5	6.60	4.04	3.35	1	9.00	4.24		6	7.00	4.12	3.61
18	3	6.67	4.20	0.34					3	6.67	4.20	0.34
	459				395				854			

* Indicates cases in which the observed variance exceeds the expected variance

Symbols: L =litter size; N =the number of litters of size L in the sample; M =the average number of males per litter, in litters of size L ; exp=the expected binomial variance of the number of males in litters of size L . Given that m (Table 1) is the probability of being a male, $\text{exp}=Lm(1-m)$; obs=the observed variance of the number of males per litter

(the sex ratio) and its variance is $Lm(1-m)/L^2 = m(1-m)/L$. Hereafter we will focus on the *number* of males per litter, rather than the *proportion*, because this simplifies the presentation of data and conforms to the usual practice in discussions of binomial distributions.

The variance of number of males per litter is consistently less than the binomial variance based on m , implying that each member of a given litter does *not* have the same probability of being a male. Table 3 shows expected and observed variances of number of males per litter for each litter size and parity type. Nulliparous females produced 16 litter sizes, and the observed variance is less than expected in 15 cases ($P < 0.0005$, two-tailed sign test). Multiparous females produced 17 litter sizes, but for only 14 of these can observed and expected variances be compared, because $N=1$ in two cases and $m=1$ in another. In 11 of the 14 meaningful cases the observed variance is less than expected ($P < 0.06$). For females of both parity types combined, the observed variance is less than expected in all 16 meaningful comparisons ($P \approx 0.00003$). These sign tests establish the significance of the pattern, but they use only part of the information

contained in the data. A more efficient test is described in the "Appendix".

In magnitude, the observed variances are, on average, less than 70% as large as expected on the hypothesis of independent sex determination for each member of a litter. For example, the average ratios of observed to expected variance, weighted by number of litters of each size, are 0.66 for nulliparous females, 0.71 for multiparous females, and 0.69 for all females combined. If the sex ratio of each litter is predicted from the bivariate linear models already described (which are functions of time of mating as well as of litter size), then the ratios of observed to expected variance are 0.64, 0.69, and 0.67, respectively.

The probability of being conceived a male appears to vary with the order of conception

Table 4 shows the complete distribution of litter compositions by litter size, for all females combined. A striking feature of this distribution is that 11 litters consist entirely of males, but none consist entirely of females. Thirty litters consist of males and just one female, while only 5 consist of females

Table 4. Distribution of the number of males in litters of different sizes, for all females combined

<i>L</i>	Number of males																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	–	1																	
2	–		2																
3	–	2	5	3															
4	–		5	8	1														
5	–	2	5	8	4	1													
6	–		5	14	11	6	2												
7	–		4	19	30	13	4	1											
8	–		3	10	23	14	10	1	–										
9	–		1	12	33	30	19	6	2	–									
10	–		2	24	28	25	16	9	5	–									
11	–			15	24	38	40	28	7	1	–								
12	–			4	13	39	10	34	6	1	–								
13	–			2	6	14	25	25	12	3	–								
14	–				3	7	11	3	6	2	–								
15	–					2	4	7	7		–								
16	–				1	1	2	4	2	2	1	–							
17	–					2	1	1	1	2		–							
18	–						1	2				–							

Symbols: *L* = litter size; – indicates the minimum (0) or maximum (*L*) possible number of males

and just one male, even though the overall sex ratio is very close to one-half. This observation immediately suggests a way to explain both the observed dependence of average litter sex ratio on litter size, and the observed reduction of sex-ratio variance below that expected on the hypothesis of independent sex-determination events.

Formally, this explanation requires that *m* (the probability of being conceived a male) be adjusted from high values for the first few offspring conceived, to lower values for subsequent offspring. A mechanism that brought about such an adjustment of *m* would cause relatively large litters to have relatively low sex ratios, and it would also cause the observed sex-ratio variance to be lower than the binomial variance produced under an equivalent constant value of *m*.

The mean number of males per litter is plotted as a function of litter size in Fig. 4. A regression fitted to the 854 individual litter compositions is superimposed on the 18 litter-size means. The line has a slope of 0.38, and it passes far above the origin. When taken together with the detailed distribution shown in Table 4, this unusual pattern implies that litters are assembled according to the following rule: let the first two (“early”) offspring be male with a very high probability, and let each subsequent (“late”) offspring be male with a much lower (but approximately constant) probability, which for this combined sample would be about 0.38.

Under this rule, each sex-determination event would still be a fully probabilistic Bernoulli trial – the toss of a weighted coin. Considered separately, the sex-ratio distribution of early and late offspring would be binomial. But the overall sex-ratio distributions for typical litters consisting of some early and some late offspring would *not* be binomial. They would show apparently reduced variance, in a pattern much like the one seen here. Likewise, mean litter sex ratios would decline with increasing

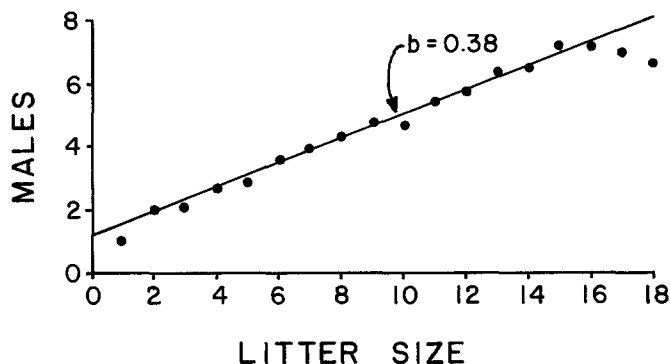


Fig. 4. Average number of males per litter as a function of litter size. Each point shows the average number of males in litters of a given size for all females combined (see Table 3). The line is a least-squares fit to the underlying data (Table 4), not to the 18 means shown here ($a=1.23$, $b=0.38$). For nulliparous females the regression is $a=1.28$, $b=0.36$, and for multiparous females it is $a=0.99$, $b=0.42$; the difference between these two slopes approaches but does not attain significance at the 0.05 level

litter size, in a pattern also much like the one seen here.

Is the sex-ratio variance of the hypothetical "late" offspring actually binomial? To answer this question we modified the data for all females (Table 4) by removing two hypothetically "early" males from each litter. (In five cases we could remove only one male.) Then we ran the tests described above on this modified data set, which contains 3 fewer litters than the original and a maximum litter size of 16. Meaningful comparisons between the observed and expected variances can be made for the 15 litter sizes between 2 and 16. In ten cases the observed variance is smaller than expected, and in five cases it is larger (not significant by the sign test). But the weighted mean ratio of observed to expected variance is still less than unity (0.92). This difference is much smaller than for the actual data, but it is still highly significant according to the test described in the "Appendix". Thus the simple formal model described above can successfully explain the main qualitative features of the variance reduction seen in these experiments, but it can not explain the full magnitude of the reduction.

Discussion

Of the patterns just described, the decrease in litter size with time of mating is the easiest to explain by means of known mechanisms. Hamster ova remain viable for only a short time after ovulation (Ward 1946). Yanagimachi and Chang (1964) found that 86% of golden hamster ova were fertilizable 3 h after ovulation, but less than half were fertilizable at 6 h, and only 9% at 9 h. Spermatozoa that have been in the female reproductive tract for less than about 3 h are unable to fertilize ova (Yanagimachi and Chang 1964; Yanagimachi 1969). It follows that the ova of females mated at 0300 hours (around the time of ovulation) would begin to be fertilized about 3 h after ovulation, while the ova of females mated at 1000 hours would begin to be fertilized about 10 h after ovulation, by which time most of the ova would already have degenerated.

The increase in the sex ratio with time of mating has several possible explanations. Positive relationships between the time of mating relative to ovulation and the subsequent birth sex ratio have been seen in many species of mammals, and there has been much speculation concerning their possible adaptive significance and underlying mechanisms. (For entries into this literature see Werren and Charnov 1978; Verme and Ozoga 1981; Trivers 1985.) One possible mechanism is based on differences in the behavior of X- and Y-bearing sperma-

tozoa. Rohde et al. (1973) report that human X-sperm tend to live longer than their Y-bearing counterparts, but that Y-sperm tend to swim faster. If the same were true of golden hamster spermatozoa, then matings that occurred before ovulation should produce an excess of females, because X-sperm would do better than Y-sperm at surviving to the time of ovulation, while matings that occurred after ovulation should produce an excess of males, because Y-sperm would generally be the first to arrive at the waiting ova. This mechanism is simple, and it is open both to adaptive and to nonadaptive interpretations. From the karyotypes of pre-implantation blastocysts collected $3\frac{1}{2}$ days after mating, Sundell (1962) found 64% males (1.8:1) in a sample of 98 golden hamster embryos. Unfortunately, neither the light cycle nor the time of mating was controlled in Sundell's study; the mean number of corpora lutea was slightly less than nine.

Another obvious (but more complicated) possibility is selective resorption of excess embryos. In principle, this mechanism could act as a second level of control, after differential fertilization. Maynard Smith (1980) considers the evolution of the investment ratio when the primary sex ratio is fixed at unity and the only method of sex-ratio adjustment is premature termination of investment.

Of the patterns seen in the present study, the reduced sex-ratio variance is by far the most difficult to explain by means of known physiological mechanisms. But if X- and Y-bearing spermatozoa behave differently, then a process having some of the needed features might easily arise. As previously discussed, the variance of primary litter sex ratios would be less than binomial if the probability of being conceived a male changed systematically within each litter, with the order of conception. Assume that ovulation occurs over a period of hours, with ova being released into the fallopian tubes one at a time rather than all at once. (This assumption seems plausible to us, but we can find no published evidence for or against it.) Then over the course of the period of ovulation, more and more of the functional spermatozoa in the fallopian tubes would be X-bearing, and fewer and fewer would be Y-bearing, because the Y-bearing spermatozoa would have arrived earlier, but then died at a faster rate than the X-bearing spermatozoa. Each successive ovum would tend to have a higher probability than those before it of being fertilized by an X-bearing spermatozoon and thus of giving rise to a female embryo.

This simple model generates a dependence of sex ratio on time of mating and on litter size, and it generates a reduced sex-ratio variance. But if

ovulation proceeds at a steady pace, then the probability of being conceived a male will tend to change smoothly between the first ovum and the last, rather than in the stepwise pattern implied by Fig. 4 and discussed near the end of the "Results" section. Some degree of control might be gained by modifying the temporal pattern of ovulation. For example, a few ova could be ovulated early, followed by a lull, and then by the rapid ovulation of a large number of ova. Under the assumptions discussed, this would generate two distinct probabilities of being conceived a male, rather than a smoothly changing range of probabilities.

Pratt et al. (1987) find negative correlations between vaginal pH at the time of mating and the sex ratios of litters subsequently produced, for matings that occur early or late in the receptive period, but not for matings that occur during mid-estrus. Perhaps vaginal pH (or some correlate of pH) unequally affects the longevity, motility, or fertilizing ability of X- and Y-bearing spermatozoa, or perhaps it reflects other physiological changes that occur during the receptive period that somehow affect litter sex ratio.

As interesting as these possibilities may be, they do not add up to a plausible mechanism that fully explains the patterns described here. In this sense our findings raise more questions than they answer.

The process that reduced the litter sex-ratio variance in these experiments may have had to overcome unknown and uncontrolled environmental, developmental, or genetic differences among the females. If such differences affected the females' average litter sex ratios, then they would have *increased* the observed sex-ratio *variance*. On the null hypothesis of constant probabilities within litters, differences among females would lead to observed sex-ratio variances that were *greater* than binomial. The variances seen here are less than 70% as large as the expected binomial variances, but it is possible that they would have been even smaller in the absence of unknown and uncontrolled differences among the females used in the study; such differences would be analogous to the experimentally induced differences in parity and in time of mating that are known to have caused heterogeneity in the average sex ratios produced by various groups of females within the experimental population.

This appears to be the first convincing case of reduced sex-ratio variance in a vertebrate. But does it fully meet Williams' (1979) criteria for evidence of *adaptive* control of the sex ratio? The hypothetical mechanisms that we just discussed could have evolved because they tended to increase the fitness

of their bearers, in which case the patterns described here would be adaptive. But the mechanisms could equally well be incidental side-effects of other processes that have nothing to do with the sex ratio as such.

There is good experimental evidence that undernourished female hamsters (and their daughters and granddaughters) tend to produce female-biased litters (Huck et al. 1986a, 1987; Labov et al. 1986). Not only do undernourished females (and their female progeny) tend to produce relatively few sons, they also tend to produce relatively small sons, as compared to well-nourished controls (and their female progeny). If the eventual reproductive success (RS) of a male depends more strongly on juvenile size than does the RS of a female, then lowering the sex ratio under food stress could be an adaptive response of the kind envisioned by Trivers and Willard (1973; also see Bull 1981). If the members of large litters were less well-fed, on average, than the members of small litters, and if male RS were more strongly size-dependent than female RS, then a mechanism that tended to make small litters male-biased and large litters female-biased could be favored by selection.

Lions may exhibit a related (but opposite) pattern. Litters containing three males are more frequent than expected in litters of three and four cubs (Packer and Pusey 1987). Packer and Pusey suggest that trios may be better at establishing and maintaining coalitions than are pairs or singleton males, and that the resulting local resource enhancement (Clark 1978; Toro 1982; Emlen et al. 1986) could select for the overproduction of trios of males within large litters. According to the test described in the "Appendix", the sex-ratio *variance* within litters of three and four cubs is *larger* than binomial ($t = 2.3$, 98 *d.f.*, $P < 0.05$). Within litters of two cubs the sex-ratio variance is *smaller* than binomial, but this difference only approaches significance ($t = -1.8$, 70 *d.f.*, $P < 0.1$). We find it interesting and somewhat puzzling that the *mean* sex ratio is close to $\frac{1}{2}$ for each litter size between one and four (range: 0.49 to 0.55).

In principle, many kinds of ecologies and population structures that give rise to differentially non-linear returns on cumulative investment in the two sexes can favor the evolution of sex ratios that vary with litter size (e.g., Charnov 1982; Seger and Charnov 1988). The possibilities include temporal variation of the population recruitment rate (e.g., Werren and Taylor 1984) and local mate competition (LMC; Hamilton 1967) with variable fecundity (e.g., Suzuki and Iwasa 1980; Yamaguchi 1985; Frank 1987; Stubblefield and Seger, *submitted*).

Hamsters could experience significant LMC if juvenile females migrated farther, on average, than juvenile males. In laboratory settings female *Mesocricetus* tend to be very aggressive toward each other (Takahashi and Lisk 1984), but nothing is known about their migration in nature. If they do migrate farther than males this would be contrary to the usual pattern for mammals (Greenwood 1980). The tendency of late matings to produce male-biased litters (independent of litter size) is potentially consistent with the idea that delayed mating indicates a local scarcity of males (Werren and Charnov 1978), but again, nothing is known about the distribution of mating times in nature. Thus, the adaptive significance (if any) of the patterns reported here will remain obscure until we know much more than we presently do about the life histories and demographics of natural populations of *Mesocricetus*.

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Appendix

Here we describe a simple but efficient way to compare the observed variance of litter sex ratios to the variance expected under the assumptions of the binomial distribution, where the litters are of different sizes, or of different expected sex ratios, or both.

Define the standardized squared deviation $Y_i = (x_i - \mu_x)^2 / \sigma_x^2$. Then $E(Y) = 1$, and $\sum Y_i$ is distributed approximately as chi-

square with n degrees of freedom if x is distributed approximately normally. If μ_x is replaced by the sample mean \bar{x} , then there are $n-1$ degrees of freedom. Because the Y_i are standardized, we can break a sample into parts having different means and parametric variances and then add together the sums of Y_i for each of the subsamples. The resulting grand sum is distributed approximately as chi-square with $\sum (n_k - 1)$ degrees of freedom, where k indexes the subsamples.

Here the subsamples are litters of different sizes. The mean number of males would differ in litters of different sizes, even if the sex ratio were independent of litter size. But we know that the mean sex ratio does differ among litters of different sizes. Because we are interested in the variation among litters after such sources of systematic variation have been removed, we define the standardized squared deviations as

$$Y_{iL} = (M_{iL} - \bar{M}_L)^2 / L p_L (1 - p_L), \quad (1)$$

where M_{iL} is the number of males in the i th litter of size L , \bar{M}_L is the mean number of males, and $p_L = \bar{M}_L / L$ is the sex ratio. On the null hypothesis that the number of males is distributed binomially within litters of each size, the sum of the Y_{iL} over all litters will be distributed approximately as chi-square

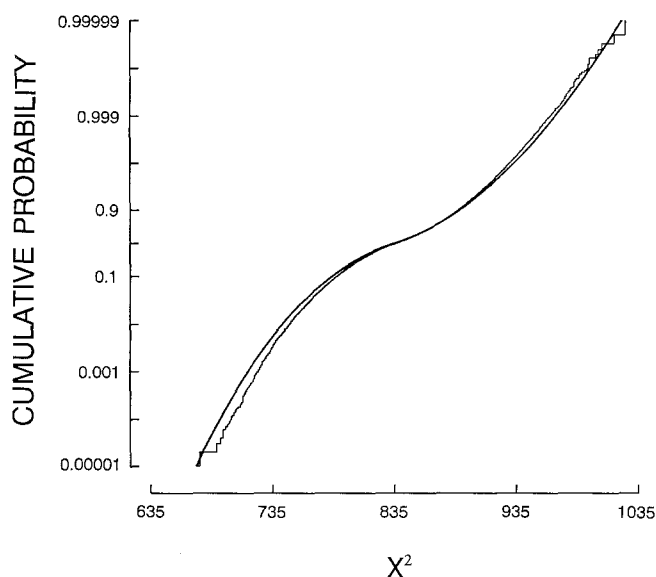


Fig. 5. Cumulative probabilities of observing given values of X^2 . The smooth curve shows the cumulative chi-square distribution for 835 degrees of freedom, calculated using double-precision versions of the routines described by Press et al. (1988). The jagged curve shows the observed distribution of X^2 for 100,000 randomly constructed data sets having the same marginal totals as the real data. Note that the y axis is scaled logarithmically in both directions from $\frac{1}{2}$.

with $\sum (n_L - 1)$ degrees of freedom, where n_L is the number of litters of size L .

Excluding the three litters of sizes one and two ($p_1 = p_2 = 1$), there are 851 litters of 16 different litter sizes (3 to 18). Thus the expected value of $\sum Y_{iL}$ is $851 - 16 = 835$ (its degrees of freedom). The observed value is 574.2, about 69% as large as expected. Almost all published chi-square tables end at 100 degrees of freedom, so we used double-precision versions of the routines described by Press et al. (1988) to calculate the probability of observing a chi-square of 574.2 (or less) with 835 degrees of freedom; this probability is extremely small ($P \approx 3 \times 10^{-13}$).

An alternative approach to the derivation of this test is to view it not as one concerning variances, but rather as one concerning the heterogeneity in a set of $2 \times N$ contingency tables. Let there be one such table for each litter size; columns correspond to litters ($N = n_L$), and rows correspond to sexes; thus the (i, j) th cell contains the number of offspring of sex i in the j th litter. The column marginals are all equal to L , and the row marginals are the total numbers of male and female offspring in litters of size L . $\sum Y_{iL}$ (as derived above) is mathematically identical to the sum of chi-square over a set of such $2 \times N$ tables. Each table has $(n_L - 1)$ degrees of freedom, so in the present case there are again 835 degrees of freedom in all. An observed $X^2 (\sum Y_{iL})$ smaller than the degrees of freedom indicates less heterogeneity in the table than expected; this is equivalent to the previous interpretation, which was cast in terms of variances.

Having seen that the test can be viewed as an analysis of contingency tables, we are naturally prompted to ask whether there might be a problem concerning the number of expected observations per cell in columns corresponding to small litters. Obviously, for litter sizes smaller than 10, one of the two cells in each column must have an expected value smaller than five, which is the value commonly cited as a minimum required for

safe use of the chi-square approximation. On the basis of extensive simulations, Lewontin and Felsenstein (1965) concluded that the chi-square test is *conservative* (the risk of falsely rejecting the null hypothesis being *less* than the formal significance) at the 5%, 2%, and 1% significance levels, when applied to $2 \times N$ tables with $N > 5$, even if many cells have expected values less than unity. To see if the test is also conservative in the *lower* tail of the distribution, and to confirm the overall logic of our method and its implementation, we generated 100000 artificial data sets with marginal totals identical to those of the real data, but with sex assigned independently within litters.

The jagged curve in Fig. 5 is the cumulative distribution of X^2 (or $\sum Y_{ij}$) for these artificial data sets. The smooth curve is the cumulative distribution of chi-square for 835 degrees of freedom. The simulated distribution of X^2 closely approximates the theoretical distribution. The error is in the conservative direction in the conventional rejection region of the upper tail of the distribution, as it was for the smaller tables studied by Lewontin and Felsenstein; in the lower tail of the distribution the error is somewhat larger, but still in the conservative direction.

If we ignore the fact that mean sex ratios vary among litters of different sizes, and put all litters of size 3 or greater into a single 2×851 table, then $X^2 = 627.0$ ($d.f. = 850$, $P \approx 1.3 \times 10^{-9}$). In this case the observed variance is about 74% as large as expected.

If we conceptually remove two males from each litter of size 4 or greater, we are left with 841 litters of sizes 2 to 16. (Only one male can be removed from two of the litters of size 5.) The sex ratio of these hypothetical "late" offspring is very close to 0.38 overall, and there is no longer any relationship between litter size and sex ratio. If all the litters are placed in one 2×841 table, then $X^2 = 769.3$ ($d.f. = 840$, $P \approx 0.039$), and the observed variance is about 92% as large as expected. If we successively remove from the sample the smallest litters (2 through 6, which were 4 through 8 in the original data), this pattern remains qualitatively unchanged and the probability values range between 0.020 and 0.046. If each of the 15 litter sizes is placed in its own $2 \times N$ table (as in the original form of the test), then $X^2 = 759.6$ ($d.f. = 826$, $P \approx 0.048$). Thus the formal model discussed in the text can account for most (but not all) of the observed variance reduction.

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