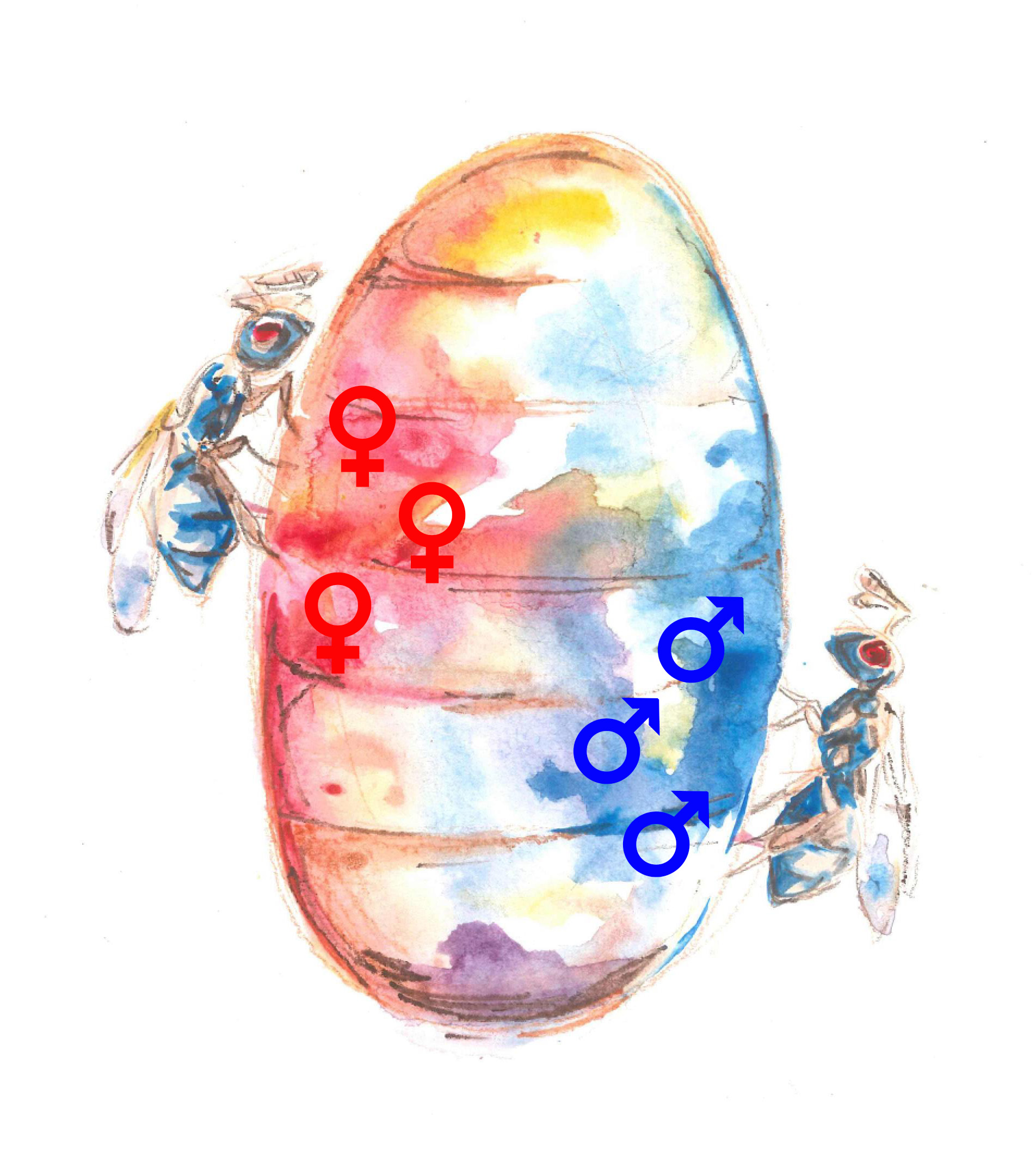
**Sex Allocation**

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**Outline**

**Note to instructor:** If the practical is being conducted with pre-existing data only and a physical experiment will not take place, in the student version delete sections that are in red and add the sections that are in blue.

**Activities**

Part 1: Read background of sex allocation biology. Answer questions about background. Write a design for and set up an experiment on sex allocation.

Part 2: Collect experimental data. Analyse individual group data and pooled data. Answer questions about sex allocation using inferences from data.

**Materials**

For each team of 3-4 students:

1 work laptop with Microsoft Word and Excel installed

1 dissection microscope

3-4 small paintbrushes (for transferring wasps)

10-20 small vials

Fresh cotton (for plugging small vials)

3-10 large vials (this depends on the experimental design of students)

3-10 large vial plugs (this depends on the experimental design of students)

5 small vials of *Nasonia vitripennis* with 10 females and 3 males that have been given 24 hours to mate under standard conditions (it is highly recommended that you use a genetically variable strain such as HVRemix to simulate more natural populations, as inbred strains such as AsymCx can have abnormal offspring sex ratios)

1 large vial of fresh blowfly pupae (~100 hosts). Commercially purchased *Sarcophaga bullata* or *Calliphora* spp. are recommended

1-2 racks for small tubes

1 rack for large tubes

3-4 micro picks (for opening hosts to collect offspring)

1 marker for labelling tubes and racks

Rolls of masking tape for labelling racks and euthanizing wasps (as many as needed, to share)

Squirt bottles of 70% ethanol for cleaning and euthanizing wasps (as many as needed, to share)

Paper towels for cleaning

**Objectives**

-Learn *Fisherian* and *Hamilitonian* concepts of sex allocation

-Learn background of *Nasonia vitripennis* system

-Learn concept of haplodiploid sex determination

-Learn experimental handling of *Nasonia vitripennis* as a laboratory model

-Learn analyses of experimental data

-Answer questions on biological knowledge based on literature and experimental data

**Goal**

To enhance the knowledge of students on sex allocation, experimental design, and data analyses.

**Feedback**

Instructor will provide feedback on: interpreting primary literature of given materials to answer questions, following the scientific method to design and conduct an experiment, and drawing conclusions from experimental data.

**Sex allocation – part I**

Sex allocation is the allocation of resources to male versus female reproduction. Sex allocation theory has proved extremely successful at predicting under what circumstances individuals will adjust the sex of their offspring. R.A. Fisher (1930) was the first to show why most species show an equal sex ratio (sex ratio = proportion males = 0.5). Fisher assumed that, within a population, each male would have an equal chance to mate with each female and vice versa, a so-called “panmictic” population. Bill Hamilton (1967) realized that for many organisms, this assumption does not hold. For example, small animals often have their food distributed over isolated patches, so the only option is to mate with those animals that are present on the same patch. How does sex ratio evolve under these (more realistic) conditions?

|  |  |
| --- | --- |
| Ronald Fisher | Bill Hamilton |

Let's start with the simplest situation: a female finds a suitable patch to lay her eggs. She is the only female on that patch and no other females will find the patch after her. Her daughters will have to mate with her sons (their brothers), i.e. they mate locally. Her sons will therefore have to compete with each other for mates. How can this female maximize her fitness?

In order to maximize fitness, one first has to define fitness, which is not an easy task. There are several different definitions of fitness. For this practical, an appropriate fitness definition is the number of grandchildren per female.

Suppose that two females each lay four eggs in a patch (i.e. N=2). A female can behave according to Fisher and produce two daughters and two sons, or she can follow Hamilton’s prediction and produce three daughters and one son. A *Fisherian* female therefore produces a balanced sex ratio (sex ratio = 0.5) and a *Hamiltonian* female a female-biased sex ratio (sex ratio = 0.25). Grandchildren are counted through offspring produced by daughters and inseminations obtained by sons. Consequently, fitness is measured as the number of gene sets that are inherited through sons and daughters to the grandchildren.

The *Nasonia* system

Biased sex ratios are often produced by animals with haplodiploid sex determination (Figure 1). In these animals, the males are haploid (single set of chromosomes) and the females diploid (double set of chromosomes). This system is mainly found in the order of the Hymenoptera (the ants, bees and wasps). The system works like this: a male will inseminate a female. The female then stores the sperm in a specialized organ, the spermatheca. When she lays an egg, she can decide whether or not to open her spermatheca and release a sperm to the egg. When she lays an unfertilised, haploid egg, it will develop into a male. When she fertilizes the egg, it will develop into a diploid female. Because of this system, Hymenoptera are able to adjust the sex ratio of their offspring to the (environmental) conditions.

Adjustment of the sex ratio is harder in organisms that have specialised sex chromosomes. In those organisms, these sex chromosomes might pose a genetic constraint on the adjustment of sex ratios. But even these species show sex ratios that differ from 0.5. Some mammals for instance are thought to adjust the sex ratio of their offspring with selective abortion. Many parasitoid wasp species, fig wasps and mites show sex ratios as predicted by Hamilton's model.

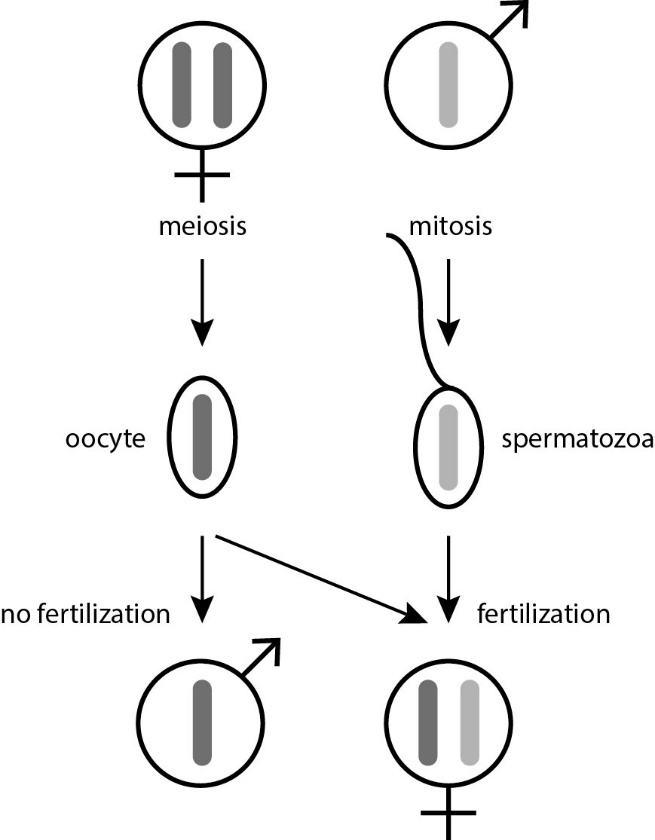
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Figure 1. Haplodiploid reproduction. Males develop from unfertilised eggs and are haploid. Females develop from unfertilised oocytes (eggs) and are diploid. The spermatozoa (sperm) females receive during mating is stored in a spermatheca. Females can decide whether or not to release a sperm to the egg during oviposition. Through this mechanism females can determine the sex ratio of their offspring.

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| **Box 1 - *Nasonia vitripennis* biology**    *Nasonia* are small parasitoid wasps (Hymenoptera: Pteromalidae) that parasitize blowflies and fleshflies (see Figure 2 for the life cycle). These wasps have become a popular model organism for evolutionary biologists and geneticists. They are easy and quick to culture (generation time of 14 days at 25ºC), their gender is easy to determine (males have short wings, females have long wings) and they can be handled without anesthesia. Like all wasps, *Nasonia* has haplodiploid reproduction (see Figure 1 on page 5). Males develop from unfertilized eggs and are haploid, while females develop from fertilized eggs and are diploid. Sons only have a mother and no father. Furthermore, females decide whether or not to fertilize eggs, so mothers control production of daughters versus sons.  *Nasonia* uses different fly species as its host (mainly *Protocalliphora)*. The fly larvae feed on blood of bird chicks and pupate in the bottom of nests. After young birds have fledged, *Nasonia* appear in the bird nests to parasitise the fly pupae. The flies are also found on animal carcasses where the fly larvae feed and pupate on meat. Bird nests and carcasses are only suitable as for fly larval feeding and pupation for a short time, and only on a local scale. This means that *Nasonia* populations and their hosts are actually made up of many small, isolated subpopulations (demes). *Nasonia* males have a slightly shorter development time than females. In contrast to the females, *Nasonia* males cannot fly and mate locally with females from the same patch. Depending on the number of females (foundresses) that laid eggs in that deme, the males often mate with their own sisters. Females can fly, and so disperse and migrate to colonize new hosts.    There are four closely related species in the *Nasonia* genus, *N. vitripennis*, *N. longicornis*, *N. giraulti and N. oneida*. *N. vitripennis* is found throughout the world, but the other species are endemic to North America.    Figure 2. Life cycle of the parasitoid wasp *Nasonia* *vitripennis* and their *Protocalliphora* fly hosts. The fly larvae live inside bird nests and carcasses. After pupation they are vulnerable to parasitisation by *Nasonia*. A *Nasonia* female lays about 10-40 eggs per fly pupa, the wasp larvae will feed on the fly pupa. The wasps will emerge after approximately two weeks at 25ºC (unless they are in developmental diapause, a winter survival strategy that occurs when mothers are exposed to shorter photoperiod, in which case they can be kept in 5ºC storage for two years), The males emerge first. The males have short wings and cannot fly. They will mate with the emerging females, which will then disperse and colonize new hosts. The flies are ~10 mm, the wasps are 2-3 mm. |
| Box 2 – How to handle Nasonia Even though *Nasonia* are wasps, they cannot sting people. Males and females of *N. vitripennis* are easily distinguished: males have short rudimentary wings, while females have larger wings that extend over the tip of the abdomen (see Figure 3). Males also have yellow antennae and legs females have black antennae and darker legs. Additionally, females have an ovipositor at the ventral side of the abdomen.  Adult wasps can be shaken in small numbers from the main vial onto the working surface. The wasps are more visible on a light-coloured background. When the wasps are walking around on the working surface, you can isolate them by placing a small vial over them. Most of the times the wasps will walk up the vial. If necessary, the wasps can be pushed by using a small paintbrush. The vials are closed off with cotton wool and placed in styrofoam trays. The wasps are cultured in a climate-controlled room at 25ºC (Figure 4). You may euthanize loose wasps with masking tape or spraying and wiping with 70% ethanol, and wasps still in tubes by freezing overnight.  Differences between *Nasonia vitripennis* ♂ and ♀   |  |  | | --- | --- | | ♂ | ♀ | | Short wings, shorter than abdomen | Large wings, longer than abdomen | | Yellow antennae and legs | Black antennae and partially black legs | | Tip of abdomen rounded | Tip of abdomen pointed with ovipositor |   Description: femaleDescription: male  Figure 3. Morphological differences between the *Nasonia vitripennis* sexes. Photo by P. Koomen    Figure 4. *Nasonia* cultures incubated in plastic vials in an incubator |

**Questions**

**Table 1** The number of grandchildren as a function of the sex ratio under Fisher’s Equal Sex Ratio model and Hamilton’s Local Mate Competition model. Each female produces 4 offspring, F = female, M = male.

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| --- | --- | --- | --- | --- | --- | --- |
| Patch (deme) | 1 | | 2 | | 3 | |
| Type of female | Hamilton | Hamilton | Hamilton | Fisher | Fisher | Fisher |
| Offspring | 3F + 1M | 3F + 1M | 3F + 1M | 2F + 2M | 2F + 2M | 2F + 2M |
| Grandchildren per child | 4 12 | 4 12 | 4 6.67 | 4 6.67 | 4 4 | 4 4 |
| Total gene sets per foundress | 12 + 12 = 24 | 12 + 12 = 24 | 12 + 6.67 = 18.67 | 8 + 13.34 = 21.34 | 8 + 8 =  16 | 8 + 8 = 16 |
| Overall gene sets per group | 48 | | 40 | | 32 | |

Assume for a diploid organism that there is random mating and each female produces 4 offspring. If N=2 there are three possible patch (deme) compositions (Table 1). In deme 1 both females use a *Hamiltonian* strategy and they have the highest production of grandchildren. In deme 3 both females use a *Fisherian* strategy and have the lowest production. At the patch level, there is selection for the *Hamiltonian* strategy, because this results in the largest contribution to the next generation. When we consider the patch where both females use different strategies (deme 2), one sees that the *Fisherian* female is better off. Her sons can mate not only with their own sisters, but also with the daughters from the *Hamiltonian* female. The single *Hamiltonian* son has to compete for mates with the two *Fisherian* sons. Therefore, when *Fisherian* females are present producing a *Hamiltonian* sex ratio is not a stable strategy, because selection within patches will act against *Hamiltonian* females.

**Question 1:** In **Table 1** sample calculations are given that show the different outcomes in the theoretical cases of two females laying 4 eggs per generation, each, where either both play the same sex-ratio strategy or one follows Hamilton’s and the other Fisher’s strategy.

*Please explain the calculation with your own words!*

Hamilton calculated the evolutionary stable sex ratio for two females laying eggs on a single patch, for three females on a single patch, etc. His conclusion was that the evolutionary stable sex ratio for a diploid organism with N founding females on a patch is given by: (N-1)/2N.

**Question 2:** *What would be your strategy (Hamilton or Fisher) if you were a female in a N=2 situation?*

If there is competition in a patch, Fisher is the logical choice. If the other female uses a *Hamiltonian* strategy, the *Fisherian* female has the advantage. If the other female uses a *Fisherian* strategy, there is equal competition.

Also good: The best strategy is the strategy that delivers on average, the most grandchildren regardless of the strategy of the other female. This is the *Hamiltonian* strategy.

Also good: According to the formula of 25% males, the *Hamiltonian* would be optimal in a deme of two founders

Correct answers here vary because the optimal strategy depends on what strategy the other female uses.

**Question 3:** *What is, according to Hamilton's equation, the optimal sex ratio for a mated female that is laying eggs alone at a patch? How would you interpret this biologically? What would be a better solution for such a female?*

Hamilton’s equation N-1/2 N with N = 1 gives a sex ratio of 0, i.e., only females (daughters). This is not evolutionarily advantageous because all females remain unmated, and the deme dies out because the next generation will only have males produced by virgin mothers. Biologically, you can interpret it as, the best solution for females is to have just enough sons to mate with all daughters.

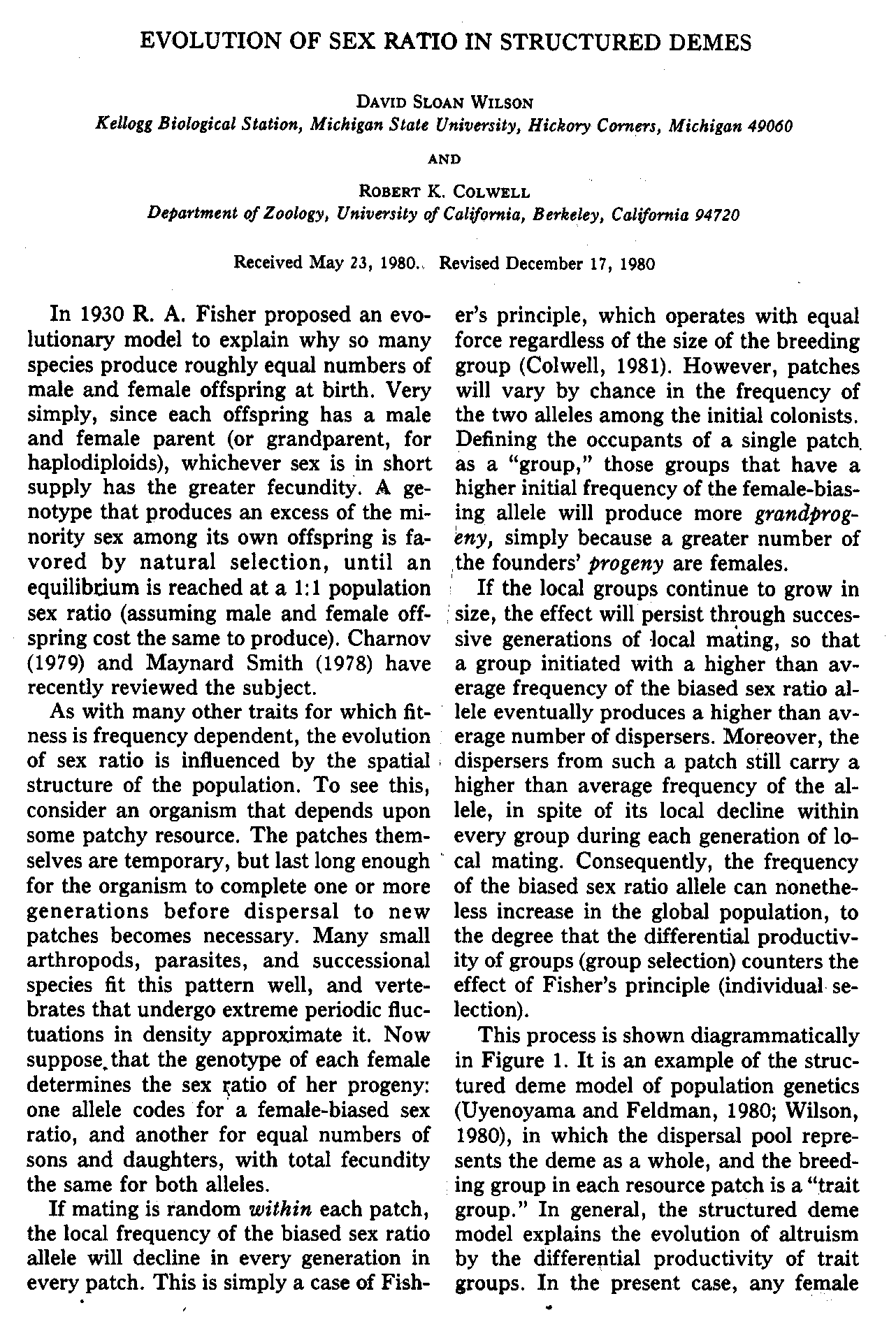
**Question 4:** *Plot Hamilton's equation in a graph. Use the formula for a diploid organism, as this one is simpler than for a haplodiploid organism and the difference between the two formulas is small. Describe in your own words what the graph shows.*

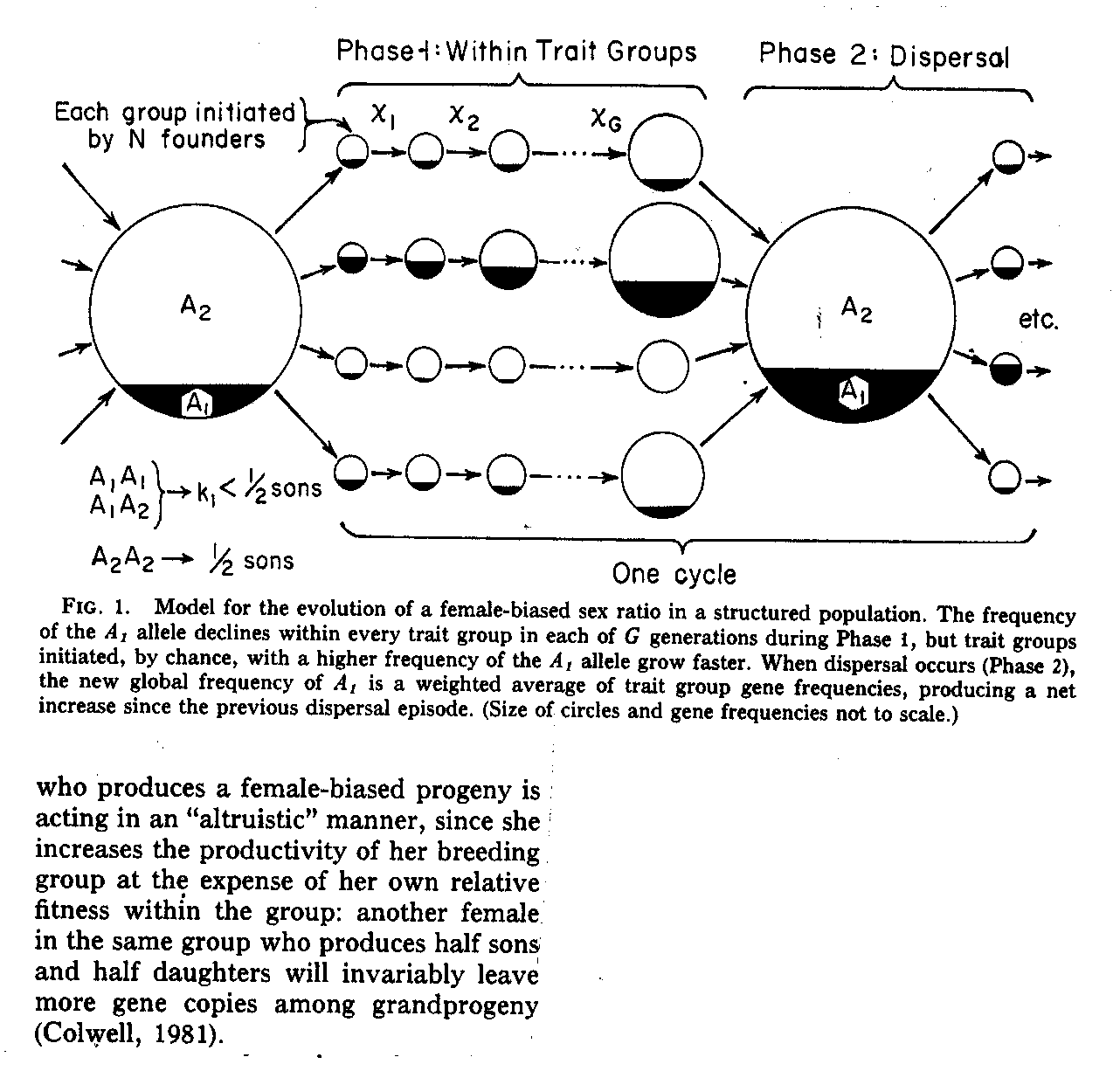
In the graph, you should see that when more females use the same patch, they have to produce more sons to compensate for the increased competition with sons of the other females. For large values of N, the optimal sex ratio approaches 0.5, which brings us back to Fisher's theory.

The conclusion is that, if populations are subdivided in isolated patches of reproducing females, selection will favour female biased sex ratios. A prerequisite for this is that males born within a patch only mate with females from the same patch.

Thus far, we have considered an evolutionary explanation for the effect of Local Mate Competition, i.e. how it affects individual fitness through the number of produced progeny. There is however also an ecological effect. The figure from the paper by Wilson & Colwell (Appendix 1, page 10) gives another reason why selection favours biased sex ratios in small animals with isolated patchy resources (allele A1 is *Hamiltonian*, allele A2 *Fisherian*).

**Question 5:** The A1 allele increases in the total population, while you have just seen that within a patch the A2 allele has a selective advantage. Explain the increase of the A1 allele. How could one call this form of selection on the A1? (Hint: read the following excerpt from Wilson and Cowell, 1981)





Demes with relatively large-sized *Hamiltonian* females (A1 alleles, A1 dominant to A2) produce more offspring and these demes therefore grow faster (size of circles). While inside the demes, the *Fisherian* females (A2 alleles) still have an advantage and the relative frequency of the Hamilton allele decreases. As a result, in spite of the selective advantage of the A2 gene, there is still an increase in the A1 gene in the total population. This type of selection is called group selection, population selection, or, according to the authors, interdemic group selection.

**Exercise: Design an experiment to test Hamilton's theory**

Design an experiment to test whether the sex ratio behavior of the haplodiploid wasp *Nasonia vitripennis* fits Hamilton's theory. Note that Hamilton’s formula in question 1 was developed for diploid organisms but that the formula is slightly more complicated for haplodiploids. We will use the diploid formula as outcomes are similar to that of haplodiploids.

Above you will find two boxes describing the biology of *N. vitripennis* (Box 1)and how to handle the wasps (Box 2). Before you start designing your experiment, **read these two boxes.** Divide yourself into groups of 3-4 students. Each group will receive vials with mated females, and hosts (fly pupae). Use these to test Hamilton's theory. Discuss using these reference materials how you would design the experiment (i.e. wasp individuals, hosts, and trials you would use).

***Do not start with your experiment before you have discussed your experimental set up with the supervisors!***

**Question 6:** Give a short description of your experiment.

Ideally, students will test a range of foundress numbers spanning N=1 to N=10 (up to N=15) to fully capture Hamilton’s curve. For smaller foundress numbers they may choose to use the smaller vials, and for larger foundress numbers, the larger vials. The fresh hosts provided should be consistent for all foundress groups.

**Submit your answers to the questions (as a group of 3 or 4).** Put your **names** in the file name and email subject, and email to your instructor (or upload through your academic site).

### **Sex allocation – part II** (two weeks later)

Count the number of males and females in the broods. Enter your data in the computer so we can combine the results of all groups. Answer these questions using the provided dataset (generated from experiments of previous years)

**Question 1:** *Plot your results, calculate and add standard deviations to your graph. Add the expected curve based on Hamilton's theory. Make the same plot for the group data. Explain your experimental set up (number of foundresses, replicates, included) and compare your results to the expected curve (Hamilton’s theory). Take also the group data into account. Do the wasps follow Hamilton's Local Mate Competition theory?*

In general, the sex ratio increases with the number of founding females and that is in accordance with LMC theory. However, some quantitative deviations are expected.

**Question 2:** *Give some biological reasons why the data might not fit the theoretical predictions. Think of other factors that might influence the sex ratio.*

Unmated females only produce sons, which can be seen for the N = 1 patches. This may explain why the sex ratios are above the curve.

Other possibilities:

- Non-natural conditions (limited space, limited number of fly puppets, not natural hosts, females cannot migrate, etc)

- No stress factors such as predation

- Not well distinguished from females and males

- Not enough replicates/coincidence

- Incorrect estimation of the number of founding females by the wasps

- Males used instead of females

- Reminder of large number of wasps in the origin culture tubes

- Females have received too little sperm

**Question 3:** *How do you think females can recognise how many other females are present on the patch? How would you test this?*

It is possible they recognize each other through physical encounters; visual cues; or meeting each other in the adult stage.

To test this, blind wasps can be used. No difference with a sighted control group would suggest that females detect each other through pheromones or other chemical signals. To further test this, pheromones can be artificially administered. If they underlie how females recognize the presence of other females, then in an experiment with no other females present, individual control females that were not exposed to pheromone should have a lower sex ratio than test females exposed to pheromone.

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**Question 4:** Consider the situation of two founding females. Suppose the first female does not lay eggs on all fly pupae, and the second female arrives on the patch after the first one has left. The first female will behave according to Hamilton's theory and produce a sex ratio based on N=1. *What sex ratio will the second female produce and why? How would you test this?*

Several answers are possible

1. The second female does not perceive that eggs have already been laid. She behaves as if she is alone and lays eggs according to *Hamilton* N=1.

2. The second female recognizes that eggs have already been laid in a fly pupa and therefore behaves like N=2 and thus lays 25% males.

3. The second female recognizes that eggs were laid according to *Hamilton* N=1, and compensates for this by laying more males than you would expect at N=2.

To test this, with no other females present, females can be offered either fresh hosts or hosts that were previously parasitized by another female. If females use pre-parasitised hosts at a lower rate than fresh hosts, this may indicate that detection of other females is through assessment of host quality. You may also use two females simultaneously. In either case, score the sex ratio of male to female offspring. A difficult aspect of this design is determining which offspring are from which female. One means of doing this is using females with different heritable physical markers than test females, so their respective offspring can be distinguished

**Hand in your answers to the questions (as a group of 3 or 4).** Put your **names** in the file name and email subject, and email to your instructor (or upload through your academic site).