

Figure 2. Examples of spite. (A) Two strains of the bacterium *Photorhabdus luminescens* (pink versus orange) engage in chemical warfare. Note the inhibition zones (bacterial no-man's land) that appear when colonies of the opposing strains meet. (Photo by R. Massey.) (B) The bacterium *Wolbachia* causes males to spitefully sterilize females in many species of insects, including the parasitoid wasp *Nasonia vitripennis*, pictured. (Photo by D. Shuker and S. West.) (C) Red fire ant workers execute a queen who carries the wrong genes. (Photo by J. All and K. Ross.) (D) A suicidal soldier of the parasitoid wasp *Copodisoma floridanum* murders and eats her embryonic hostmate (Photo by J. Johnson, D. Giron, and M. Strand.)

large mandibles, that pass their time in the caterpillar by killing regular embryos — including their siblings — before dying themselves. Usually, it is the females who are most spiteful, and their attacks are concentrated mainly on brothers and unrelated males. Hence, there are important implications for sex ratio evolution in this system.

#### Are there other ways of explaining these behaviours?

Spiteful behaviours have sometimes also been interpreted as a form of altruism or indirect altruism. Mutually harmful behaviours are favoured if they result in a benefit for some third party that is sufficiently related to the actor. Here, there is no requirement for negative relatedness to one's victims, and so a distinction has been made between so-called Hamiltonian spite that does rely on negative relatedness and Wilsonian spite that does not. However, this distinction may be more semantic than real, as one can often

switch between these two views when considering a particular example of spite, depending on how one chooses to measure genetic relatedness between the perpetrators and the victims of spiteful behaviours.

#### Where can I find out more?

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## Primer

# Sex ratios and social evolution

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When we think of modern biology, an image that does not usually come to mind is of an entomologist squinting over mounds of wasps, sorting out the males from the females, and assiduously tallying them up. This work requires no fancy machines, no chemicals, no molecular techniques. But what it does rest on is a theory, and such seemingly pedestrian work has tested and confirmed one of the most elegant and successful theories in modern biology.

The ratio of females to males in a species is a topic that interested Darwin, but how such ratios evolve left him puzzled. The basic solution to the problem has led to a body of work that has informed nearly every important area of social evolution: group selection, kin selection, parent–offspring conflict, evolutionary stable strategies and game theory, and within-genome conflict.

#### The Düsing–Fisher model

The solution of the sex ratio problem has traditionally been attributed to Sir Ronald A. Fisher's 1930 classic *The Genetical Theory of Natural Selection*. But it is now known that a German biologist, Carl Düsing, got the solution more than four decades earlier. Fisher's book was the pipeline through which the theory flowed into the modern era, but his research reputation will now have to rest on other accomplishments, such as inventing the analysis of variance and deriving the fundamental theorem of natural selection.

The sex ratio argument, modernized and simplified a bit, goes like this. Natural selection is about reproduction. A gene affecting sex ratio does not affect your number of offspring;

it simply shifts those offspring between the categories of male and female. But this allocation can affect your number of grandchildren if sons and daughters have different average reproduction. Düsing's key insight was that the total reproduction of all sons in the population has to equal the total reproduction of all the daughters, which follows from the fact that each grandchild has a father (one of the sons) and a mother (one of the daughters). If we let the total number of grandchildren, sons and daughters in the population be  $G$ ,  $S$  and  $D$ , respectively, then the average reproduction of a son is  $G/S$  and the average reproduction of daughter is  $G/D$ . This means if there are more daughters than sons in the population ( $D > S$ ), the average daughter will be less successful and mothers will be selected to make more sons (Figure 1). Conversely, if there are more sons than daughters ( $S > D$ ), their average success will be lower, and selection will favor mothers that produce more daughters. Because the total reproduction of each sex is equal, individuals of the rarer sex have greater average success, and parents who produce more of the rare sex will have more grandchildren. This produces a stable equilibrium at  $S = D$  and accounts for the widespread occurrence of 1:1 sex ratios in nature.

Düsing's result eventually became important for social evolution in two ways. First, it showed how to handle cases in which individual fitness depends on what other individuals are doing. The value of producing a daughter is high if the population is male-biased and low if it is female-biased. Düsing showed that one could solve for an equilibrium point at which no alternative strategy would do better. The work of W.D. Hamilton and John Maynard Smith later formalized this approach as evolutionary game theory, which has been very widely and successfully applied to understanding other social behaviors.

Second, by showing how individual selection operates on the sex ratio, Düsing set the stage for fruitful tests on the level of selection. In principle, selection could work on genes, individuals, groups or species. Each such unit can possess the properties of reproduction and heritability that make evolution work, but which is most powerful? One way to decide is by seeing what unit is adapted. It is instructive to revisit Darwin's thinking on the subject. In *The Descent of Man*, he wrote: "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". Apparently Darwin was uncomfortable with good-of-the-species theories, and with good reason. Sex ratios now provide some of the best evidence against species-level selection. A species might sometimes do best with a 1:1 sex ratio, as when both a male and a female are needed for adequate parental care. But in the majority of species, females do all the heavy lifting of nourishing the next generation. Males provide only sperm and put most of their effort into competition with other males that is unproductive for the group or species. Under these conditions, species reproduction would be increased by having many of the productive females and just enough of the males to provide sufficient sperm. And yet sex ratios in polygamous species tend to be about 1:1, just like in monogamous species. This failure of the species selection model also applies to group selection, but the result is exactly as predicted by Düsing's individual-selection model.

#### **Separating proximate and ultimate: the Hymenoptera**

At this point an alert reader might raise an objection to Düsing's account. Sex is often determined by chromosomal mechanisms that produce a

1:1 sex ratio simply because of Mendelian segregation. An evolutionary biologist would reply that this is a proximate (or mechanistic) explanation and Düsing's argument was an ultimate (or evolutionary) explanation. The two are not necessarily competitors; indeed Düsing's ultimate theory is a likely explanation for why sex is often determined in this simple chromosomal manner. Nevertheless, we would be more confident if we could separate the two and show that Düsing's theory works in the absence of chromosomal sex determination.

This is where the haplodiploid Hymenoptera enter the picture. The Hymenoptera are an insect order consisting of the ants, bees and wasps. Along with a few smaller taxa, they have a haplodiploid genetic system. In haplodiploids sex is determined by whether the mother chooses to release sperm from her sperm storage organ to fertilize an egg. Fertilization produces a diploid female and lack of fertilization a haploid male. Sex ratio control is therefore behavioral and not determined by Mendelian segregation of chromosomes. Düsing's theory must be modified in minor ways for haplodiploids, but it turns out that the modifications cancel and it still predicts 1:1 ratios (daughters will pass on genes to both sexes of grandchildren with 50% fidelity, sons to only one sex, but with 100% fidelity). And for the most part, 1:1 ratios are what we see in the Hymenoptera, so Düsing's theory succeeds even without the assist from Mendelian segregation.

However, there are exceptions that do not have 1:1 sex ratios. Does this mean the model is wrong? At one level it does; the simplest model does not fit all the facts. But at another level, these exceptions have provided the means to truly probe the logic of the theory. Düsing's model made a number of implicit assumptions. Hamilton's 1967 paper on extraordinary sex ratios began a tradition of changing those assumptions and following out the logic to get altered

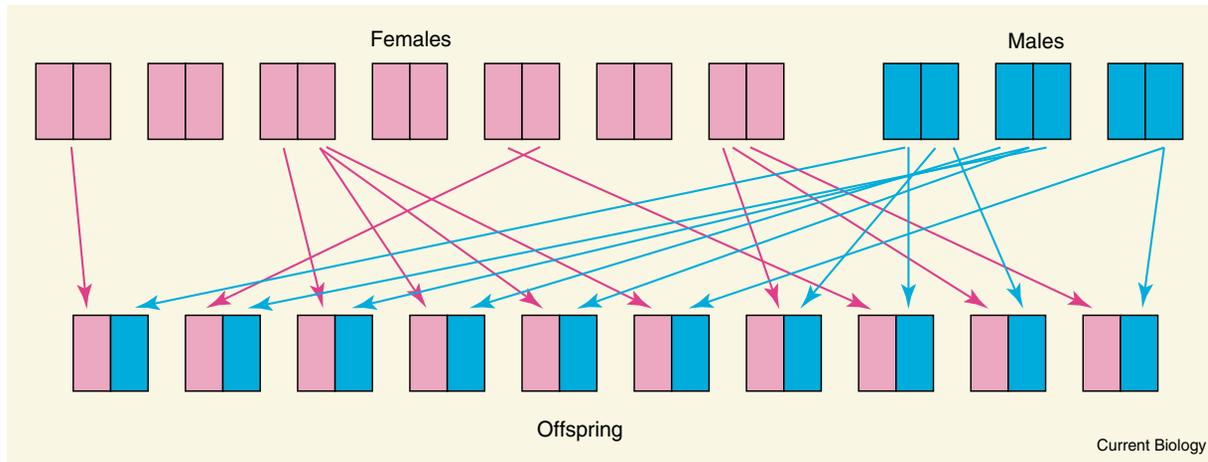


Figure 1. An example of a population with more females (two pink genes) than males (two blue genes).

No matter what the breeding system is, the offspring genes in the next generation must come half from female genes and half from male genes, so the average reproduction of the majority females is lower, and genes for producing females are therefore being selected against. The converse is true if males outnumber females.

predictions. Then, by searching for organisms where the changed assumptions hold, one can see if their sex ratios are successfully predicted.

It turns out that two highly successful guilds of Hymenoptera have been particularly instrumental in this endeavor: social insects and parasitoids. Social insects such as ants live in colonies with one or a few individuals specializing in reproduction while the others support them as sterile workers. Parasitoids, which include thousands of Hymenopteran species as well as some other insects, lay their eggs on a host, usually another insect, and the young develop by feeding on that host, eventually killing it.

#### Unequal costs

An assumption that Düsing made (though Fisher did not) was that sons and daughters are equally expensive to produce. If one sex is less expensive, it will tip the equilibrium in favor of production of that sex. In the model above, we can divide the average success of sons and daughters by their respective costs,  $C_s$  and  $C_d$ , and ask when the two payoff/cost ratios are equal ( $G/SC_s = G/DC_d$ ). The equilibrium occurs not at equal numbers of sons and daughters in the population, but at equal parental expenditure on each

( $SC_s = DC_d$ ). Thus, the ubiquity of 1:1 sex ratios occurs because sons and daughters are usually about equally costly. But we would like to test the altered prediction under unequal costs. Because it is much more difficult to estimate costs than to simply count males and females, a useful strategy is to find a species where the cost differences are clearly extreme.

Here is where certain social insects enter the picture. Honeybee colonies invest hugely in each new daughter queen. The old queen departs, leaving many of the workers, much of the food stores, and all of the hive structure to the daughter queen. Males are not similarly expensive — they simply fly off and try to mate with queens from other colonies. As expected given the cost difference, honey bees rear hundreds of males for every new queen. The same pattern occurs in other social insects that split the colony between the old queen and her daughter, such as army ants. But in most social insects, where the daughter queens are not particularly expensive because they fly off to start their own colonies alone, sex ratios are closer to the normal 1:1.

#### Non-random mating

Another of Düsing's implicit assumptions was random mating. Calculating each son's

expected success as  $G/S$  implies that all sons are roughly equal in their expected success. But this assumption fails under a certain kind of non-random mating that occurs in many parasitoids. In these species, a mother deposits a number of eggs on the host, leaves them to hatch and develop to adulthood and — here is the key feature — these offspring mate among themselves rather than joining a population-wide mating pool. In this case, as W.D. Hamilton argued in 1967, the number of grandchildren that will be produced by the entire population ( $G$ ) is irrelevant to a son's success, because he can father only the grandchildren produced by his sisters. An egg-laying mother laying eggs will get the most grandchildren if she produces as many daughters as possible and just enough sons to ensure the daughters are inseminated. Any extra sons simply compete with each other without increasing the number of grandchildren. Hamilton showed that, in agreement with this logic, parasitoids with this kind of 'local mate competition' have sex ratios highly skewed towards females.

Agreement with theory is actually rather precise. For example, Jack Werren showed that *Nasonia vitripennis* (Figure 2) mothers were very sensitive to

the presence of other females laying on the same host or patch of hosts (fly pupae). Although one mother alone produces a highly female-biased ratio, two mothers laying together produce a somewhat less female-biased ratio, because a mother's number of grandchildren can now be increased somewhat through her sons competing to mate with the daughters of the other female. As more co-laying females are added, the female bias continues to decrease until it approaches 1:1 with 12 co-laying females. Female-biased sex ratios have been documented in many other taxa with local mate competition, such as fig wasps that both pollinate and parasitize figs.

#### Control by offspring

Düsing also assumed that sex ratio was controlled by the mother, which must often be true. But remember, it is the investment in sons and daughters that counts, and in social insects it is the workers who actually feed and care for the brood. In the Hymenoptera, the workers themselves are females, but they do not normally count in the sex ratio because they are sterile (although we saw with the honey bee, workers bequeathed to the daughter queen can be considered as investment in that queen).

Incidentally, haplodiploidy is most famous as an explanation for worker sterility. W.D. Hamilton explained the evolution of the sterile worker caste by his theory of kin selection. He noted that genes in sterile workers could, under certain conditions, make more copies of themselves in future generations by rearing their siblings instead of producing offspring. One such condition, he argued, was haplodiploidy, because under that genetic system a female shares 3/4 of her genes with full sisters, higher than the 1/2 she shares with her offspring. This would appear to explain why sociality evolved so many times in haplodiploids, and why the workers in these species were invariably female. However, the specific hypothesis has not stood up very well because,



Figure 2. The parasitoid wasp *Nasonia vitripennis* on its host, a fly pupa. Photo courtesy Jack Werren.

among other things, it ignored the fact that a female is related to her brother by 1/4, so that on average, she is related to both siblings and offspring by 1/2.

Although recognition of the troublesome 1/4 relatedness to brothers undermined this famous application of Hamilton's kin selection theory, it also led to the most spectacular support for kin selection theory — through sex ratio studies. As we have noted, in the most typical colony structure with a single once-mated queen, workers are related to their sisters by 3/4 and to their brothers by 1/4. Genetically speaking, it is as if the sisters are a three-fold better bet for a worker to pass on her genes. Robert Trivers showed that, if such workers are controlling the colony's investment in reproductive females and males, they should invest three times as much in females. Data from museum specimens supported the prediction that sex ratios are indeed female-biased in haplodiploid species with this colony structure, in contrast to a variety of species without it (such as those with multiple queens, those with slave workers

unrelated to the brood, and diploid termites). Later it was shown that the same difference frequently appears within species. For example, colonies where workers are related to sisters by 3/4 specialize in females, while those where relatedness to females is lower, owing to multiple queens or multiple mating, specialize in males.

#### Control by non-autosomal genes

Düsing, writing before Mendel was rediscovered, could not have known that his theory also implied an assumption that sex ratio is controlled by the mother's autosomal genes. When that is true, simple tallies of sons and daughters will suffice because both carry a given autosome with probability 1/2. But if sex ratio is controlled by a genetic element with a different transmission pattern, then selection should reflect the altered probabilities (just as it does with differing worker relatedness to sisters and brothers). For example, Y chromosomes spend all their time in males, so production of daughters is useless to a gene on

the Y. Likewise, X chromosomes spend 2/3 of their time in females, and should be selected to produce female-biased sex ratios. Hamilton marshaled the evidence supporting biased sex ratios caused by genes on the sex chromosome. Because autosomes continue to favor a balanced sex ratio, the important implication is that individuals do not always have indivisible interests — instead there can be conflict between contending sets of selfish genes.

This particular bias will not occur in the haplodiploid Hymenoptera because sex is determined by ploidy rather than by special chromosomes, although maternally transmitted parasites can cause their preferred expected female biased sex ratios. But there is a spectacular example of a selfish sex ratio distorter in the parasitoid *Nasonia vitripennis* studied by Jack Werren. Called PSR for paternal sex ratio, it causes fathers to have excess sons, which ought to be puzzling because haplodiploid males cannot normally have sons (if the egg is fertilized, it becomes diploid and hence a daughter). It works because PSR is inherited on a B chromosome. B chromosomes are small inessential chromosomes that exist in one or more copies in some cells. They do not segregate neatly like autosomes (or A chromosomes) but copies do get transmitted in less regular fashion to offspring. When one or more copies of this B chromosome are transmitted via sperm, they cause the father's A chromosomes to condense into chromatin and be lost. What remains then, are the maternal autosomes, whose haploid state directs the development of a male, along with the successfully transmitted B chromosome. Models that incorporate the details of PSR's effects into sex ratio theory show that PSR can spread only when sex ratios are otherwise female-biased, but that it can spread at all is remarkable. It is an ultimate selfish element; in each generation, the B chromosome succeeds only by completely destroying its fellow

traveler A chromosomes and joining a new set.

### Conclusion

Düsing's basic insight into how sex ratios evolve has been developed into a wide-ranging and successful theory that predicts both the conventional 1:1 sex ratios and numerous extraordinary ones. A surprising amount of the evidence has come from haplodiploid Hymenopteran insects, though many of these effects can also be found in other organisms, even where chromosomal sex determination mechanisms would seem to lock them into 1:1 sex ratios. Most important, this seemingly arcane topic has played very prominent roles in the development of game theory, in the debate over group selection, in supporting kin selection and parent-offspring conflict, and in the field of within-organism conflict. Many biological advances will require high tech instrumentation and deeper probing into molecular mechanisms of model organisms. But there remains room for a good theory, for adroit choice of non-standard organisms, and even for simple counting.

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## Correspondences

### Road crossing in chimpanzees: A risky business

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During group movements, monkeys may cooperate to reduce the risk of predatory attacks through adaptive spatial patterning. For example, adult males move toward the front of the group when travelling towards potentially unsafe areas such as waterholes, and bring up the rear when retreating [1–4]. Comparable data on progression orders in moving groups of great apes are lacking.

We hypothesised that chimpanzees evaluate risk when crossing roads, and draw on a phylogenetically-old principle of protective socio-spatial organization to produce flexible, adaptive and cooperative responses to risk. Progression orders were studied in the small community of chimpanzees (*Pan troglodytes verus*) at Bossou, Guinea, as they crossed two roads, one large and busy with traffic, the other smaller and frequented mostly by pedestrians. We found evidence that the degree of risk, estimated in terms of the width of roads and the amount and type of traffic they carried, influenced the waiting time before crossing the roads and the order in which the chimpanzees crossed.

The home range of the 12-strong chimpanzee community at Bossou (7° 39' N; 8° 30' W), covers about 15 km<sup>2</sup> of mixed forests surrounded by abandoned and cultivated fields. It is dissected by a narrow road (3 m wide) which is used by pedestrians, and a recently widened larger road (12 m wide at the crossing point), which carries trucks, cars, motorbikes and pedestrians. The Bossou chimpanzees have to cross