Maternal Diet & Sex of Offspring

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In most vertebrate and invertebrate species the number of male and female individuals in a population is close to 1:1, even though the genetic mechanisms that determine sex are often distinct.

Sex Ratio of Humans

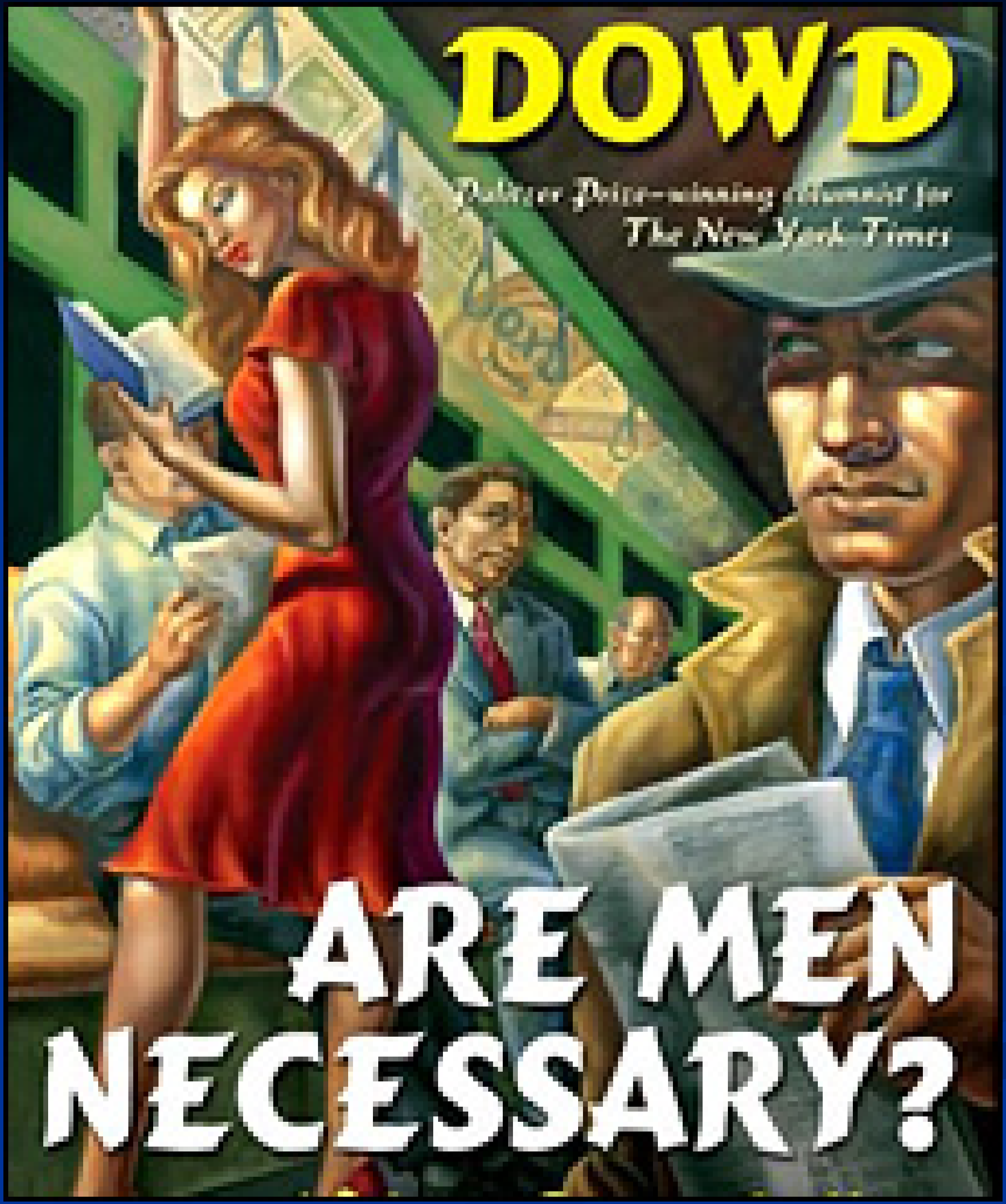
- In general, 105 boys are born for every 100 girls
- Females generally experience lower mortality during childhood, and so the ratio corrects itself in adulthood
- Exceptions to the above rule occur, e.g. in India where mortality of females in childhood and as mothers is unusually high
- In older age, females outnumber males (for people over age 70, there are only about 65 males per 100 females)

Why does the sex ratio remain at about 50:50?

Seems inefficient. Most species are not monogamous and one male can fertilize several females.

Maureen Dowd:

'Are Men Necessary?'



Why does the sex ratio remain at about 50:50?

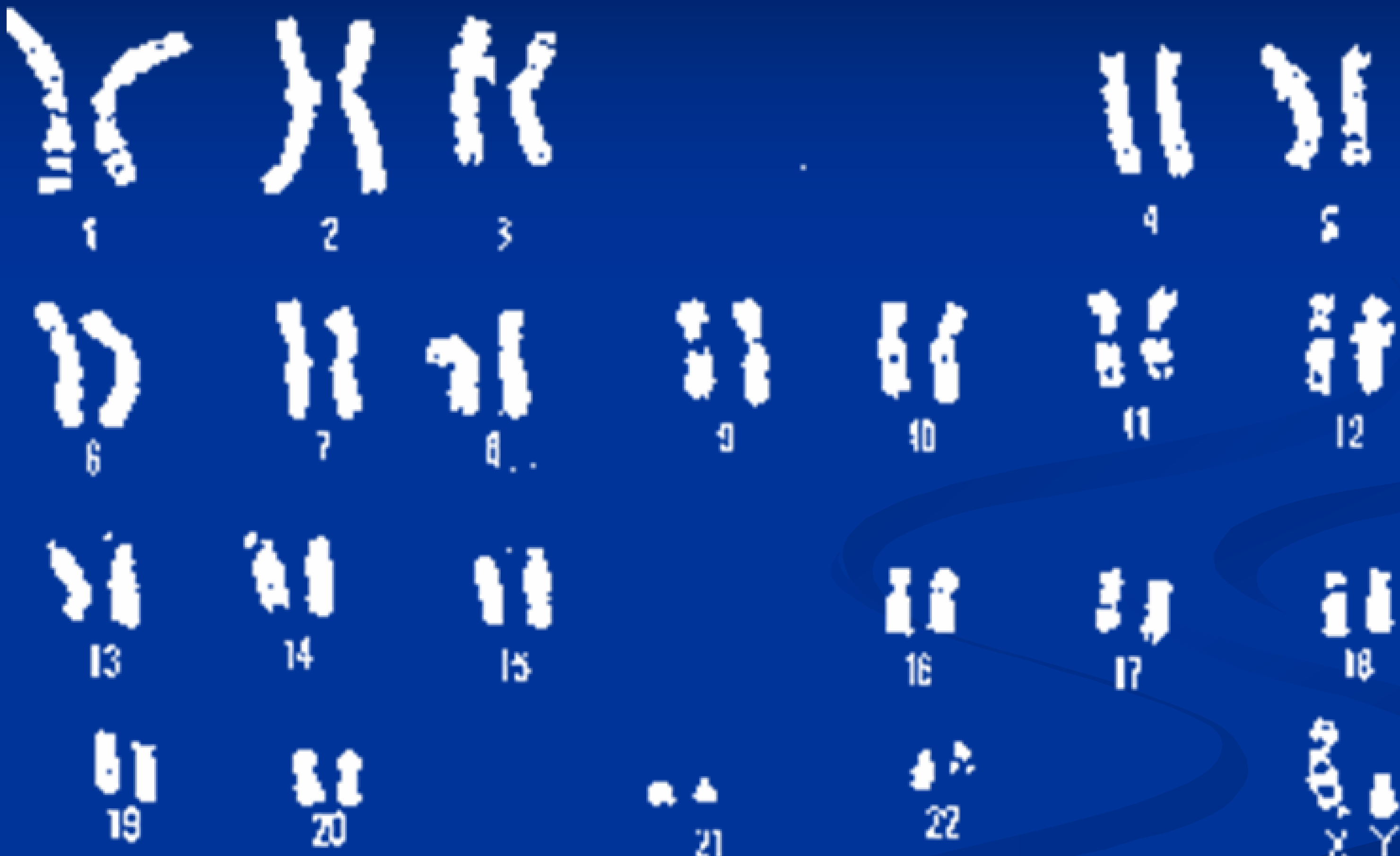
- If there are fewer males than females, then males have a higher chance of mating. There is clearly an advantage to being a male. However, there is also an advantage to being a female that produces more sons than daughters.
- Natural selection will favor females that produce more sons.
- The deviation in the sex ratio will be corrected.
- The rule generally holds regardless of the method of sex determination.

The strategy of producing equal numbers of sons and daughters is an evolutionary stable strategy

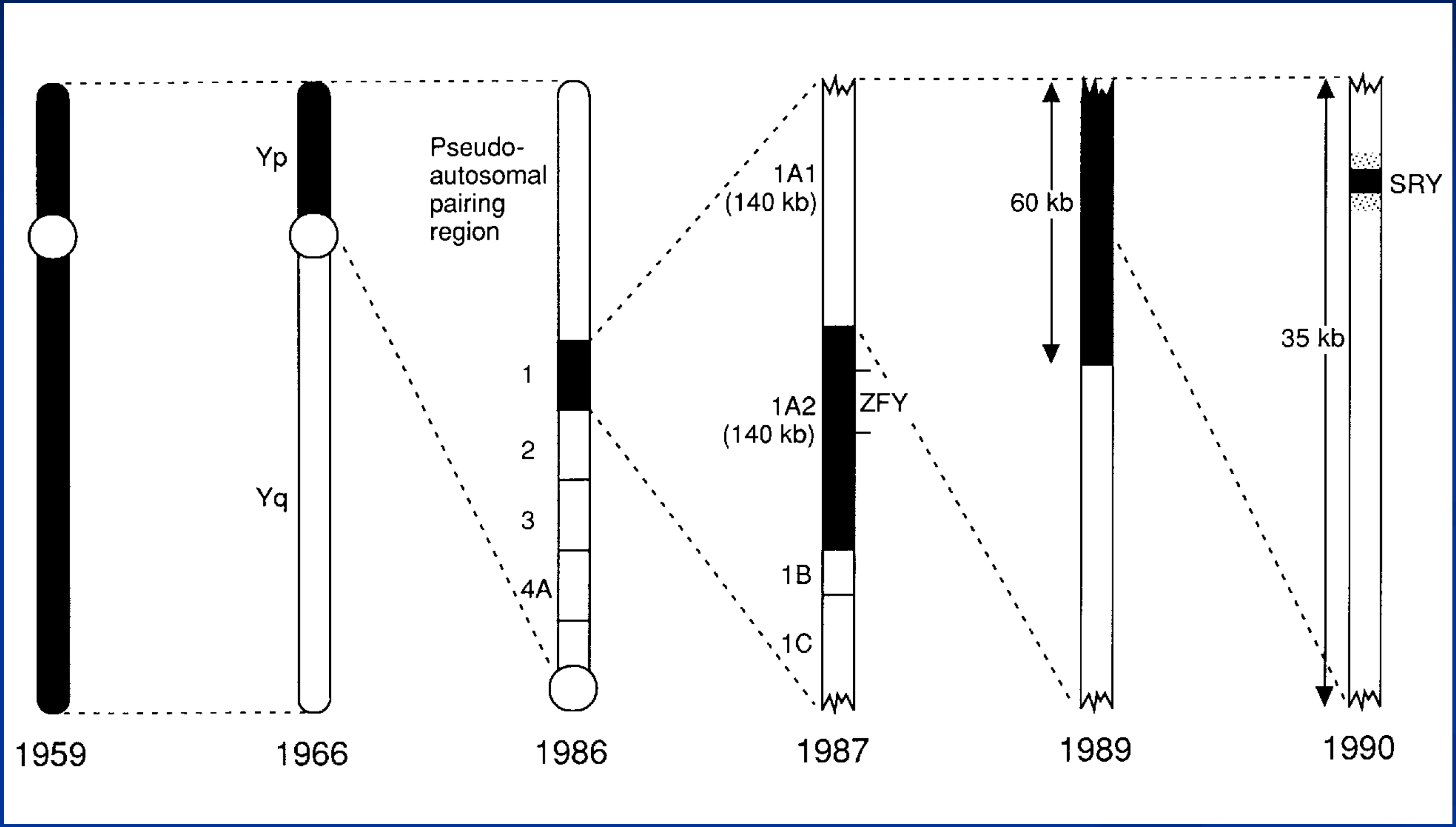
What are the mechanisms underpinning sex determination?

- Mammals, the XX/XY model: male produces X and Y gametes.
- Birds, the ZW/ZZ model: female produces Z and W gametes. Also, butterflies, some reptiles, some fish
- Amphibians, several models: 20 out of 1500 species examined show sex chromosome heteromorphism, but most may have microscopically similar sex chromosomes
- Reptiles: many show temperature-dependent sex determination, but snakes display a ZW system like birds

Chromosomes and Gender



The Search for the Testis-Determining Factor (TDF) on the Y Chromosome

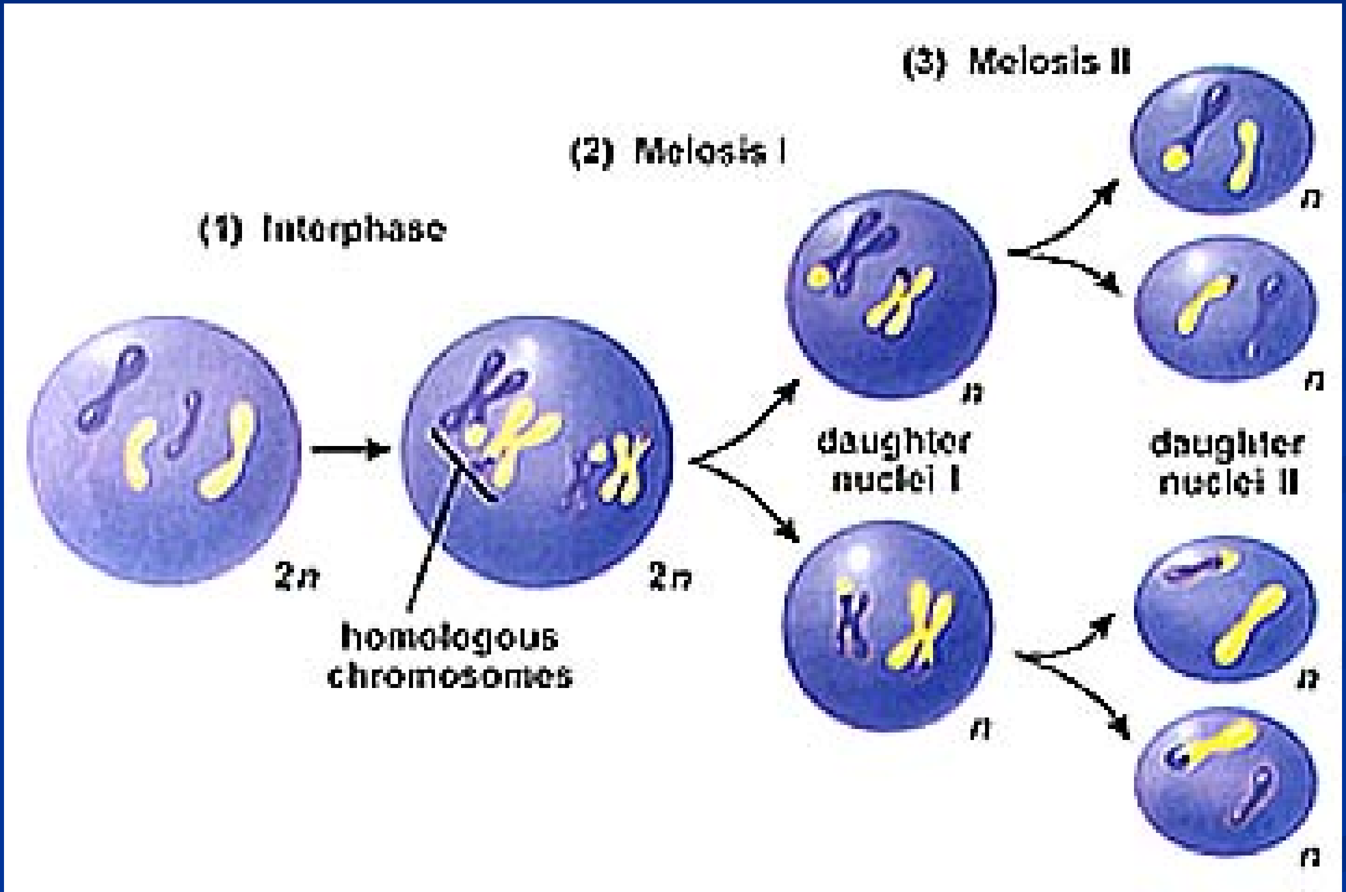


Thirty-one years of hunting the testis-determining factor. The chromosomal region thought to include the elusive factor is shaded. The search has narrowed from 30-40 million bases (1959) to less than to 250 bases encoding for the conserved 80-amino-acid motif of SRY (1990). See text for further explanation. Source: McLaren, A. (1990): *Nature*, 346:216-217.

Meiosis

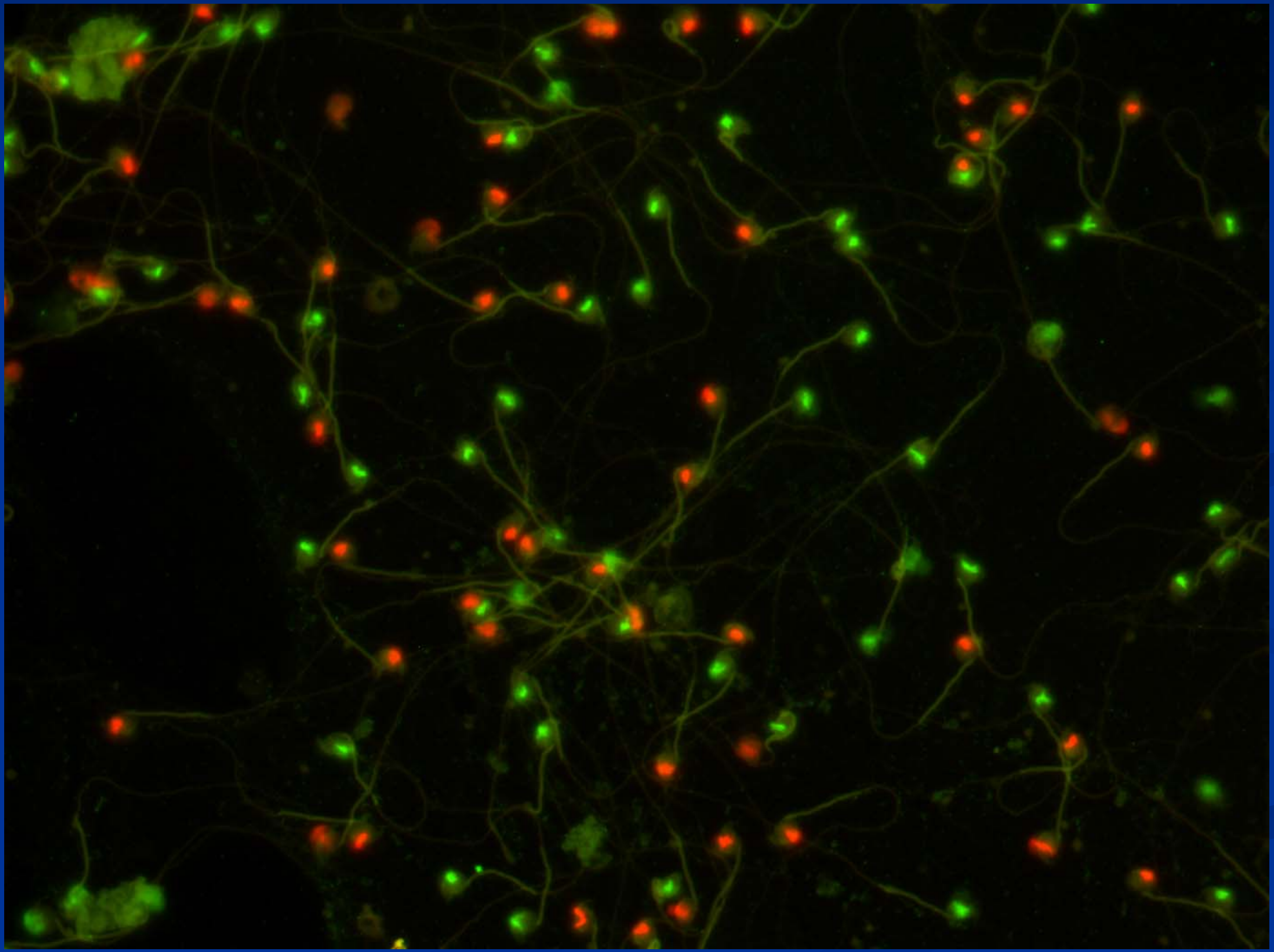
- Required for the formation of gametes
- Pairing of homologous chromosomes
- Crossing over and recombination
- Production of haploid cells that will mature into sperm or egg
- Oocytes will contain only an X-chromosome
- Sperm will contain either an X- or a Y-chromosome.

Overview of the major events of meiosis

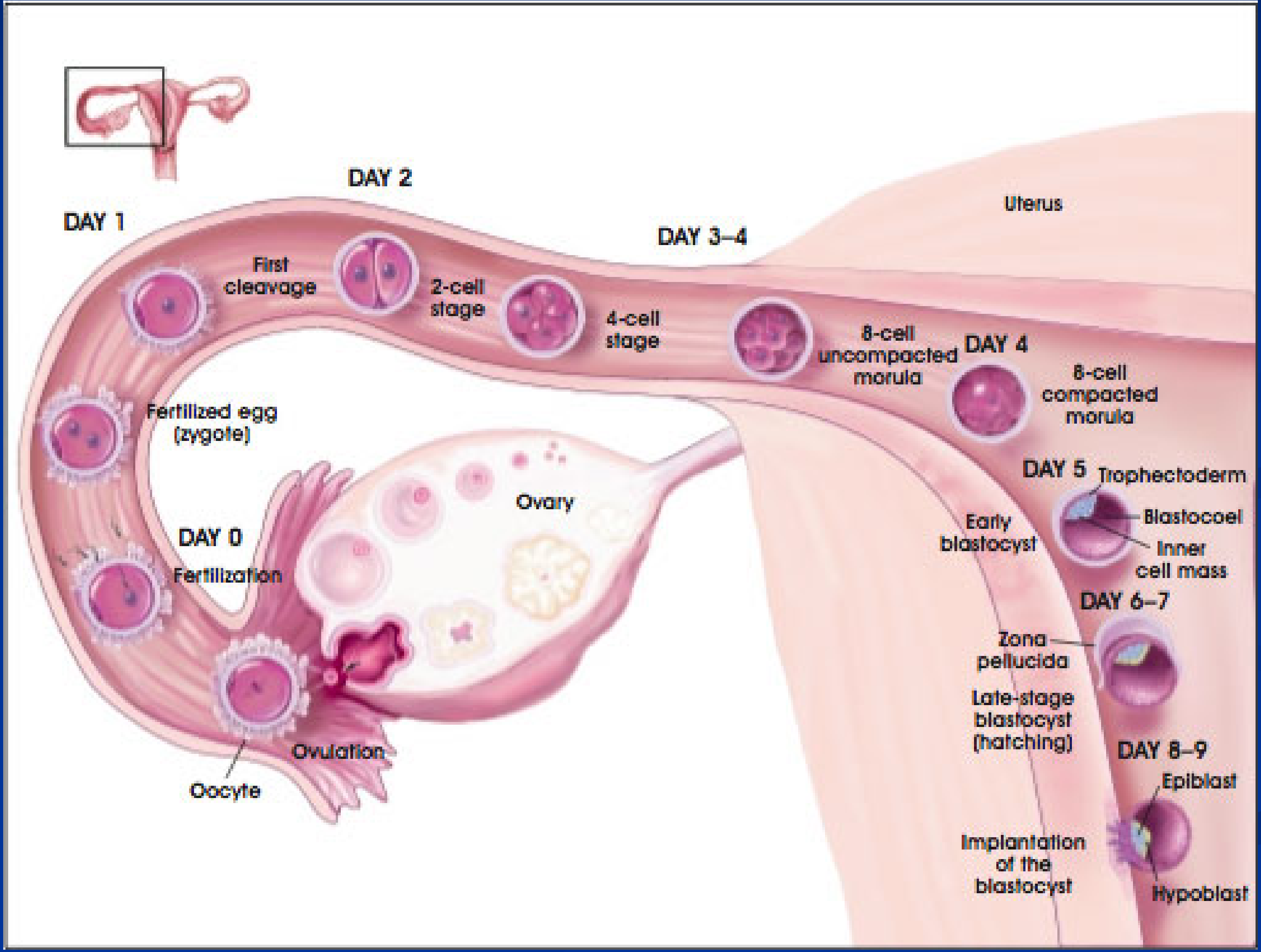


X- and Y-sperm are usually present in approximately equal numbers in ejaculates

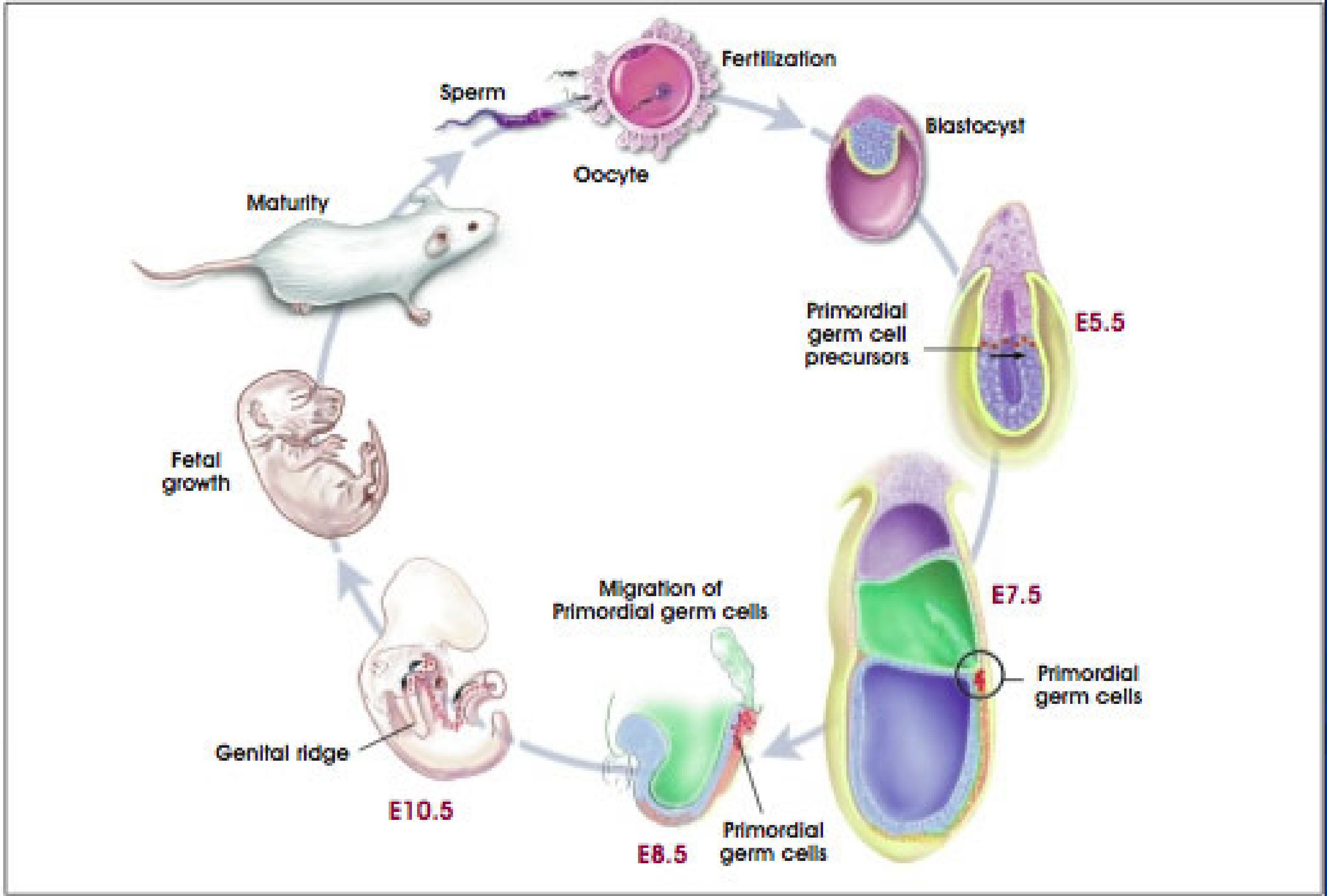
(Photo courtesy of Jeff Whyte)



Development of the preimplantation embryo in humans



Life Cycle of a Mouse



Sex Ratio Deviation in Mammals

"I formerly thought that when a tendency to produce the two sexes in equal numbers was advantageous to the species, it would follow from natural selection, but I now see that the whole problem is so intricate that it is safer to leave its solution to the future."

Charles Darwin, The Descent of Man, 2nd Edition, 1871.

Sex Ratio Deviation in Mammals Is Influenced by Diet

Elk and red deer

(*subspecies* of *Cervus elaphus*)

are highly polygamous.

The sex of the calves varies according to the body condition of the mother and her social status.

Polygyny

- A mating system in which a male has a more or less stable breeding relationship with more than one female, but the females are only bonded to a single male.
- Probably the most common mating system among vertebrates, and is especially common among mammals
- Sexual dimorphism, particularly of size, with males being bigger, more aggressive, better equipped for fighting, and more colorful than females
- Uniparental care of the young, with males contributing less than females or nothing at all

Trivers and Willard Hypothesis

In polygynous mammalian species, females in better body condition will enhance their reproductive success by investing in male offspring.

Red Deer

Dominant hinds, under non-crowded conditions deliver more male than female offspring (65:35).

Female calves born to subordinate hinds is 50% or less.

(Clutton-Brock et al., 1984, 1986, 1999)

Trivers/Willard Hypothesis

If size or vigor influences the reproductive success of one sex much more than the other, then natural selection may favor parents that produce the more expensive sex when they (the parents) are in good condition, and hence can afford to invest heavily in each offspring.

Maternal nutrition and sex ratio of offspring in polygonous species

In some reports, high ranking, better fed, females tend to produce more male than female offspring in:

- Deer (Red deer, Roe deer)
- Reindeer
- Sheep
- Pigs
- Cattle?
- Opossums
- Mice?
- Humans?

Not all reports have been consistent, however.

Can the sex ratio of offspring be influenced by maternal diet in rodents? If so, there will be a better opportunity to study the phenomenon experimentally?

Factors affecting sex ratio of offspring of rodents

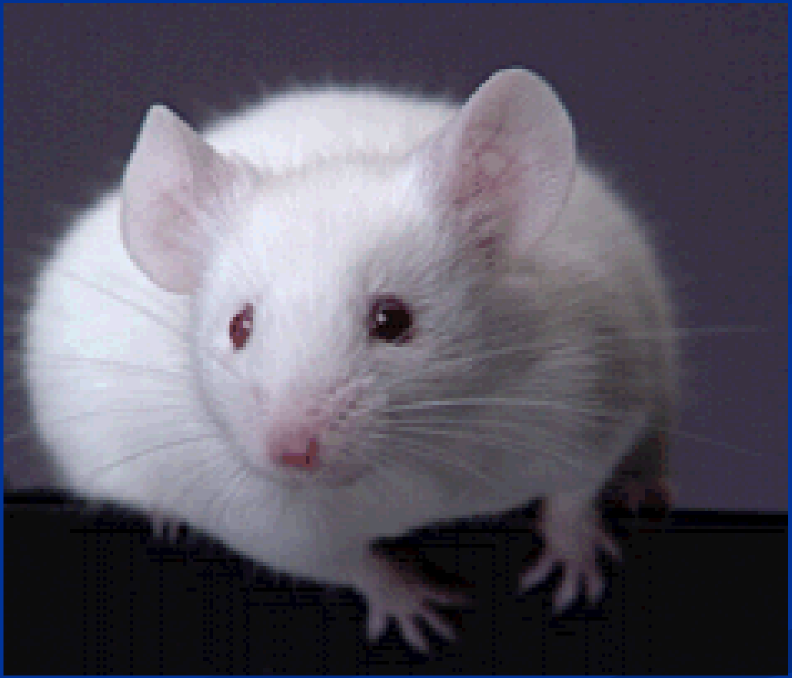
Maternal nutrition: food-deprived mothers bias litters towards females.

Stress: crowded cages and other stressful conditions favor female-biased litters.

Timing of insemination: early insemination gives rise to female biased litters (rats). This observation also holds for mice, deer and sheep, and possibly cattle.

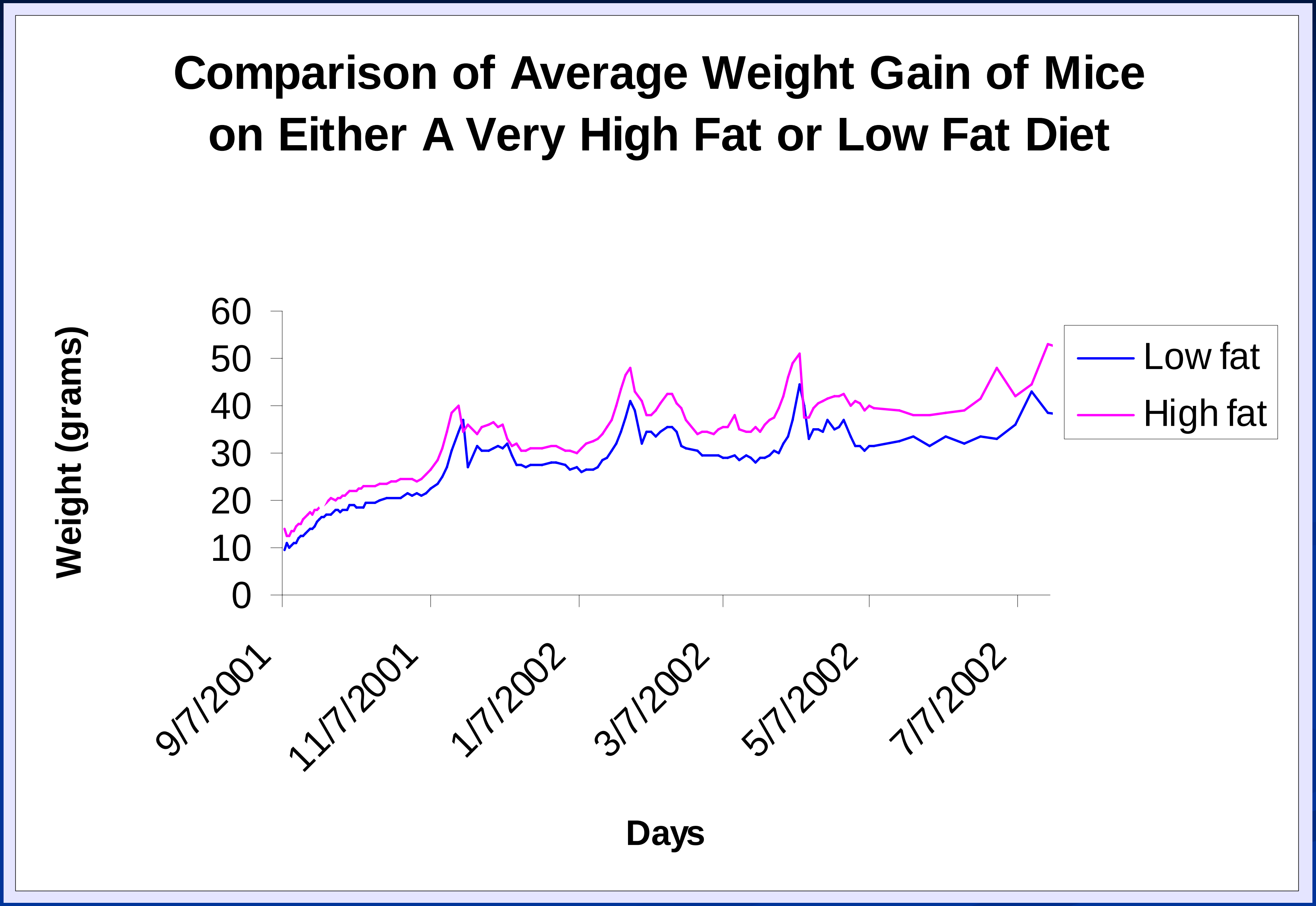
In Vivo Approach to Determine if Feeding a High Caloric Diet to Females Can Alter the Sex Ratio of their Offspring

- Four-week-old NIH Swiss females were placed either on the Very High Fat (VHF) (n=16) or low fat (LF) Diet (n=16) (Research Diets).
- They were bred at 10 to 12 weeks of age.
- The mice were re-bred 2 to 3 weeks after weaning their pups.
- The mice gave birth to their fourth and final litter when they were ~ 45 weeks of age



Relative energy content (Kcal%) of major nutrients in mouse diets.

Diet	D12450B (LF)	D12492 (VHF)	Purina 5015 (CLC)
Protein:	20	20	18
Carbohydrates: Starch Maltodextrin Sucrose	 31 4 35	 0 13 7	 51 NS 1
Total Carbohydrates:	70	20	56
Fats: Soybean oil Lard	 6 4	 6 54	 NS NS
Total Fat:	10	60	26


Comparison of Average Weight Gain of Mice
on Either A Very High Fat or Low Fat Diet
Weight (grams)
60
50
40
30
20
10
0
9/7/2001
11/7/2001
1/7/2002
3/7/2002
5/7/2002
7/7/2002
Days
Low fat
High fat

Weight at Time of Breeding for the Mice Fed the Two Diets

Treatment	Litter Number	Weight at Time of Breeding (g)
LF (n=16)	1	20.8
	2	26.7
	3	29.4
	4	30.8
VHF(n=16)	1	23.1
	2	30.6
	3	35.7
	4	38.0

Litter Size and Gestation Length in Mice Fed the Two Diets

Treatment	Litter Number	Litter Size	Length of Pregnancy (d)
LF (n=16)	1	9.4	20.0
	2	10.8	19.8
	3	9.1	19.3
	4	9.1	20.0

Treatment	Litter Number	Litter Size	Length of Pregnancy (d)
VHF(n=16)	1	9.5	19.6
	2	10.7	18.8
	3	9.9	20.0
	4	8.6	19.9

Male Pups per Litter and Litter Skewing in Mice Fed the Two Diets

Treatment	Litter Number (n, number bred)	Fraction of Male Pups	Number of Male-Biased Litters
LF	1 (n=15)	0.48	3
	2 (n=14)	0.45*	4
	3 (n=15)	0.35**	1
	4 (n=10)	0.38*	0
VHF	1 (n=16)	0.51	10
	2 (n=15)	0.66**	12
	3 (n=14)	0.65**	12
	4 (n=9)	0.71**	7

Why is there no skewing of sex ratio at litter 1?

Parity Effect?

Age of mothers?

Effect of diet on sex ratio of first litter born to mature mice, aged 20-27 weeks before breeding

Diet	Conception Weight (g)	Litter Size	Gestation Length (d)	Sex Ratio	Number of male-biased litters
LF (n=14)	31.0±4.9	9.2±3.6	20.4±1.5	0.38[a]	2
VHF (n=11)	41.4±7.4	9.1±3.4	20.4±1.7	0.64[a]	10

Summary of mouse feeding experiments

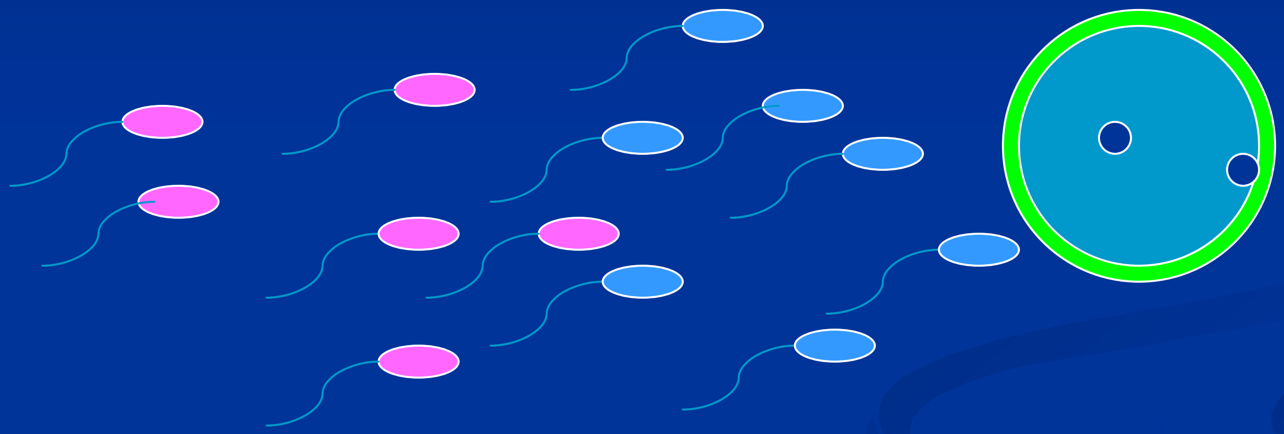
- Mice on VHF and LF diets did not differ in gestation length or in numbers of pups born.
- After their first pregnancy, VHF mice produced male-biased litters, LF mice female-biased litters.
- The sex bias effects were the result of the diets and were not related to the weights of the mice.
- The sex bias effects resulting from the VHF and LF diets were also observed in first litters of (virgin) mice that were older than 25 weeks of age.

What are the causes of the sex bias observed in mice?

Possible Basis for Sex Ratio Skewing *in Utero*

- Differences between X- and Y-sperm in their abilities to reach the egg
- Differences between X- and Y-sperm in their ability to penetrate the zona
- Differences in fitness of male and female pre-implantation embryos
- Differences in fitness of male and female post-implantation embryos

Do X- and Y-sperm have equal fertilizing ability in the oviduct?

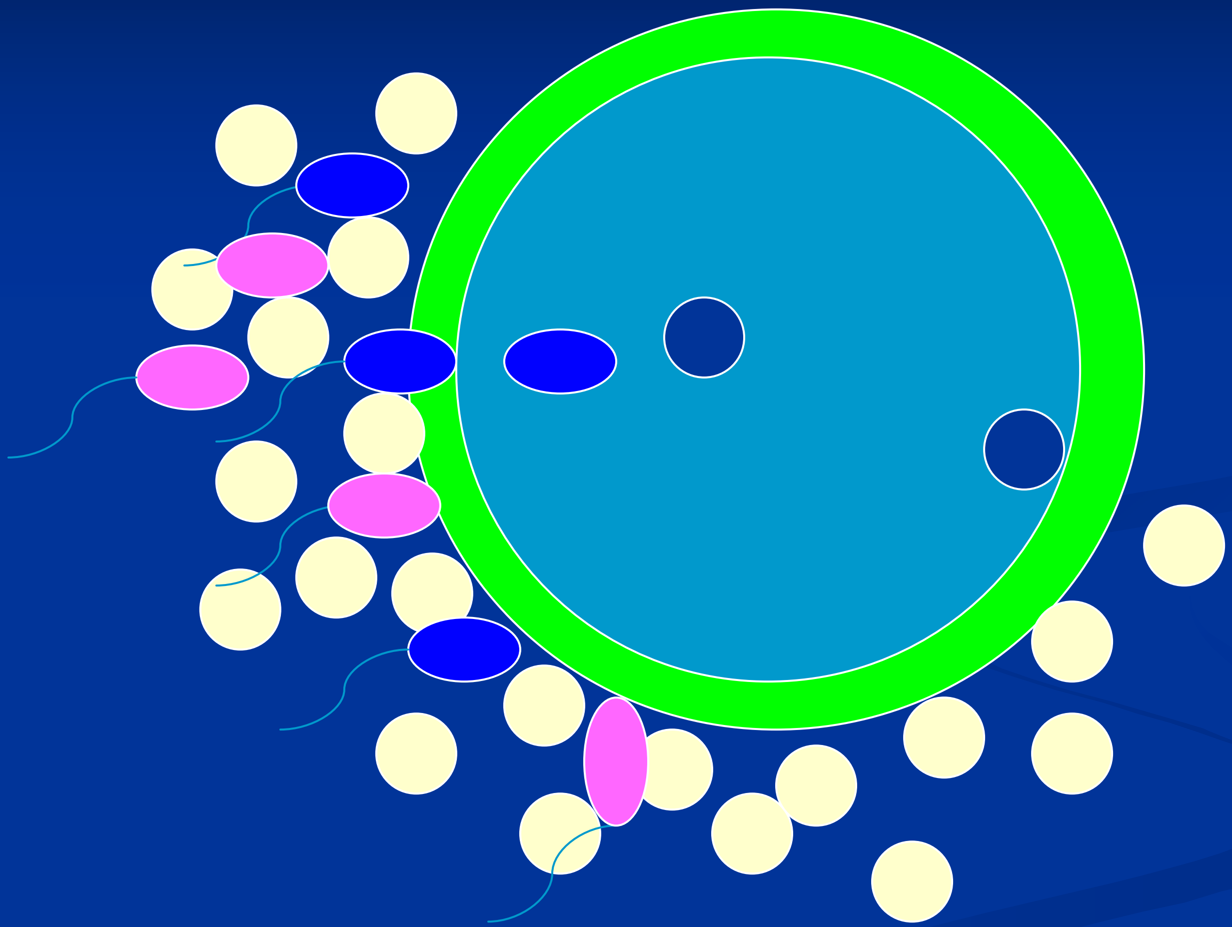


Y-sperm reach the egg more quickly than X-sperm

Possible Basis for Sex Ratio Skewing *in Utero*

- Differences between X- and Y-sperm in their abilities to reach the egg
- **Differences between X- and Y-sperm in their ability to effect fertilization**
- Differences in fitness of male and female pre-implantation embryos
- Differences in fitness of male and female post-implantation embryos

Do X- and Y-sperm have equal fertilizing ability in the oviduct?

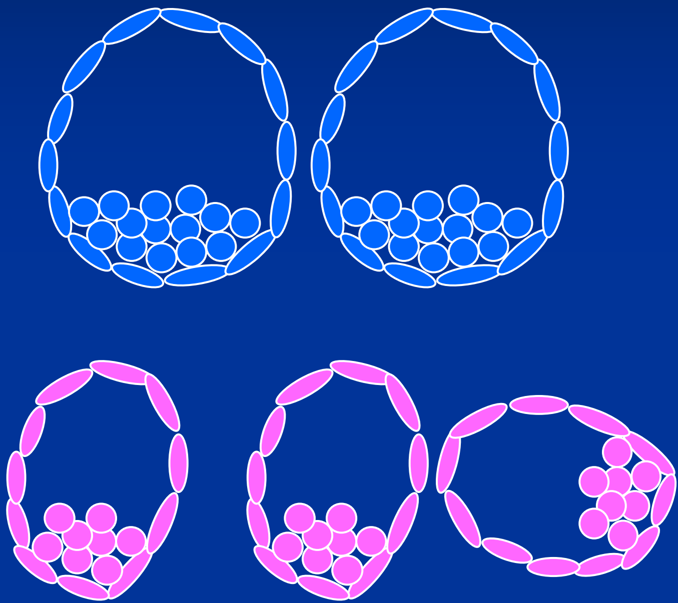


Y-sperm penetrate the cumulus and zona more efficiently than X-sperm

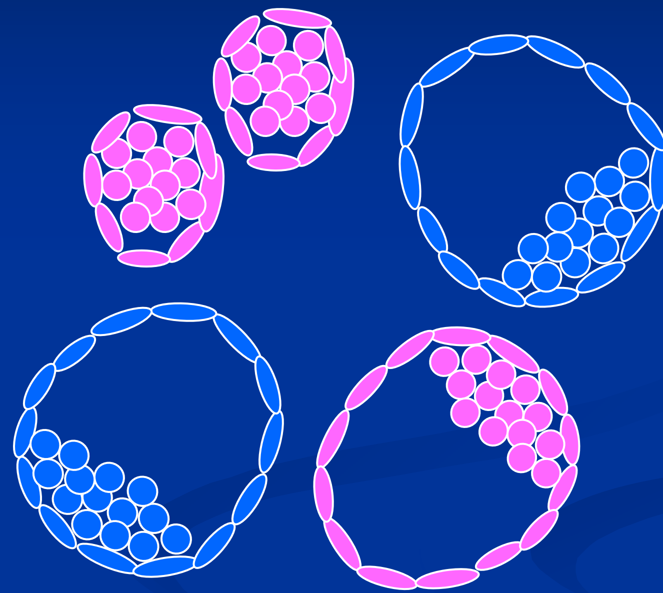
Possible Basis for Sex Ratio Skewing *in Utero*

- Differences between X- and Y-sperm in their abilities to reach the egg
- Differences between X- and Y-sperm in their ability to penetrate the zona
- Differences in fitness of male and female pre-implantation embryos
- Differences in fitness of male and female post-implantation embryos

Sexual dimorphism among embryos may lead to more developmental failure among embryos of one sex than the other



Embryos of one sex grow and develop faster than embryos of the other sex, depending upon conditions in the uterine tract, and are at an advantage at implantation



A subset of embryos of one sex fail to make an appropriate transition, e.g. from morula/early blastocyst to expanded blastocyst as a result of conditions in the uterine environment

Possible Basis for Sex Ratio Skewing *in Utero*

- Differences between X- and Y-sperm in their abilities to reach the egg
- Differences between X- and Y-sperm in their ability to penetrate the zona
- Differences in fitness of male and female pre-implantation embryos
- Differences in fitness of male and female post-implantation embryos

Implications?

Agriculture

Reproduction in the 21st Century Dairy Cow

Muranda Oscar Lucinda-ET
67,914 pounds milk 2x 365-day

Is there a bias towards production of bull calves on well managed dairy farms?

Years: 1997-2001

Milk production/cow: ~23,000 lb

Number of calves born to cows: 466 (male, 59%; $p < 0.05$)

Number of calves born to heifers: 282 (male, 50%)

Cows' feed supplemented with Hershey's chocolate

Data provided by Dr. Jennifer Juengel

Endangered Species?

Maternal Manipulation of Offspring Sex Ratio in the Kakapo

From Sutherland WJ. Science, sex and the kakapo. Nature 2002; 419:265-266.

- Flightless parrot.
- Sex ratio of offspring determined by the nutritional condition of the mother.
- Food supplementation avoided until after egg production → 15/24 young fledged were female (Sutherland, 2002).

What about humans?

Diet effects on sex ratio in humans

- Sex ratio falls during famine (Dutch famine after world war II, and examples from Africa)
- Italian women in the lowest quartile of pregnancy body weight (less than 54.6 kg) produce more girls than boys (0.497 versus 0.525) (Cagnacci et al. 2004)
- Changes are generally attributed to greater attrition of male fetuses in women with non-optimal reproductive conditions.

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Maternal condition influences sex ratio in house wrens (Whittingham et al., 2001)

- Females in good condition produce more male offspring than female
- Correlation was strongest in the second brood
- Positive relationship between female body condition and proportion of sons
- Females in good body condition also provisioned their young better
- Extra pair mating common in wrens
- Males in good condition may be preferred breed

Polygamy in Elk

- There are generally fewer bulls than mature females
- Dominant bulls form harems
- Young bulls must compete with larger, formidable bulls for access to females
- Female choice?

Potential Problems with Polygamy

- A genetic bottleneck and loss of diversity
- Older males may produce sperm with more mutations
- Inbreeding and expression of deleterious recessive alleles

Human Polygyny

- The majority of human societies have probably permitted polygyny. It was accepted in ancient Hebrew society, in classical China, and in Islam. It was accepted in many traditional African and Polynesian cultures. In India, polygyny was practiced from ancient times onward, though historically only kings were polygynous in practice
- Polygyny was not accepted in ancient Greece or Rome, and has never been accepted in mainstream Christianity (although it was practiced in the early Mormon church and survives in certain Mormon sects). The political and economic dominance of (at least nominally) Christian nations from the sixteenth to the twentieth century has meant that on the world scale polygyny is legally recognised in very few nations. Although many Muslim majority countries still retain traditional Islamic law which permits polygyny, certain liberal movements within Islam continue to challenge its acceptability.

Human Polygyny

Since the number of human males and females born is approximately equal, if some men have more than one wife, that necessarily deprives other men from obtaining even one wife.

How could polygyny work in a stable society?

- Men marry late (30+ years) and women marry early (mid teens, or earlier). This limits the number of men who can marry and prohibits the younger men from interfering. At the same time, because of mortality, the number of women available is always larger than the number of men seeking spouses. Some societies have formal age grades for males, and no man may marry until he succeeds into the highest grade. Younger age grades are used as a military force or for labor details.
- High male mortality from warfare, feuding, occupational accident, and disease. Not only are the men too involved in these activities to consider marriage, but the number arriving at the marriageable age is reduced. Again, this means that fewer men than females are marriageable.

- Bride price or bride service.

Stress and Sex ratio

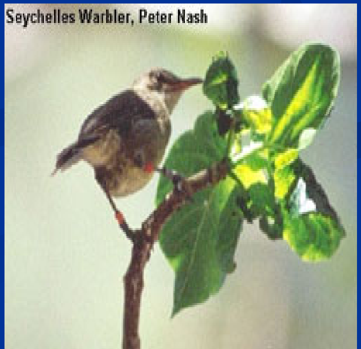
- Sex ratios in the two Germanies: sex ratio in East Germany lowest in 1991 when economic stress was high (Catalano, 2003)
- Conflicting data on the effects of war
- Ambient stressors as common as increasing unemployment elevate the risk of fetal death among males: again controversial
- Environmental toxins, e.g. dioxin, and fewer males born

Explanations for the effects of stress

- Stressed females abort males selectively
- Stressed males exhibit reduced sperm motility, presumably of their Y-sperm
- Stress effects on sperm formation

Quality of the territory and number of helpers influences sex ratio of offspring of the Seychelles warbler

- Parents on high quality territories tend to have few female helpers from previous broods and produce female-biased broods
- Conversely, parents on poor quality territories tend to have more helpers and produce male-biased broods
- Switching territories reverses the sex ratio
- Removing helpers drives sex ratio towards females



Adaptive Sex Allocation in Fig-Pollinating Wasps

Extreme sex ratio adjustments are noted in in fig-pollinating wasps and confirm many of the tenets of evolutionary theory. There are many species of fig-pollinating wasps, and in each case, female wasps pollinate and lay eggs in the enclosed fruit of their own host fig species. Mating occurs between the wasps that develop in the same fruit, before the females disperse. Typically, if only a single female lays eggs in a fruit, she produces an extremely female-biased sex ratio (only 5 to 10% of the offspring are males). As the number of females laying eggs in a fruit increases, i.e. food is scarcer, the sex ratios in the broods become less biased. Although there are deviations between observed sex ratios and those predicted by theory, the fit is often very close.

Sex Ratio Distortion in Birds

- In general, birds produce roughly equal numbers of male and female offspring
- Changes in sex ratio have been observed in response to food availability, female body condition, and local ecology
- The heterogametic sex in birds (and also butterflies and fish) is the female
- Z-W system: female gamete can either carry a Z- or W-chromosome. Males are ZZ

The effect of diet on the number of CL and number of one-cell zygotes recovered from CF1 females the morning after copulation.

Diet	**Number of CL***	**Number of Zygotes***
5015	14.2 ± 4.5 (n=7)	11.9 ± 5.2 (n=7)
LF	14.5 ± 2.6 (n=4)	14.3 ± 3.0 (n=4)
VHF	14.5 ± 1.0 (n=4)	15.3 ± 2.5 (n=4)

*Neither number of CL nor number of 1-cell zygotes differ across groups. All mice were >20 weeks of age.

Number of CL versus number of 1-cell embryos for CF1 mice.

Mean number of CL	14.6 ± 3.8[a] (n=15)
Mean number of zygotes	13.4 ± 4.0[a] (n=15)
Mean number of pups VHF diet	10.1 ± 3.7[b] (n=27)
Mean number of pups LF diet	10.6 ± 3.4[b] (n=25)

Values with different superscripts a, b differ significantly ($p<0.01$).

All mice in these studies were >15 weeks of age.

Effect of low fat (LF) and a very high fat (VHF) diet on sex of pups in three successive litters of CF1 mice bred at approximately 10, 17 and 24 weeks of age.

Treatment	Litter	Number of mice bred	Number of pups	Fraction male pups
	1	8	106	0.56
LF	2	8	88	0.55
VHF	3	8	88	0.42*
	1	8	102	0.57*
VHF	2	8	102	0.66*
	3	6	61	0.64**

* Deviated significantly from 0.5 ($p<0.05$).

**Deviated significantly from 0.5 ($p<0.01$).

Differences between treatments (LF versus VHF) were significant ($p<0.01$) at litters 2 and 3.

Comparison of three diets (5015, LF and VHF) on sex of pups born to CF1 mice first bred at 10 weeks of age.

Treatment	Number of mice bred	Number of pups	Fraction male pups
5015	12	145	0.54
LF	12	136	0.43*
VHF	12	137	0.58*

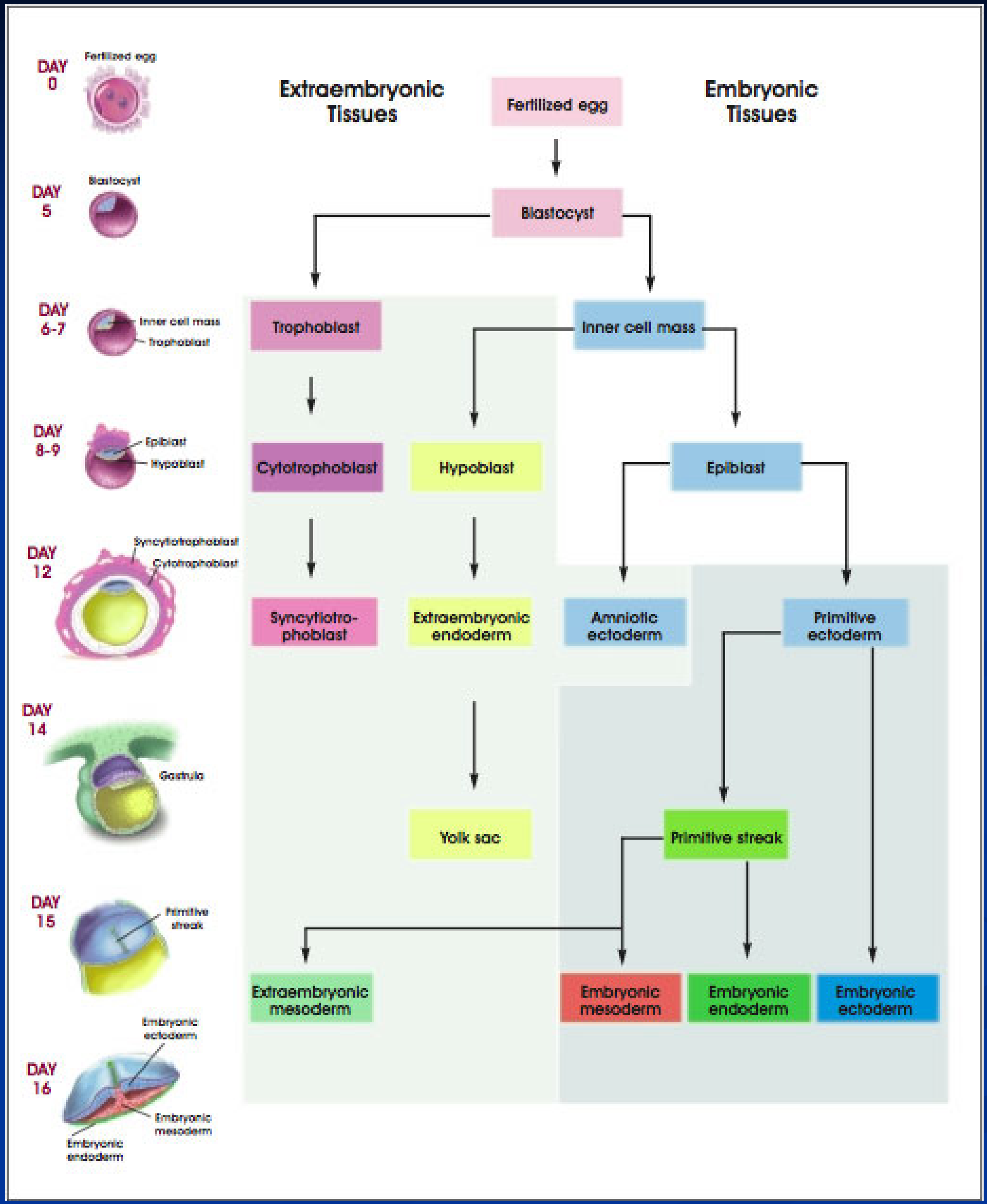
*Deviated significantly from 0.5.
LF and VHF diet groups differed significantly ($p<0.01$).
LF and 5015 diet groups differed significantly ($p<0.01$).

Effect of low fat (LF) and a very high fat (VHF) diet on sex of pups born to NIH Swiss mice bred at 15 weeks of age.

Treatment	Number of mice bred	Number of pups	Fraction male pups
LF	13	109	0.39**
VHF	13	110	0.62**

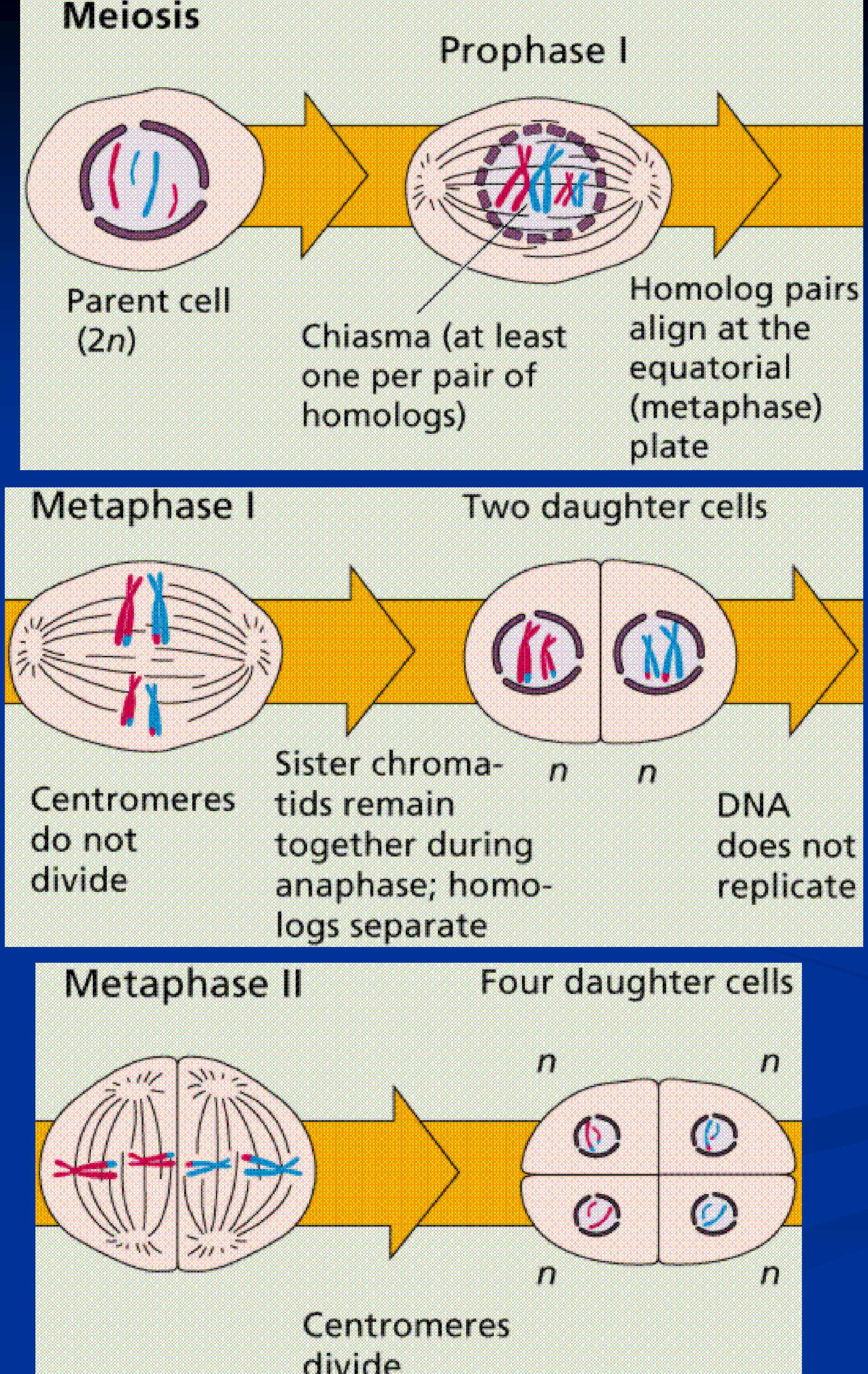
**Deviated significantly from 0.5 ($p<0.01$).

Sex ratio of LF and VHF diets deviated significantly ($p<0.001$).

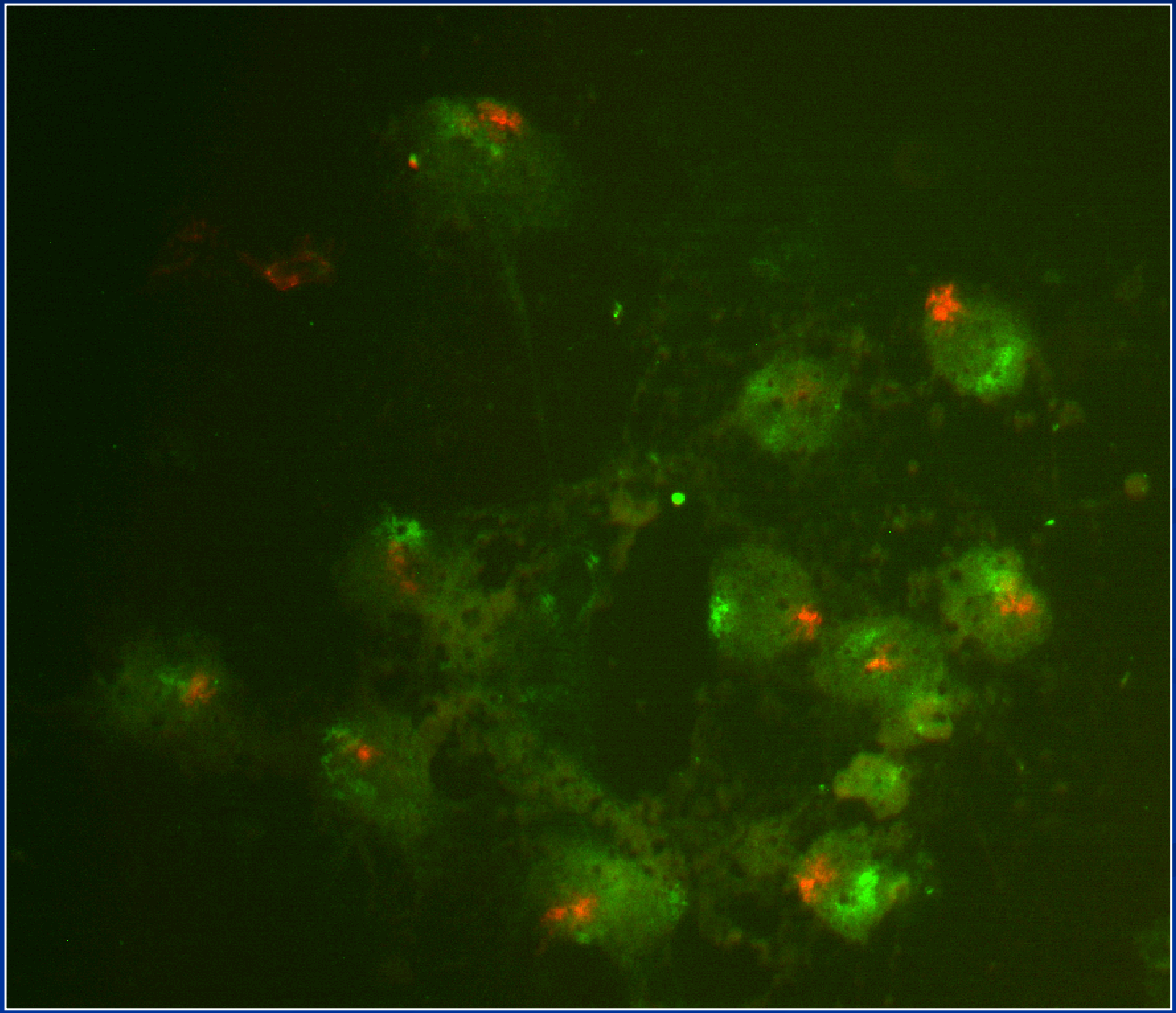


DAY 0
Fertilized egg
DAY 5
Blastocyst
DAY 6-7
Inner cell mass
Trophoblast
DAY 8-9
Epiblast
Hypoblast
DAY 12
Syncytiotrophoblast
Cytotrophoblast
DAY 14
Gastrula
DAY 15
Primitive streak
DAY 16
Embryonic ectoderm
Embryonic mesoderm
Embryonic endoderm
Extraembryonic Tissues
Fertilized egg
Embryonic Tissues
Blastocyst
Trophoblast
Inner cell mass
Cytotrophoblast
Hypoblast
Epiblast
Syncytiotro-phoblast
Extraembryonic endoderm
Amniotic ectoderm
Primitive ectoderm
Yolk sac
Primitive streak
Extraembryonic mesoderm
Embryonic mesoderm
Embryonic endoderm
Embryonic ectoderm

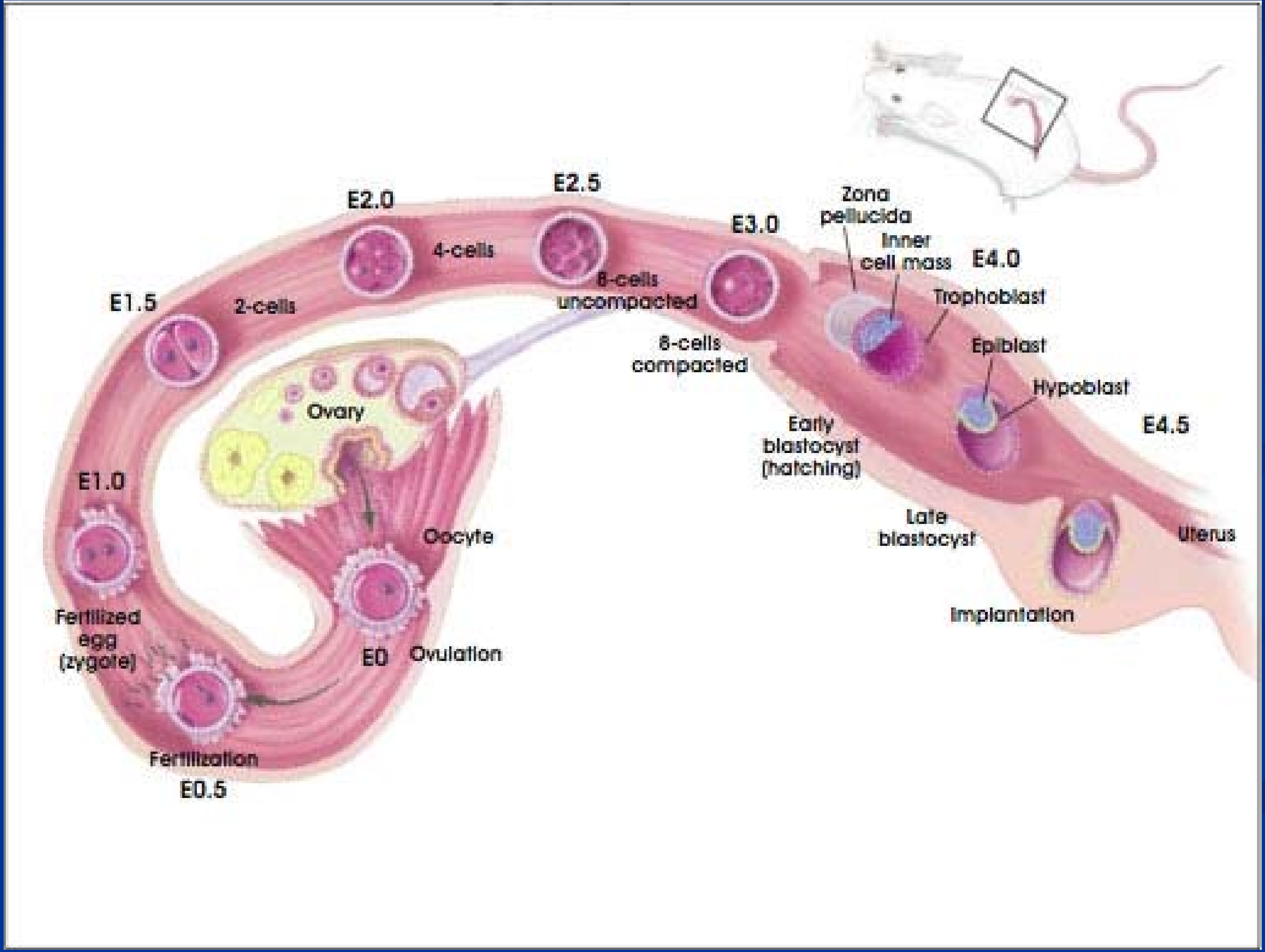
Meiosis. Images from Purves et al., Life: The Science of Biology, 4th Edition, by Sinauer Associates (www.sinauer.com) and WH Freeman (www.whfreeman.com), used with permission.



Nuclei from male embryos have an X- and a Y-chromosome



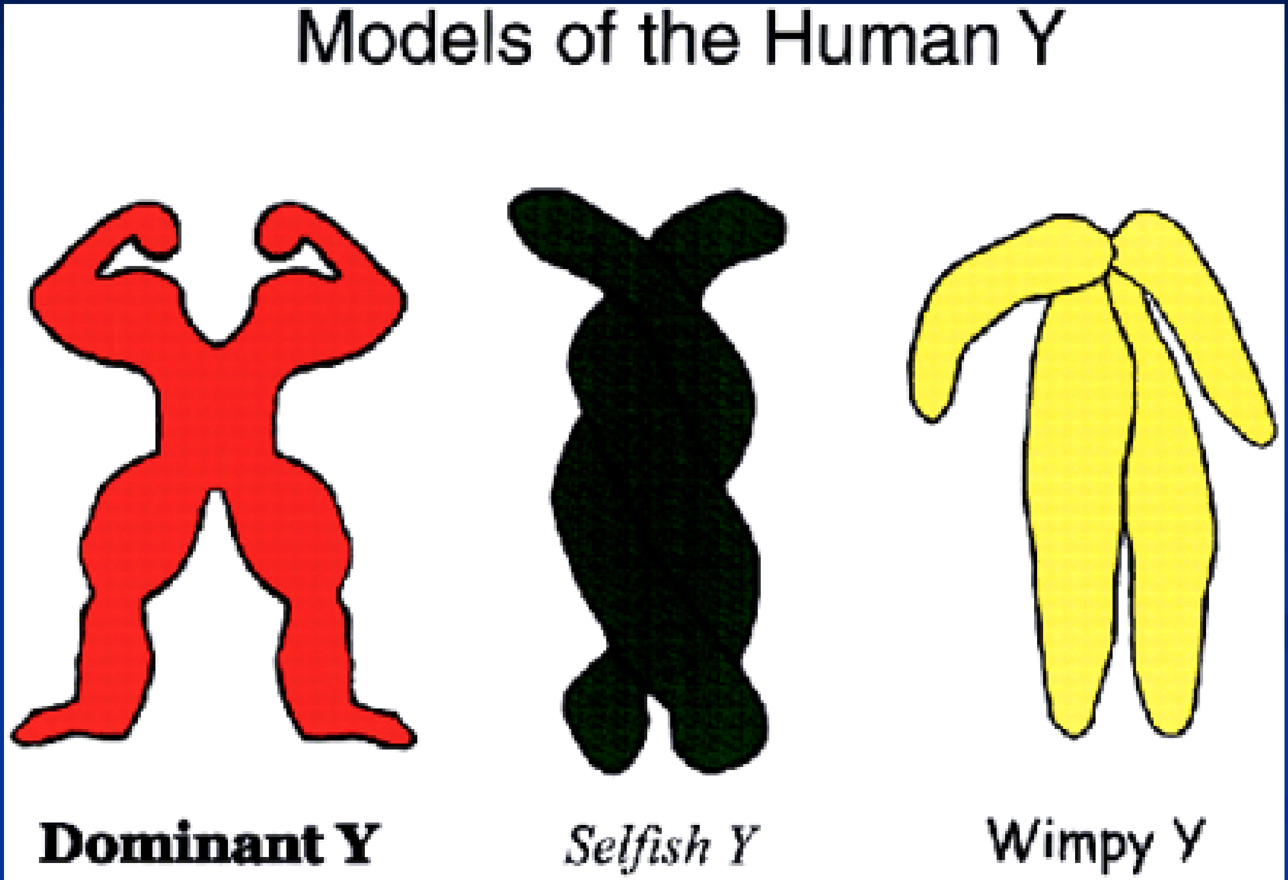
Development of the preimplantation mouse embryo



Sex Ratio Divergence

- Reptiles and nest temperature
- Parasitic wasps
- Ants, bees, wasps (males develop from unfertilized eggs)

- Fig wasps
- Seychelles warblers Komdeur et al.)
- Lesser black-backed gull (Nager et al. 1999) Produced more eggs of sex with greatest survival prospects


Models of the Human Y
Dominant Y
Selfish Y
Wimpy Y

Comparison of the X and Y chromosomes

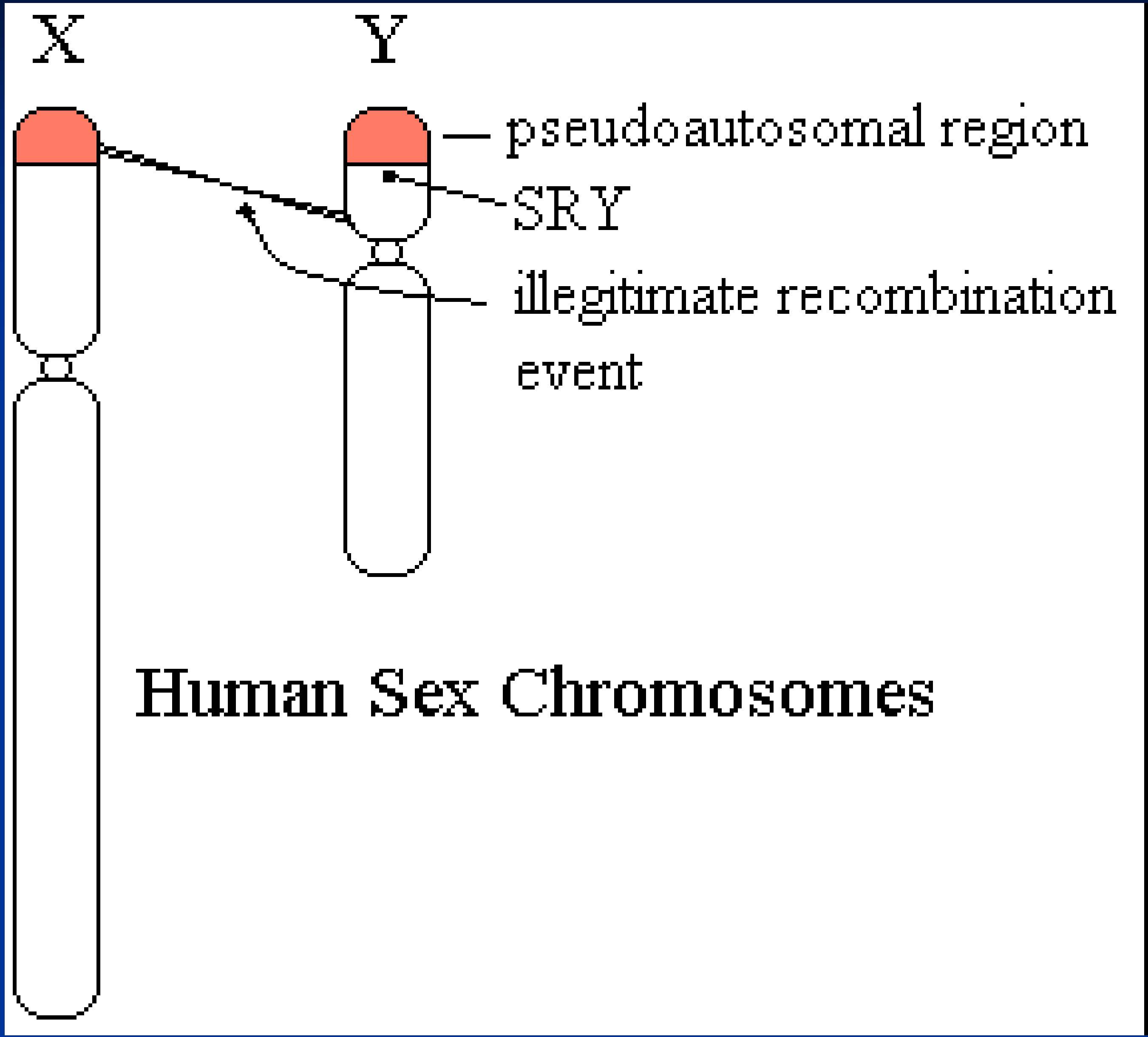


Table 7. Embryo recovery and development, and sex of blastocysts cultured from the zygote stage, recovered from CF1 mice on the 5015 (control), LF and VHF diets.

Treatment	Number of mice bred	Zygotes collected	Number of 2-cell embryos	Number of Blastocysts*	Male	Female	Fraction male pups	Number male-biased litters
5015	**14**	**173[a]**	**146**	**121 (70%)**	**69**	**48**	**0.59**	**7**
LF	8	123	83[a]	76 (62%)	43	30	0.59	5
VHF	**8**	**119**	**103[b]**	**86 (72%)**	**56**	**29**	**0.66****	**7**

[a] Includes one flush of 13 zygotes that failed to cleave.
[b] Includes one flush of 10 zygotes that failed to cleave.
* Sexing failed on four 5015, three LF and one VHF blastocyst.
** Sex ratio deviates significantly ($p<0.05$) from 0.5.

Do Implanted Conceptuses Of One Sex Survive To Term Better Than The Other?

- **Competition for space and maternal resources?**
- **Maternal nutrition favors one sex over the other?**
- **Maternal stress, such as crowding, temperature, and low energy intake, favors one sex over the other?**

Possible Effects of Diets on Maternal Physiology

- pH or ionic conditions in the reproductive tract (hamsters)?
- Time of mating (rats, sheep, cattle)?
- Circulating steroid hormones: progesterone/testosterone/corticosteroids?
- Glucose and other metabolites in the tract?
- Endocrine disruptors?

Androgenized Mice

- Dominance effects
- Aggressive towards introduced males
- Poor nesting instincts
- Produce more sons than daughters
- Subsequent generation effects?
- Dominance and testosterone in women

Power analysis for determining the number of embryos or pups needed to provide a significant skewing of sex ratios.

Number of pups/embryos		
Sex Ratio (fraction male)	$p<0.01$	$p<0.05$
0.30	30 (4)	24 (3)
0.35	38 (5)	32 (4)
0.40	58 (7)	48 (6)
0.45	114 (13)	94 (11)

These calculations, based on a one-tail test, assume that each pup or embryo within a litter is an independent sample. Figures in parentheses are the approximate number of NIH Swiss litters needed to provide significance assuming a litter size of ~9.

Richard Dawkins: The Selfish Gene

The argument of this book is that we, and all other animals, are machines created by our genes.