

Curious Sex Ratios and Cytoplasmic Genes

Microbes Can Distort the Sex Ratio of Populations

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Evolutionary theory predicts that most populations should consist of roughly as many males as females. Recently, a variety of populations have been described which show very different proportions of the two sexes, especially in invertebrate species that contain individuals whose offspring are almost all female. Some of these instances can now be explained by the diverse actions of various 'selfish' elements residing in the cytoplasm of the individuals, which are transmitted exclusively through the female line. These instances of curious sex ratios exemplify an important principle: the fitness of genes, rather than of individuals, is the primary currency of evolution.

Introduction

Typically there are as many men as there are women, more or less, and this ratio is found in many species. However, it is not so in all organisms. In ladybirds, for example, we find strains of females whose sons all die, producing populations in which there are many more females than males. Similarly there exist wasps that reproduce asexually (i.e. without fertilisation of the eggs) also producing only daughters. Curiously, however, when fed antibiotics, both the ladybirds and the wasps reproduce 'normally': the ladybirds' sons survive and the wasps require their eggs to be fertilised. To understand why this is so, it is necessary to look closely at the theory of evolution and to realise that natural selection cares neither about the fitness of species nor the fitness of individuals, but only about genes (see *Box 1*).

In his book of 1930, *The Genetical Theory of Natural Selection*, Sir Ronald Fisher suggested "the sex ratio will so adjust itself under



Box 1 The Concept of Evolutionary Fitness

The ecologist Steven Stearns has described 'fitness' as "that which everyone understands but no one can define". Phrases such as 'survival of the fittest', which have slipped into everyday language, are testament to its intuitive nature: on the other hand, widespread misuse and misunderstanding of such phrases bear witness to the subtlety of the concept.

Charles Darwin's theory of evolution through natural selection rests on four tenets:

- (i) that any given species reproduces more individuals than can possibly survive.
- (ii) that these individuals vary in all sorts of characteristics.
- (iii) that such variations are heritable (i.e. are passed on to offspring).
- (iv) that individuals whose particular variations cause them to leave more offspring than their counterparts will come to dominate the population over successive generations.

Although much of this article illustrates that this view of evolution, centred on the individual, is oversimplified and can be misleading, Darwin's description provides us with a rough working definition of fitness: it is a measure of the net effect of heritable characteristics on reproductive success. A gene which causes its carrier to avoid predation better than its counterparts (e.g. by coding for better camouflage), will spread through a population over time because its carriers, as a result of the fact that they are less likely to die from predation, will tend to leave more offspring.

the influence of natural selection that the total parental expenditure incurred in respect of children of each sex shall be equal". In many cases, this suggests that an evolutionary pressure should exist for sexual species to maintain a stable 1:1 ratio of females to males. (See *Box 2*)

The beguiling simplicity of this assertion belies the fact that it rests on several major assumptions: among the most obvious are that populations are composed of separate sexes, that two parents are required for sexual reproduction, and that individuals mate randomly within a large, unstructured population. In fact, an entire field of evolutionary biology, sex allocation theory (see *Box 3*), has grown up to explain how deviations from these assumptions lead to very different predicted sex-ratios.

Natural selection cares neither about the fitness of species nor the fitness of individuals, but only about genes.

Box 2: Fisher's theory of Sex Ratios

To understand Fisher's predictions for a stable 1:1 sex ratio, we need only concentrate on the fact that in a population which reproduces sexually, every individual will carry and transmit the genes of one father and one mother.

To investigate the implications of this, first consider the fate of genes carried by individuals in a population which comprises 1 male for every 10 females (a 1:10 sex ratio). Let us suppose that this sex ratio bias is heritable such that the next generation will again show a 1:10 sex ratio. On average any given male will have ten times as many offspring as a given female (i.e. males will have a substantially higher fitness than females). Note at this point that although males and females have a very different fitness, all males have the same fitness and all females have the same fitness, on average.

Now consider a newly mutated gene (capable of being transmitted by both males and females) that causes carriers to produce only sons. The gene will rapidly spread through the population i.e. its carriers will have far more grandchildren than their counterparts (as a result of having all male children, each of which experiences higher reproductive success than it could have expected as a daughter). In other words the 'all male' gene will initially confer a strong selective advantage to its carriers. As the 'all male' gene spreads, however, the population as a whole will approach a 1:1 sex ratio. The closer the population gets to a 1:1 sex ratio, the smaller becomes the difference in the expected reproductive success (fitness) between males and females. As a consequence, the closer the population gets to a 1:1 sex ratio, the less selective advantage the 'all male' gene will confer. At a population sex ratio of exactly 1:1, a given male and a given female will have exactly the same reproductive success. At this point, for an individual to produce an excess of one sex or the other becomes disadvantageous: to produce more sons and less daughters (or vice versa) will cause the over-represented sex to experience lower reproductive success. Carriers of such genes, whether they be existing carriers of the 'all-male' gene or carriers of alternative new mutations, will have fewer grandchildren (by the same argument as above) than their non-carrying counterparts. The 'all male' gene will therefore be lost from the population, and no other such genes which arrive in the population will be able to spread.

One can equally well start the population with a 10:1 sex ratio, in which case the opposite is expected to occur, i.e. selection will favour those individuals capable of making a female biased sex ratio.

A further and rather subtle assumption is that the genes controlling the sex ratio are equally likely to be transmitted by fathers and mothers. You have 46 chromosomes, 23 each from your mother and father. The genes contained on these chromosomes are said to exhibit Mendelian Inheritance, i.e. the chance that a given gene will be transmitted to progeny is the same as for all other genes regardless of the sex of the parent. Some genes



Box 3 An example of Sex Ratio Theory

Sex Ratio Theory uses Fisher's theory (*Box 2*) as a starting point, but considers the effect of varying his underlying assumptions in order to explain anomalous sex ratios observed in the natural world. Take the following example.

In most natural populations, males are potentially able to inseminate many females, such that females represent the limiting factor in reproductive output for the population as a whole (consider, for example, the reproductive output of a population containing 10 females for every male compared to that of a population containing 1 female for every male). In large randomly mating populations, this is irrelevant because the individuals comprising that population are unrelated: the way for any one individual to maximise its expected reproductive success is to produce the rarer sex until an equal ratio is attained (see *Box 2*).

Bill Hamilton was the first to consider one type of alternative: a species wherein all offspring of a given individual inbreed (i.e. a female will only mate with her brothers). In this case, all the individuals involved in a particular bout of mating are closely related. Why should this affect the sex ratio? Imagine a female that produces a 1:1 sex ratio under this circumstance. Her sons will be competing amongst themselves to mate with their sisters. Males, however do not invest in the production of young and hence the number of grandchildren will be determined by the number of daughters. Consider then an alternative strategy to produce just one son and as many daughters as possible. Under such circumstances related males will not be competing and there will be many more grandchildren. Hence, if competition between males for mates is between related males, then it is in the parents' interest to produce more daughters than sons. Although this argument is most easily seen from the point of view of the individual, it works just as well from the point of view of a gene. This exposes an implicit assumption in Fisher's argument (see *Box 2*), namely that he assumes there is open competition between unrelated males for access to females.

Although these conditions may seem bizarre, they are in fact well described in a number of species of insects and mites. A particularly good example is that of the fig wasps. Here, a single female will lay eggs within a fig. When the eggs hatch, the son will fertilise all daughters before any individual leaves the nest (indeed, the male often dies without ever leaving the fig!). In the case of a single family within a fig, observations show a resulting sex ratio very close to that predicted by Hamilton's model. What makes these creatures yet more interesting however, is that sometimes more than one (but still only very few) female will lay eggs in a single fig. Hamilton's model predicts that, as the competition between unrelated males becomes more common, the sex ratio produced by any one mother should get closer and closer to 1:1 as more families are present in a single fig and this is what is found.

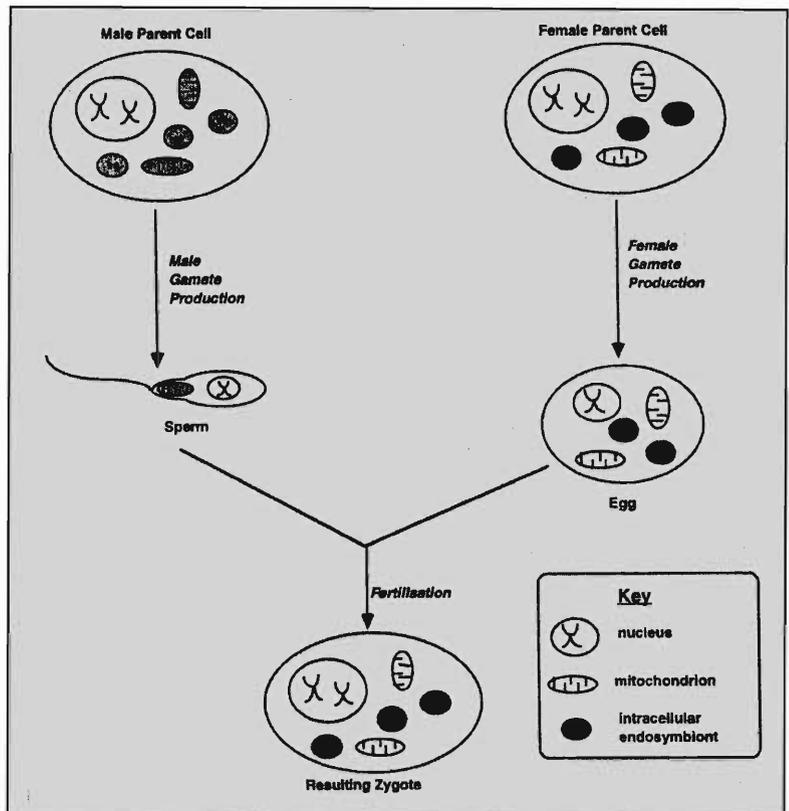


however are not contained on these chromosomes and are not transmitted by fathers: it is these genes that hold the secret to the curious sex ratios of wasps and ladybirds.

Cytoplasmic Genes

In general, sperms are much smaller than eggs. The additional space within eggs is termed the *cytoplasm*. Genes existing here are called *cytoplasmic genes*. Such genes reside within cell organelles such as mitochondria and chloroplasts. Many insects and crustaceans also harbour endosymbionts such as bacteria and single celled eukaryotes within their cells and these microbes, obviously, carry their own genes. Although these extra passengers usually occur within the cells of both males and females, the difference in size between sperm and egg means that these 'intracellular endosymbionts' find their way into the egg cells but not into the much smaller sperm (see *Figure 1*). For this

Figure 1 Uniparental Inheritance. During the formation of sperm from mature male germline cells most cytoplasm is lost, including all endosymbionts. In contrast, when eggs are formed from mature female germline cells, they retain a significant amount of cytoplasm, and this is likely to contain endosymbionts. As a consequence, individuals appearing at each new generation will contain endosymbionts inherited from mothers only: endosymbionts located within fathers are at an evolutionary dead end.



reason cytoplasmic genes, endosymbionts included, are typically only inherited from mothers (sperms do contain a few mitochondria, but their genes are too few to make a difference).

In contrast nuclear genes (on our 46 chromosomes) are inherited from both parents. The difference in transmission from parent to progeny between these two classes of genes is important because different selective forces act on each. Imagine a cytoplasmic factor that is in an egg which is about to be fertilised. Where would it be advantageous for it to end up in a son or a daughter? Naturally, the answer is that ending up in a daughter would be advantageous in terms of fitness. Imagine what would happen if a new cytoplasmic factor appeared in a population that could somehow ensure that it ended up in a male. Within the first generation this factor would go extinct - it would remain in its male host during the latter's lifetime but would not be transmitted to further generations. Cytoplasmic genes which find themselves within males are, thus, at an evolutionary dead-end.

On the other hand, if a cytoplasmic factor could ensure that it always ended up within a female, this would be a very successful strategy. A factor with no control over its own destiny would, by chance, occur within females only 50% of the time, on average. Hence we would expect our hypothetical mutant to be at a strong advantage. In more formal terms cytoplasmic genes are under strong selective pressure for increased frequency of location within female offspring of their hosts. Successful mechanisms for achieving this goal have now been described for a variety of intracellular endosymbionts: the strategies are diverse and ingenious.

Cytoplasmic Sex Ratio Distorters

- **Feminisers**

The sex of an organism is usually determined by its nuclear genes: humans use the 'X' and 'Y' chromosomes: men contain a

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copy of each (i.e are 'XY') and women contain two copies of the 'X' (i.e. are 'XX'). Perhaps the most obvious strategy for a cytoplasmic factor to ensure that it always occurs within a female would be to override the (nuclear gene) sex-determining system, such that a zygote develops into a female regardless of the nuclear chromosomes present. Exactly this effect is now known for intercellular endosymbionts occurring within a variety of crustacean species.

The best example has been described in the common woodlouse *Armadillidium sp.* which harbours the bacterium *Wolbachia sp.* Uninfected woodlice have a similar genetic sex-determining system to humans except that the pattern is reversed: males are 'XX' and females 'XY'. Individuals infected with *Wolbachia*, however, develop as females regardless of their sex chromosomes. Something very similar occurs within the shrimp *Gammarus duebeni*, except that the endosymbiont is not a bacterium but a microsporidian a very early form of eukaryote.

Feminisation has not been confirmed in non-crustaceans. It seems sensible then to ask whether there is something about crustaceans which predisposes them to this sort of manipulation. Clues come from the mechanisms by which the feminising microsporidian and the bacterium work: both seem to interfere with the development of a gland whose secretion makes males male. Intriguingly, if this gland is prevented from forming, the crustacean develops into a perfectly normal female. The endosymbionts hence need only interfere with the development of one small gland to be able to cause a genetic male to develop into a female that this may be quite easy to do probably underlies the fact that feminisers are so common in crustaceans.

An interesting point about the process of feminisation, and sex ratio distorters in general, is that it is most definitely not in the interests of the host organism to produce an excess of female offspring. Return to Fisher's original sex-ratio argument, and consider an uninfected population with a stable 1:1 sex ratio. If



a feminising agent arises within this population, then the females in the population would experience a lower reproductive success than if they had developed as males. In addition, the physical pressure of the feminizing agent may be bad for females, much as parasitic infections in humans make us ill. Either way, the endosymbiont reduces the evolutionary fitness of the host organism. It is for this reason that in *Armadillidium* the host seems to have bounced back. In some French populations the spread of the feminising factor has resulted in the host counter-adapting to redress the sex ratio. Just as Fisher noted, nuclear genes can typically spread by allowing the production of the rarer sex. In the French population this has resulted in a remarkable transition - the whole means of sex determination has changed. No longer is sex determined by X and Y chromosomes, but instead a nuclear gene controls the transmission of the bacterium: eggs that receive the bacteria become female and those that do not become male.

Evolution does not care about the long term fate of genes - the only issue is: will the gene spread here and now?

But what if such counter-adaptations had not occurred? It is perfectly possible for all individuals to become infected with the feminising agent. At this point, no males will be produced and the population will go extinct. This clearly illustrates the fact that evolution does not care about the *long term fate* of genes - the only issue, and the only question worth asking is: will the gene spread here and now? The feminisers are acting in their own selfish and immediate interest, and we can only understand the system in these terms.

● Parthenogens

Given that cytoplasmic genes can spread by making their hosts female, but can also go extinct by so doing, we might ask whether there is an evolutionary nirvana for cytoplasmic factors in which they are always transmitted by females that never need males to reproduce? Such a state does exist if cytoplasmic factors could make females reproduce asexually and only produce daughters (a process known as *parthenogenesis*), they could

both spread and not go extinct. A different form of *Wolbachia* that has been shown to do just this is a tiny wasp, *Trichogramma*: infected individuals form strains of parthenogenetically reproducing females.

Again, the mechanism by which *Wolbachia* achieves this goal is simple and elegant. Uninfected populations of *Trichogramma* reproduce sexually, being comprised of haploid males (containing a single copy of each chromosome) and diploid females (like all humans, containing two copies of each chromosome). Individuals appear to 'know' what sex they are to develop into by counting the number of chromosomes present within their nucleus: two sets of chromosomes indicates diploidy and hence leads to development of female characteristics. The bacterium interferes with the early mitotic cycle of its host. Whereas a normal zygote splits in two to produce a pair of haploid cells, the bacterium prevents this splitting process, thus leaving a single cell with two chromosome sets. All subsequent divisions of the infected individual are normal. This forced diploidy causes progeny to develop into females. One means by which this form of cytoplasmic sex-ratio distortion can be 'cured' is to feed the wasp antibiotics, just as antibiotics cure bacterial disease in humans (*Figure 2*). With no bacteria present the wasps revert to normal, sexual reproduction.

- **Male Killers**

In the above two examples, the benefit to the cytoplasmic symbiont is self-evident; the symbionts simply increase the number of daughters. Somewhat more subtle is the action of so-called 'male killers'. For example, in the ladybirds a cytoplasmic factor which finds itself within a male simply kills that male (*Figure 3*). It does not escape, and thus effectively commits suicide. The number of daughters produced by the mother is not altered. How could such a gene, not increasing the number of females and committing suicide, ever spread and persist within natural populations?





Figure 2 (left) Organisms which harbour bacterial intra-cellular endosymbionts may be 'cured' by feeding them antibiotics. In this picture, a ladybird harbouring Male Killer endosymbionts is being cured by feeding it antibiotic dissolved in syrup.

Figure 3 (right) A brood of lady-bird eggs showing a 50% hatch rate. Those eggs which have hatched are all females. Those which have not hatched would have been male, but have been killed by Male Killer endosymbionts.

In order to understand this strategy, we first need to recognise that because they share the same mother, the sisters of the dead males carry genetically identical ('clonal') relatives of the bacterium which causes the deaths. In addition, remember that the bacteria located within males were never going to go anywhere: effectively they already had zero fitness. Thus under certain circumstances, suicide is the best strategy. If the death of the males increase the fitness of the infected sisters, then overall the fitness of the bacterium can only increase. In broader outline, this is an elegant example of the action of 'kin selection': the evolutionary rationale tendency for organisms to help their relatives. Genes may spread by increasing the fitness of any copy of themselves.

A Sex Ratio Distorter in Humans?

So far we have discussed curious sex ratios only in insects and crustaceans. But what about humans? Perhaps most suggestive of all is the curious case of Mme B, reported by French doctors in 1947. This anonymous French lady comes from a lineage of 3 generations of 72 offspring in which only daughters have been

Genes may spread in populations by increasing the fitness of any copy of themselves, even if such copies reside in related individuals.

Suggested Reading

- ◆ Hamilton W D. Extraordinary Sex Ratios. *Science*. Vol.156. pp. 477-488, 1967.
- ◆ Dawkins R. *The Extended Phenotype*. WH Freeman. Oxford, 1982.
- ◆ Bull J J and Charnov E L. How fundamental are Fisherian sex ratios? *Oxf. Surv. Evol. Biol.* Vol.5. pp. 96-135, 1988.
- ◆ Hurst L D. The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. *Biological Reviews* Vol.68 . pp.121-193, 1993.

produced: very few further details have been researched but the possible links to some form of feminisation process are very intriguing.

Discussion

The theory of evolution by natural selection is both the simplest but also one of the most misunderstood and subtle of ideas. Selection, you will often hear said, is for the 'good of the species'. It isn't. It is instead a simple statement that genes can spread within populations and that the traits which we see manifested in organisms around us are those which are consistent with this spread. Often when we think of this, we consider a gene coding for a trait which increases the 'fitness' of the organism in which it is found: a gene coding for a better eye spreads in the population because bearers of the gene will, on average, leave more progeny than bearers of the alternative gene. It may be advantageous to the survival of a species but this is a *consequence* rather than *cause* of the spread.

Although the idea of evolution by natural selection at first sight appears to be trivial, the subtlety of the idea is easy to overlook. In particular, as we see in the case of cytoplasmic sex ratio distorters, genes may spread despite the fact that they decrease the fitness of the organism in which they are found. Not only should we not be asking whether a trait is for 'good of the species', we should not even be asking if traits are for the good of individuals. Instead we should ask: is the trait good for the gene? i.e. will the trait spread. Once this is realised, we see that organisms, metaphorically speaking, are battlegrounds in which genes 'selfishly' pursue their own short term 'goals'. The cytoplasmic genes we have discussed here are fine examples where we can see these intragenomic battles going on, with the prize to the victor being increased representation in the next generation.

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