Chapter Ten
Sex Determination in Insects
Sexual Reproduction

Results in genetic exchange, variation, diversity

Meiosis → genes from different parents are combined in single descendant

Segregation and recombination during meiosis → new variability
Sexual Reproduction

Sex determination and sex allocation have both fundamental and applied implications.

Sexual reproduction has costs:

- Mating expends energy.
- Males are expensive (50%).
- Sexual selection can lead to maladaptations & competition.
Sexual Reproduction

Sexual reproduction has costs

Fixation of novel types difficult due to recombination and segregation

Sexual species have to find mates, which is difficult in sparse populations

Sexual species prone to sexually transmitted diseases (incl. TEs)
Large Advantages of Sex

Theories to explain why sex persists

- Able to incorporate and accumulate favorable mutations
- May allow accumulation of favorable mutations when deleterious mutations are present

“ability to reassort existing genes as the environment changes and in elimination of harmful mutations”
Large Advantages of Sex

Muller’s ratchet allows harmful mutations to be eliminated

Asexual populations, unless very large, all have deleterious mutations

Most fit has only one; next generation it has two and ‘ratchet’ has turned another cog

Sex can reduce mutational load
Large Sex Advantages

Sex and diploidy provides protection from somatic mutations

Diploidy requires passing thru single-cell stage each generation so soma can begin without mutations

Diploidy also permits efficient repair of ds breaks in DNA
Origin of Sex

Sex determined initially by an allelic difference at a gene located on a homologous pair of autosomes

Transformation of autosomes into heteromorphic sex chromosomes (X, Y) occurred by accumulation of mutations in neighborhood of sex-limited allele (?)
Origin of Sex

Not all species reproduce sexually; sex can be lost by mutations

*Mycocepurus smithii* thought to be asexual (thelytokous) but some populations were found to be sexual (10.3%)

This species a mosaic of sexual and asexual populations with sex being lost repeatedly in different lineages
Sex Determination

Involves soma and germ-line tissues

ovaries & testes

Important example of genetic regulation of development

Details nearly clear for *D. melanogaster*

Is it similar for other insects?

Can we learn to modify sex ratio?
Sex Determination: *D. melanogaster*

Involving identifying spontaneous mutations: relatively few genes

Maternal genes: *daughterless*, *hermaphrodite*, *extramacrochaetae*, *groucho*, *sisterless*, *runt*

Zygotic genes: *Sex - lethal*, *transformer*, *doublesex*, *intersex*, *fruitless*, *male – specific lethal*
Sex Determination: *D. melanogaster*

**Dosage compensation:** equalizes amount of gene products produced by XX and XY individuals

Achieved by **hypertranscription** of X in males

Achieved by inactivating one X in mammals and a few insects
Hypertranscription in Males

*D. melanogaster* males are aneuploid for an X

Requires autosomal genes, *male-specific lethal*, under control of *Sxl*

MSL proteins assembled in a remodeling complex (compensasome) on ca. 100 sites on X in males → histone H4 acetylation → hypertranscription
Hypertranscription in males

Females

- Sxl
- Genes: msl-2, msl-1, msl-3, mle
- Proteins: MSL-1, MSL-3, MLE
- No compensasome with X chromosome

Males

- Sxl
- Genes: msl-2, msl-1, msl-3, mle
- Proteins: MSL-1, MSL-2, MSL-3, MLE
- Compensasome associates with X chromosome

H4 and H4Ac16
Somatic Sex Determination

Primary step: Assess relative number of X chromosomes and autosomes in *D. melanogaster*

2X and 2A (ratio of 1) = females
1X and 2A (ratio of 0.5) = males

XXX : AAA = females
XX : AAA = intersexes
Somatic Sex Determination

Sex determination is cell autonomous in *D. melanogaster*

Due to lack of circulating sex hormones?

Y chromosome does NOT determine sex in *D. melanogaster*

Y is necessary for fertility (6 genes)
Somatic Sex Determination in *D. melanogaster*
doublesex locus is a double switch

When active in males, it represses female differentiation

When active in females, and the intersex product is present, male development is suppressed

If dsx is inactivated, both male and female genes are active $\rightarrow$ intersex
Somatic Sex Determination

Determination of sex during embryogenesis transmitted through a hierarchy of regulatory genes to terminal differentiation genes → sexually dimorphic traits of adult fly

Differences in males and females primarily due to sex-specific differences in RNA splicing
Somatic Sex Determination

Individual genes not only controlled at level of RNA splicing but, in turn, specify splicing pattern of genes down stream in hierarchy → cascade of RNA splicing reactions

RNA processing important regulatory mechanism in this developmental pathway
Germ - Line Sex Determination

Differs from in soma of *D. melanogaster*

Pole cells in embryo segregated into posterior pole of embryo before blastoderm

During embryogenesis, germ cells indistinguishable, but differentiation begins during larval stage
Sex - Determination Mechanisms

Appear to be diverse in insects

Genetic sex determination

Ploidy levels sometimes important

Environmental sex determination in some: temperature or host conditions determine sex of progeny

But, are they really diverse?
Arthropod Sex - Determination Diversity

Ploidy and sex determination

Many species diploid (2n, diplo - diploidy)
Some haplo - diploid (2n females, n males) (arrhenotoky)
Some diploid females only (thelytoky)
Some parahaploid (initially all 2n but males lose half their chromosomes during embryonic development → n)
Arthropod Sex Determination Diversity

Males in many species heterogametic (XO, XY, XXO, XXY, XYY) and females XX

In some higher pterygotes (Lepidoptera, Trichoptera), females are heterogametic (ZW)
Haplo-diploid Sex Determination

At least 5 models proposed

1) *Apis mellifera, Bracon hebetor*, single-locus, multiple-allele model

*Apis*: 19 alleles (?) on chromosome 8

Heterozygous individuals normal 2n females; hemizygotes (unfertilized n eggs) become fertile males

Homozygotes -> sterile 2n males
Haplo-diploid Sex Determination

Single-locus, multiple-allele model

*Athalia rosae*: when inbred produces both diploid and triploid males
Multiple alleles at a single locus determine sex in haplo-diploid *Athalia rosae*
Haplo - diploid Sex Determination

Multiple locus, multiple-allele model

Other haplo-diploid Hymenoptera

Females must be heterozygous at one or more loci, while n males are hemizygous

After inbreeding, some 2n individuals are produced and are males if homozygous for all loci
Haplo-diploid Sex Determination

Multiple locus, multiple-allele model and single-locus, multiple-allele models could be combined
Haplo - diploid Sex Determination

Genic balance model: sex in arrhenotokous Hymenoptera determined by a balance between nonadditive male-determining genes and additive female-determining genes.

Maleness genes (m) have noncumulative effects but femaleness genes are cumulative: in haploids m > f, resulting in a male.
Intraspecific Variability

Within a species, several mechanisms may occur: *Musca domestica*

1) 5 pairs autosomes and a pair of heterochromatic sex chromosomes: females XX, males XY: presence of Y determines maleness (carries \( M \))

2) Both males and females XX; have special autosome carrying male-determining factor \( A^M \) which can be on different autosomes
Intraspecific Variability

*Musca domestica*

3) Both males have M factor in homozygous state: presence of female-determining dominant factor (F) determines sex

4) Dominant maternal-effect mutation, *Arrhenogenenous*, causes females to develop into fertile males
Sex determination in *Musca domestica*
Environmental Effects

Encarsia (Hymenoptera: Aphelinidae) adjust sex ratio based on hosts parasitized

Autoparasitoids of whiteflies (1° host) produce females (2n)

n males develop as parasitoids of 2n female Encarsia (2° host)
Diversity

*Chrysomya rufifacies* (Calliphoridae)

Females produce either females only (thelygenic females)

OR males only (arrhenogenic females)

Thelygenic females heterozygous for dominant female - determining maternal effect gene

Arrhenogenic females and males homozygous for recessive allele
Diversity

Post zygotic sex determination

Collembolans have 10 chromosomes in males, 12 in females

Sex determination occurs AFTER zygote forms (not at syngamy) when 2 chromosomes are eliminated in male embryos in both soma and germ line

Mothers must regulate sex ratio?

Step toward parthenogenesis?
Diversity vs. Single Model?

Can a single model explain sex determination in insects?

All variations upon a theme (Nothiger and Steinmann - Zwicky 1985)

NEED: gene ~ Sxl
gene ~ dsx (expressed in 2 alternative forms to interact with 2 sets of male and female differentiation genes lower in hierarchy) PLUS
Diversity vs. Single Model?

Can a single model explain sex determination in insects?

All variations upon a theme (Nothiger and Steinmann - Zwicky 1985)

PLUS

A repressor (R) which inactivates Sxl
A gene which activates Sxl
A General Model for Sex Determination in Insects?
Diversity vs. Single Model?

Evolutionary analyses suggest functions of \textit{tra-2} and \textit{dsx} may be conserved throughout higher eukaryotes.

It appears \textit{Sxl} function is not conserved.
Meiotic Drive

Assortment of chromosomes during meiosis → transmission of some chromosomes more often than expected (> 50%)

Most often observed with sex chromosomes

Found often in Diptera

How often have you examined the sex ratio of your species?
Meiotic Drive

Segregation Distorter (SD) in *D. melanogaster*

Low, but stable, frequencies in field populations

Males heterozygous for SD chromosome (SD / SD\(^+\)) produce only progeny with SD (NOT half and half)

Sperm with SD\(^+\) chromosome fail to mature
Meiotic Drive

Segregation Distorter (SD) in *D. melanogaster*

SD locus consists of 2 overlapping genes
Tandem duplication necessary for effect
Meiotic Drive

Distorter in mosquitos (*Aedes aegypti, Culex quinquefasciatus*)

- Y-linked gene (*D*) results in excess males
- X chromosomes broken during meiosis in males; fewer Xs than Ys in sperm → fewer females
Meiotic Drive

Stalk-eyed flies

_Cyrtodiopsis dalmanni, C. whitei_

Female-biased sex ratios

Element on X chromosome

→ fewer females

Females prefer males with long eye span: ? indicates male lacks MD or can suppress MD → increased female fitness
Meiotic Drive for IPM?

MD an evolutionary force that leads to increase in allele frequency even if it confers disadvantage on carriers

Could it be used to drive new genes into mosquito populations?

Not yet demonstrated practical

How stable are MD systems?
Hybrid Sterility

When different species are crossed, hybrid progeny sometimes are produced. Often have altered sex ratios, with one sex absent, rare or sterile. Known as HALDANE’S RULE. Occurs in taxa with sex chromosome-based MD (Lepidoptera, Diptera).
Medea in Tribolium

New class of selfish genes

**Maternal-effect dominant**

Embryonic arrest results in death of zygotes that do not carry it.

Heterozygous females lose half of progeny if father is wild type.

*Medea* alleles differ in different populations.
Cytoplasmic Agents Distort Sex Ratios

Bacteria, Viruses, Protozoans can alter ‘normal’ sex determination in arthropods

Cytoplasmic = maternal transmission
Often reduce fitness of host, can drive populations to extinction
Seen in Diptera, Heteroptera, Coleoptera, Lepidoptera, Acari
Not always noticed unless single-pair crosses are monitored for sex ratio
Spiroplasmas

*D. willistoni* and related *willistoni* group species
Maternally inherited; different in each
Transovarially transmitted
Lethal to male embryos
Can be injected into related species via hemolymph
Viruses associated with spiroplasmas
L - form Bacteria

*D. paulistorum* complex has 6 semispecies (subgroups of a single species in process of speciation)

Do not normally interbreed

When crossed, fertile daughters & sterile sons produced

Streptococcal L - forms associated with phenotype

Different populations = different bacteria
**Wolbachia**

Rickettsial-like bacteria

Common in arthropods (76% infected in one survey)

Transovarial transmission

In CRUSTACEA, *Wolbachia*-infected isopods produce female-biased progeny because *Wolbachia* changes genetic males (ZZ) into functional ‘females’
Wolbachia and Isopods

‘Daughters’ of infected mothers produce all-female progeny → lineages that are chromosomally males (ZZ) but functional females

Speculation that some Wolbachia genes were transferred to isopod nuclear genome
Wolbachia and Male Killing, Thelytoky, Female Mortality

Some *Wolbachia* improve fertility or vigor, others decrease fitness

Some in somatic cells, others in germ line
Wolbachia and Thelytoky

Hymenoptera typically arrhenotokous

Parthenogenesis in which only females known

At least 70 spp. parasitoids; some have arrhenotokous & thelytokous strains

Some thelytokous populations produce a few males, suggesting incomplete transmission of Wolbachia
Wolbachia and Thelytoky

Hymenopteran parasitoids

Rare males sometimes fertile; or not
Related to time without selection for fertility?
Several antibiotics can induce male production in some parasitoid populations infected with Wolbachia
**Wolbachia and Thelytoky**

**Cytogenic changes**

- Restore unfertilized haploid egg to diploidy (allows thelytoky)

In *Trichogramma* meiosis → single haploid pronucleus and diploidy restored during anaphase I when identical sets of chromosomes fail to separate → 2n nucleus with 2 sets of identical chromosomes
Wolbachia Infection

How are parasitoids (all insects) infected?

Phylogenetic analysis of Wolbachia and insects indicate both horizontal and vertical transfer occurs.

Horizontal transfer from one parasitoid strain to another via a shared lepidopteran host was demonstrated.
**Wolbachia** alters sex ratio, causes incompatibility.

*Is Wolbachia involved in speciation?*
Wolbachia Affects Mating Behavior in *Acraea encedon*
Wolbachia In Acraea

In Africa, many populations produce only female progeny; others have normal 1:1 sex ratio

*Wolbachia* causes all-female progeny by killing males

*Acraea* deposit 50 to 300 eggs; larvae often cannibalize siblings → evolution of male killing favored due to antagonistic interactions
Wolbachia In Acraea

In Africa, serious shortages of males may occur in infected pops.

*Wolbachia* - infected females have altered mating behavior.

Normally, males seek and compete for females near larval food plants.

*Wolbachia* - infected females form dense aggregations in grassy areas – perhaps to attract rare males.
Haploid Females

*Brevipalpus phoenicis* (Acari: Tenuipalpidae) has females with only haploid set of chromosomes

Due to endosymbiotic bacterium called *Cardinium*

Causes feminized haploid males?

Antibiotics $\rightarrow$ haploid male progeny
Nasonia vitripennis (Hymenoptera) has 2 mechanisms to alter sex ratio

- B chromosome causes Paternal Sex Ratio: carried only by males
- PSR transmitted by sperm to fertilized eggs

After fertilization, paternally derived chromosomes condense and are lost, leaving only maternal chromosomes.
PSR and Nasonia

Origin of PSR?

Sequences in autosomes of related species homologous with PSR

Could have been present prior to divergence of Nasonia OR could have crossed species barrier (horizontal transfer) by series of interspecific crosses
Nasonia

Also infected with *Wolbachia*, which causes cytoplasmic incompatibility

Another modifier of sex ratio caused by non-Mendelian factors is called ‘son-killer’ which is a maternally transmitted bacterium preventing development of unfertilized eggs
Nasonia

Maternal Sex Ratio, cytoplasmic agent, causes females to produce nearly 100% daughters
Coccinellidae

Male killing associated with variety of microorganisms

*Rickettsia*
*Spiroplasma*
*Flavobacteria*
*Wolbachia*
Sex and the Sorted Insects

Learning how to modify sex ratio or fertility could have important applied implications for pest-management programs

Genetic control of pests
Genetic → more females
Genetic Control

Alternative to chemical control

Safe to environment
Species specific

Ex: Screwworm, *Cochliomyia hominivorax*
Medfly, *Ceratitis capitata*
Tsetse, *Glossina palpalis, G. morsitans*
Mosquito: *Anopheles albimanus*
Codling moth: *Cydia pomonella*
A Programme for the Eradication of the New World Screwworm from North Africa
Sterile Insect Release Method (SIRM)

Require only males (sterile)

Rearing females expensive

Releasing females can create problems (biting, disease transmission)

Traditional ‘sexing strains’ have been developed, but not always stable
Sterile Insect Release Method (SIRM)

Require only males (sterile)

Goal: produce only males of high quality and vigor

An all-male colony not feasible: conditional lethal linked to female-determining gene/chromosome

Transgenic methods might be useful?
Sterile Insect Release Method (SIRM)

Dipteran pests, such as *Lucilia cuprina*, Med fly and *Anastrepha* and *Bactrocera* fruit flies have *doublesex*, *transformer* and *transformer 2* homologs of *D. melanogaster*

RNAi was used to produce all male progeny in *Anastrepha suspensa* by injecting ds RNA for *transformer* and *transformer 2* genes into embryos

Nearly all XX embryos developed into males
Sterile Insect Release Method (SIRM)

*transformer* and *transformer-2* genes are essential for development of *Anastrepha* females and this method could be used to produce male-only populations for genetic-control programs.
Sterile Insect Release Method (SIRM)

Sex determination in *Bombyx mori* (females are ZW and males are WW) has been studied and it may be possible to use this information to develop sexing strains in pest Lepidoptera for genetic-control programs
Conclusion

Gempe and Bey (2010) concluded

“One common theme from these studies is that evolved mechanisms produce activities in either males or females to control a shared gene switch that regulates sexual development.

Only a few small-scale changes in existing and duplicated genes are sufficient to generate large differences in sex determination systems.”