Group Report: Cooperation and Conflict in the Evolution of Genomes, Cells, and Multicellular Organisms

Chapter from:
Genetic and Cultural Evolution of Cooperation
Edited by P. Hammerstein, 2003
ISBN 0-262-08326-4

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Group Report: Cooperation and Conflict in the Evolution of Genomes, Cells, and Multicellular Organisms

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INTRODUCTION

Most of us think of ourselves as individuals. The biological world around us contains a multitude of individuals, each of which is composed of many subunits that were separate in the evolutionary past. Some joined to form higher-level units, and others are still separately replicating but joined for life. This marriage harbored great benefits from mutualism and cooperation but also brought with it problems that arise from conflict of interest between the partners.

Conflicts between units arise when the selection pressures on some of the units favor one outcome, whereas those on other units favor another. The most basic conflict is between two units of the same species, when selection pressure on one of the units favors the survival of its own lineage over survival of the lineage of the other unit.

Conflict can exist when two units have influence over a common feature. The nucleus and mitochondrion can have an influence on the sex ratio, and thus a conflict over sex ratio can exist. A unit of a species might have another unit, but as long as it has no influence on that sex ratio, conflict will not arise. When evolution joins units into an association, then the tighter the association is, the more areas of common influence exist and the more potential for conflict exists. If the potential for conflict is lessened through the evolution of conflict mediation such as shared fate, selection pressures on the participants of the association will tend to favor similar outcomes, which will strengthen the association between the participants. Thus an association allows conflict, and lessening the conflict

allows for stronger association. To understand this process, it is instructive to classify possible kinds of cooperation, source and types of conflicts that might arise in them, and the ways in which some of these conflicts are mediated (cf. Partridge and Hurst [1998] for further review and classification of such conflicts). In our discussions we tried to understand conflict in general, without separating conflict in interaction between units of the same species from conflict between interactions of units of different species. This was partly to understand the general features of conflict and conflict mediation, but primarily because the scenarios in which conflict arises usually involve both conflict between units of the same species and conflict between units of different species. Thus in the association between mitochondria and the cell nucleus, we have conflict between the mitochondria themselves and between the mitochondria and the nucleus.

Since organisms are composed of layers upon layers of cooperation, the formation of a new association could give rise to a conflict at one of the lower layers. Thus in the cooperative association between a multicellular parent and its internally carried offspring, conflicts at some of the lower levels that make up the multicellular partners (parent and offspring) can arise: a conflict between alleles at a locus, between organelles, or between cells.

Why have we not stated matters in terms of cooperation and conflict between genes? We could, for example, have talked about a conflict between a nuclear gene and a mitochondrial gene, but instead we talk about a conflict between the nucleus and the mitochondrion. As different levels of associations form, units have a shared fate, or a shared fate at a certain level of association. In those cases, the units will also have shared interests, and it is not necessary to separate them out into separate genes. For example, in a multicellular organism, a cell that replicates faster within the organism will have an advantage over other cells and will spread, often at a fitness cost to the whole organism — it will become a cancer. In this case, all the genes in the cell, including mitochondrial genes, autosomal genes, and sex-linked genes will usually have a shared interest in faster replication of the cell. In such a case, it is convenient to talk about the conflict of interest between cells in the multicellular organism.

We note that sexual reproduction plays an important role in cooperation and conflict. Many of the conflicts described would not exist at the population level without sexual reproduction. For example, conflict mediation seems a good explanation for why mitochondria are transmitted uniparentally, but not for why organisms are not asexual. We have tried, however, to not delve too deeply into questions that involve the reasons for sexual reproduction, since those are mostly unknown, or at least not agreed upon.

Classification of Cooperation

We classify cases of cooperation according to these types: (1) interchangeable vs. non-interchangeable units, (2) level of partner association, (3) asymmetry in transmission, (4) differences in replication rate, (5) mutational space or

available strategies, and (6) type of benefit function. Using this classification, it is then possible to point out the areas of conflict that arise within associations of units. The factors that are known to be important for conflict, kinship, horizontal vs. vertical transmission, and shared fate are included in classification type (2) level of partner association.

Interchangeable vs. Non-interchangeable Units

Interchangeable units come from the same gene pool, and thus are in direct competition. Non-interchangeable units come from different gene pools and are not in direct competition. Here, partners are in conflict only as far as the selective forces that act on them are.

The symbiotic interaction between species is an example of interaction between non-interchangeable units, whereas cooperation between individuals within a species is an example of an interaction between interchangeable units.

When one type fixes in a population of interchangeable units, it also displaces all types available to the other partners in the association. On the other hand, when a type fixes in a population in which there is interaction between non-interchangeable units, then in the population of the other partners there are still different types.

Different alleles at one locus provide another example for a group of interchangeable units, whereas alleles at different loci are non-interchangeable: When a meiotic drive allele at locus **A** invades a population, it does not outcompete alleles at other loci, as it is not in direct competition with them. It may, however, have a conflict of interest with alleles at other loci: the selection process will cause a mutant to invade even if it lowers the total fitness of the organism, as long as its own drive at locus **A** increases, whereas the selection pressure on alleles at other loci favors those alleles that raise the total fitness of the organism. The selection pressure on alleles at unlinked loci is neutral with respect to meiotic drive at locus **A**. This is a conflict, since once such an allele, which lowers the total fitness of the organism and outcompetes other alleles at locus **A**, invades the population, mutants at other loci can invade if they reverse this effect, thereby raising the total fitness of the organism.

A single interaction can involve both interchangeable units and non-interchangeable units. For example, in the symbiosis between mitochondria and nucleus, we have both interchangeable units and non-interchangeable units: cooperation between the different mitochondria in a cell is a cooperation of interchangeable units, whereas cooperation between the mitochondria and the cell nucleus involves noninterchangeable units.

Even though the definition of interchangeable vs. non-interchangeable is clear cut in many cases, these are extremes taken from a continuum. Individuals in different species are non-interchangeable units and individuals from the same species are interchangeable units. It is obvious, however, that during speciation, there is a point at which individuals from the *same* species are non-

interchangeable — for example, if they cannot drive the other to extinction, maybe because they already occupy different ecological niches. In the genome, genes at different loci are non-interchangeable, and genes at one locus are interchangeable. On the other hand, transposable elements can be seen as interchangeable units even when in different loci.

Partner Association and Kinship

One of the most important factors in the evolution of cooperation concerns the time span for which partners stay together. A well-known phenomenon in simple game theoretic examples, such as the Prisoners' Dilemma (PD) game vs. the repeated PD game, is that the length of partner association can play a role in the level of cooperation (see Axelrod 1984). Here we focus mainly on the length of association over evolutionary time.

Length of association — partner permanence vs. partner change (see Figure 18.1): In some cases partners are permanently joined and can never switch to other partners in the current population. Strict asexual reproduction provides an example of this: genes are in permanent association, and thus there is perfect alignment of transmission.

At the selective level that includes both partners, there is no long-term conflict of interest; the reproductive success of one partner is identical to that of the other. However, within the organism there could still be a short-term conflict of interest. For example, a transposable element in an asexual species has no conflict of interest with any other genes in the long term, but in the short term (within the lifetime of the lineage it is in) a transposable element might be selected for a high replication rate, even if it reduces the fitness of the organism.

In many cases, cooperation partners can be changed between generations. For example, genes at different loci in the genome are not in permanent association; recombination can change genetic partners. Partner permanence is the extreme case of a slow change of partners. In general, horizontal transfer causes partner change. When partner change occurs, one can talk about the level and fidelity of the association. This level is defined as the probability that partners in

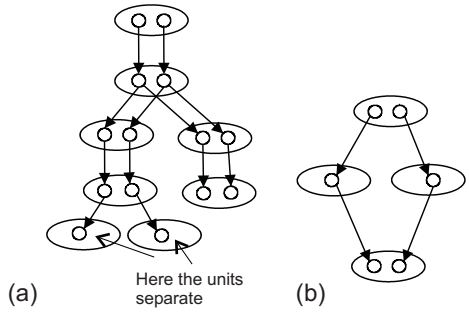

Figure 18.1 The length of association: (a) How long are partners expected to stay together? (b) How likely are they to meet again (compare also with kinship)?

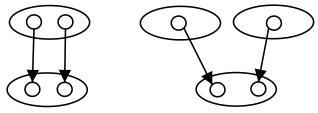

Figure 18.2 Aggregation vs. clonality: Do all units in the current association come from the same parent or from different parents?

the same cooperative group of one generation will be in the same group a certain number of generations in the future. Thus, genes on different chromosomes usually have a probability of 1/2 to stay together in the next generation after meiosis. In inbreeding populations, partners will have a high likelihood of meeting each other again, which will increase the expected length of association.

Type of formation — horizontal vs. vertical transfer (see Figure 18.2): When partners are changed, we can ask how the association was formed: through cloning or through aggregation. Aggregation is defined as the case in which partners that form a new association came from different associations in the past. Cloning is the case in which partners came from the same association. This is usually called horizontal vs. vertical transfer. When an association is formed by aggregation, horizontal transfer takes place. For example, the joining of genes from two mates during fertilization of the egg by the sperm is a case of aggregation, since the genes in the new cell come from two different cells. On the other hand, since the mitochondria in this fertilization come only from the egg, the association between the mitochondria is formed by cloning. When partner change is rare, the following is possible: an association can be formed by cloning and yet have nonpermanent partners. For example, plants can reproduce asexually by cloning, and yet the two alleles on the diploid chromosome in each cell are not in permanent association if sexual reproduction does occur from time to time.

Kinship (see Figure 18.3): In the case of cooperation between interchangeable units, we can ask not only if units that are in the same cooperative association have descended from units that were already in the same cooperative association, but also whether they have actually descended from one and the same unit, i.e., are identical by descent. If a cooperative association was formed through aggregation, the level of kinship between the units will have a strong influence on the level of conflict (Hamilton 1964).

Partner choice: Sometimes a unit can choose which other units to associate with, or can choose to leave an existing partner and find another. This choice does not have to be an active choice made by an individual. It can occur over evolutionary time. Partner choice creates the possibility of markets (see Bergstrom et al., this volume; Hammerstein, Chapter 5, this volume).

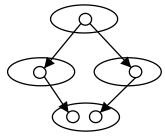

Figure 18.3 Kinship: How likely are two interchangeable units in an association formed by aggregation to be identical by descent?

Asymmetry in Transmission

Not all units that are currently in association will have the same future. It might be the case that two or more types of offspring are produced: a common example is the case of male and female offspring or offspring that are produced by unequal cell division. In the extreme case, only a subset of the units currently in association will be transmitted to the next generation — the units go through a bottleneck. The transmission of one of the two alleles at a locus to the egg nucleus and the relegation of the other allele to the polar bodies is a simple example of such a bottleneck. Asymmetric transmission can create a conflict between interchangeable units over who goes into which offspring. It can also create a conflict between non-interchangeable units as the fitness of the partners is dependent on the survival of different entities. (See Figure 18.4.)

Difference in Replication Rate

Whether partners are permanently associated or more loosely associated, non-interchangeable units can have a difference in replication rate. For example, different genes on the chromosome usually have the same replication rate. Transposable elements are one exception: the element itself replicates faster within the genome than other genes do. Such a difference in replication rate can create a conflict of interest between the units, since it allows for selection pressure that favors faster replicating units.

Mutational Space or Available Strategies

When a conflict occurs, the mutational space and strategies available to the units will strongly affect the outcome or resolution of the conflict. In a non-interchangeable association, units might have different available mutational spaces, different evolutionary rates, and different levels of phenotypic plasticity. For example, the mutational space of mitochondria and the range of influence they have on the organism is smaller than the space available to the nucleus and its influence because of the difference in the number of genes coded and because these genes effect only a limited part of cell function. On the other hand,

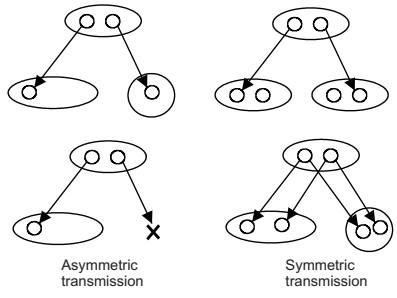

Figure 18.4 Transmission of partners in an association is called asymmetric if the offspring into which the different partners are destined can be distinguished.

mutation rates in mitochondria are sometimes higher than those in the nucleus but are, in general, highly variable (Wolfe et al. 1987; Pesole 1999). Both mutational space and mutational speed determine how fast a relevant mutation will arise when a conflict exists between the selection pressures acting on the mitochondria and on the nucleus. Mutational space is related to the concept of power, as known in economics (see Bowles and Hammerstein, this volume).

Type of Benefit Function

Fitness differences that result from the association and strategies available to the partners will affect the evolutionary outcome of the association. For example, in an interaction between interchangeable units, the benefit might be sublinear, linear (additive), or more than linear in the number of units that cooperate in an evolutionary interaction. In other cases, there might be very strong nonlinearities. Maynard Smith and Szathmáry (1995) give a good example of this effect: Imagine a group of people rowing a boat. If each person rows using two paddles, the increase in benefit, in terms of how fast the boat will get to its destination, is gradual in the number of rowers. On the other hand, if each person paddles on one side only, then removing one of the paddlers can have a catastrophic effect on this speed, since the boat will only go in circles. From this example we can see that the structure of the benefit or interaction function affects the conflict in the system, but that it can also be used as a conflict mediator: In one-sided paddling no single defector can invade since she will have a catastrophic effect on the fitness of the group and herself.

Conflict Mediation

The above classification will enable us to point to cases in which the type of cooperation is more susceptible to conflict. It also points to features that reduce conflict. Thus partner change increases the possibility for conflict, whereas partner permanence, or shared fate, reduces the possibility for conflict.

If units that have potential conflict have a large mutational space available to them, conflict is more likely; when this space is reduced, conflict is less likely. Thus, increased recombination rate reduces the mutational space available to conflict between alleles at the same locus because it reduces the total length of tightly linked loci.

In some cases specialized mechanisms for policing seem to have evolved. The immune system is such a policing agent, detecting cases of cancer in a multicellular organism. Another example is the detection and partial destruction of DNA sequences that appear twice in the genome of several fungi (*Neurospora crassa)* via mechanisms that induce hypermutation rates in repeated genes (RIP) or hypermethylation of such genes (MIP) (Selker 1999; see also Hurst and Werren 2001.) There are, however, many types of conflicts and conflict mediation, and we will expand on these throughout the rest of the chapter. For further discussion of conflict mediation, see the chapter by Michod in this volume.

ANALYSIS OF COOPERATION AND CONFLICT FOR GENES IN DIPLOID SEXUAL CELLS

Genes in the cell can be regarded as a cooperative association. In this association, many opportunities for conflict exist. We will first classify this cooperation based on the scheme described in the introduction and then discuss some possible areas of conflict and mechanisms for conflict mediation.

It should be noted that many or all of the cellular mechanisms are highly derived — thus genomes existed long before meiosis, and meiosis evolved under the background of genetic conflict. It is therefore hard to separate conflict from conflict mediation and its breakdown.

Interchangeable vs. non-interchangeable units: Within a genome, alleles at the same loci are interchangeable; alleles at different loci are non-interchangeable.

Partner association: During meiosis, partner association has a continuum from almost 1 for closely linked genes to 1/2 for genes far apart on the same chromosome or for genes on different chromosomes. The association level between alleles at the same locus between generations is close to 0. These two alleles will stay together only until the next meiosis.

Type of formation: Formation of the association is an aggregation between the genes in the sperm and the genes in the egg in the case of sexual reproduction and through cloning for asexual reproduction.

Asymmetry in transmission: Meiosis is usually a symmetric process; thus no asymmetry exists, though it is created in some cases. Sex-determining chromosomes have a different transmission pattern than the autosomal chromosomes. For example, the Y chromosome in humans is transmitted only through males.

Difference in replication rate: In cells in which all genes undergo coordinated replication, differences in replication rate do not usually exist. Such differences do exist for self-replicating units within the genome, such as transposable elements and microsatellites.

Mutational space or available strategies: When a conflict between alleles at a locus arises, the mutational space available to an allele that reduces the organism's fitness while increasing its own is limited only to the allele and to alleles tightly linked to it, whereas mutations in the whole rest of the genome could invade if they reduce this conflict and increase the organism's fitness. This idea has been termed the "parliament of genes": in cases in which there is a selection pressure on many genes to counter an effect caused by a few genes, the majority will win (Leigh 1977). One has to remember that for each particular case, the range of effects of the linked genes vs. the effect of the rest of the genome has to be considered. The mutation rate across genes is usually identical, though differences in mutation rate do exist. (One might predict that if there are mechanisms that enable local hot spots for mutations in certain genes, then "selfish genes"

with a higher mutation rate would be more successful in countering the "parliament of genes.")

Benefit function. In the association between genes in the cell, there is a complex network of interactions. These include, in many cases, apparent high redundancy but also highly essential genes. In general, there could be cases in which turning off one gene would have huge consequences, and cases in which the consequences are very small.

Conflicts

From the above classification we can see that direct competition — where we would expect the highest level of conflict — exists between alleles at the same locus. Since the association between the alleles at meiosis is zero — they will end up in different organisms — an allele would be favored if it increased its own fitness even at the cost of reducing the fitness of its sister cell, while reducing the total fitness of the organism. This can happen only when meiosis is asymmetric. Although meiosis is generally fair, in that it results in equal transmission of both homologous chromosomes to the gametes, some genes have evolved into segregation distorters. These are overrepresented among the gametes in heterozygous individuals. Several such distorters are currently known (see Hurst and Werren 2001). Examples include the *t*-haplotype in mice, segregation distorters and sex-ratio distorting chromosomes in fruit flies and mosquitoes, as well as supernumerary chromosomes in a wide range of plants and animals. Current studies suggest that segregation distorters are likely to be more commonly found as we continue to investigate the genetics of organisms (Jaenike 1996).

When segregation distortion does occur, only genes that are tightly linked to the distorter have a shared interest in killing the sister cell. Unlinked genes will suffer reduced fitness as a result and thus are in conflict with the distorting genes. Recombination is a force that reduces the size of the linked loci, and thus the size of the group of genes that have shared interests. When a segregation distorter does evolve, mutational space of the genes which disfavor the drive is bigger and this is thought to cause the drive eventually to cancel. The concept of the "parliament of genes" then claims that in this conflict, the majority present in the unlinked genes eventually gains the upper hand and restores fairness to meiosis.

Because of asymmetry between the transmission patterns of sex chromosomes and autosomal chromosomes, conflicts exist between these non-interchangeable partners. For example the Y chromosome, which is transmitted only through males in an XY sex determination system, gets its fitness only through the male offspring of the male it is in, whereas the autosomal chromosomes get their fitness from both male and female offspring. Hamilton (1967) pointed out that segregation distortion, if it occurred on the Y chromosome, could drive a population to extinction, since eventually it would fix to have only males. Cosmides and Tooby (1981) extensively discuss these conflicts. Most known examples of segregation distortion are sex-ratio distorters.

A similar effect to segregation distortion is caused by converting elements: these alleles increase their representation among offspring, not by distorting segregation but by converting the sister allele. Homing endonucleases encode an endonuclease that introduces a double-stranded break at 15–20 bp recognition motifs. The break is not repaired by direct re-ligation, but by using the sequence that contains the homing endonuclease gene as a template. The end result is a conversion of the target sequence to one that contains the converting element. Repair also splits the recognition motif, thus preventing future self-cleavage. Thus, the homing endonuclease sequence is overrepresented among the gametes of heterozygous individuals and will increase in frequency, often to fixation (Gimble and Thorner [1992], taken from Hurst and Werren [2001]). In this case linked genes do not benefit from the overrepresentation, and thus the mutational space of the unit is limited to the sequence of the homing endonuclease itself. On the other hand, conflict with other genes is lower than in the case of segregation distortion: direct competition is only with the target sequence, not with any linked sites. Whereas segregation distorters usually destroy half the gametes, converters do not cause such a big direct fitness effect on the organism and thus are in less conflict with other genes in the genome.

Conflicts can arise between genes that have different replication rates in the genome. Gene replication is usually coordinated with each other and with cell division, so that the relative number of copies of the genes stays constant. When a certain gene overcomes this restriction, it can spread through the genome of the cell. A conflict with other genes will then arise, insofar as it reduces the total fitness of the cell.

When the organism is asexual and there is no horizontal transmission of genes, then, since all genes have a shared fate, there is no conflict of interest at the higher level between the copies of the replicating elements, and no conflict between the replicating elements and other genes in the cell. In this case, in the long-term, lineages in which the replicating elements are very harmful will be weeded out. At steady state the population will reach a mutation-selection balance between replication of the element within the cell and the disappearance of lineages in which it has a high copy number.

When the organism is sexual, the association between the replicated elements can be low and, since cells form by aggregation, the replicating elements can spread through the population. As a result, there is a lower selective pressure at the level of cell lineages for transposable elements to cooperate among themselves, and with other genes in the organism, to increase the fitness of their current lineage. In such a case, conflict between the transposable elements and the rest of the genome exists because of this difference in replication rate. Again, this conflict exists only insofar as the transposable elements reduce the fitness of their lineage. It is interesting to note that once a transposable element inserts itself into a position in the genome, at that position it will spread through the population of cells faster if it does not reduce the fitness of the cells it is in, even at the cost of losing its replicating ability. From the point of view of the population of

transposable elements, such a mutant could be considered a "selfish element," even though from the point of view of the cell it is beneficial.

Examples of autonomous replicating elements are transposons and homing endonucleases. Sequences derived from transposons and other mobile elements make up over 45% of the human and 50% of the maize genome and are found in virtually all prokaryotes and eukaryotes. They are characterized by the ability to replicate and make additional copies of themselves so that they can accumulate within the genome.

Considerable evidence indicates that eukaryotic genomes are selected to repress autonomous-replicating elements or accommodate their presence. An example mentioned earlier is the detection and partial destruction of DNA sequences that appear twice in the genome of several fungi (*N. crassa)* via mechanisms that induce hypermutation rates in repeated genes (RIP) or hypermethylation of such genes (MIP; Selker 1999; cf. Hurst and Werren 2001).

ANALYSIS OF COOPERATION AND CONFLICT IN THE ASSOCIATION BETWEEN MITOCHONDRIA AND EUKARYOTIC CELL NUCLEUS

It is hypothesized that at some point in the evolution of the eukaryotic cell, a parasitic aerobic proteobacterium became an endosymbiont of an anaerobic host (Sagan 1967; Margulis 1981; Whatley et al. 1979; Cavalier-Smith 1981). Current knowledge suggests that such transitions, in which an endosymbiotic bacterium becomes an organelle, occurred only a handful of times. When such a tight association between the cell nucleus and endosymbiont occurs, many potential conflicts arise. A major force that seems to have reduced the number of potential conflicts is the transfer of many genes of the endosymbiont to the nucleus. In the hydrogenosome, which seems to have originated from a mitochondrion, all genes have been lost, which possibly removes all potential conflicts.

Many conflicts that occur between the nucleus and cytoplasmic elements have been discussed extensively by Cosmides and Tooby (1981), especially with respect to conflicts that arise in the production and fertilization of gametes. Here, we concentrate only on mitochondria as an example. (Rand [2001] also studies the various levels of conflicts between mitochondria in a population.) We will classify this cooperative system and then examine potential conflicts. As noted in the analysis of conflict between genes in diploid cells, it should be remembered that many of the features discussed here are highly derived.

Interchangeable units: In a single cell there are many mitochondria. These mitochondria within a single cell are interchangeable units in their association. The association between the mitochondria and the nucleus is an association between non-interchangeable units.

Partner association: During cell division the association between the different mitochondria in the cell is approximately 1/2, since that is the chance for two mitochondria to end up in the same daughter cell. The association between mitochondria and chromosomal genes is 1 during asexual cell division or mitosis and 1/2 during meiosis. Kinship depends on the mode of transmission. When mitochondria are uniparentally transmitted, kinship will depend on the relative probability that mitochondria have to advance to the next generation. If uniform, then all mitochondria will come from a common ancestor on average $1/2n$ generations ago, where n is the bottleneck size for mitochondria. If mitochondria are biparentally inherited, then the chance that two mitochondria descended from one mitochondrion in the same fashion (i.e., descended through the same individuals) is $a/(n+a)$, where n is again the bottleneck size and a is a constant that depends on the variance in replication rate of the mitochondria; a is 1 if mitochondria replicate randomly through a Poisson process and is 1/2 if each replicates exactly once per cell division.

Formation of association: In asexual reproduction and in sexual reproduction with uniparental inheritance of mitochondria, formation is through clonality. In sexual reproduction with biparental inheritance of mitochondria, during fusion of the gametes, the formation of the association of mitochondria is through aggregation.

Asymmetry of transmission: In uniparentally transmitted mitochondria, the mitochondria that end up in the fertilized egg of the sex that does not transmit the mitochondria will not continue to the next generation. In multicellular organisms, transmission bottlenecks of mitochondria do exist: not all mitochondria present in the fertilized egg will enter the germ line for several reasons: (a) programmed cell death of the oocytes, (b) nonreplication of mitochondria during division of the oocytes, and (c) high variance among mitochondria in different cells of the organism (Krakauer and Mira 1999).

An obvious asymmetry of transmission exists when mitochondria are transmitted uniparentally, between mitochondria that are in an offspring of the sex that transmits mitochondria to the next generation and those that do not.

Difference in replication rate: In single-celled organisms, mitochondria in general will replicate on average once per cell division (otherwise their number per cell would explode or dwindle), though the replication of these mitochondria is not coordinated with the replication of the nucleus, or with the replication of other mitochondria in the cell.

Mutational space: Mitochondria lost most of their original genes. For example, mammalian mitochondria retained only 13 of the protein-coding genes (Scheffler 1999) and thus have a limited range of mutations and strategies available to them. Post-transcriptional modification (e.g., RNA editing; Scheffler 1999) may further limit mitochondrial mutational space. The number of

mitochondrial genes presumably has changed over evolutionary time, and thus the mutational spaces available to the mitochondria have also changed. In contrast, the mutational space available to the nucleus is large. Mutation rate in the mitochondria is sometimes much higher than that of the nuclear genome, although the ratio of mutation rate of mitochondria to those of the genome is variable (see Wolfe et al. 1987; Pesole 1999).

Type of benefit function: Fitness benefit with an increased number of mitochondria is gradual within the cell. Loss of a single mitochondrion is not catastrophic: if a single mitochondrion suffers a mutation that renders it nonfunctional, the adenosine triphosphate (ATP) supplied by the rest of the mitochondria will keep the cell as a whole mostly functional. This is further enhanced by the fact that many of the functions of the mitochondria are encoded by the nucleus, and thus the expression of those genes is not affected by the loss of a single mitochondrion in the cell.

Conflicts

As can be seen from this classification, the main conflict in this case is among mitochondria in the cell, since they are in direct competition, and between the mitochondria and nucleus when they are transmitted differently. There is a difference between the type of conflicts that arise in biparental transmission of mitochondria and in uniparental transmission. Therefore, we discuss these two cases separately.

Biparental Transmission

Since mitochondria are transmitted through both parents, several things occur: mitochondria in a fertilized egg form through aggregation, and thus there is horizontal transmission of mitochondria. The relatedness between mitochondria in that cell will also vary, being zero for mitochondria that came from different unrelated parents and higher between mitochondria from the same parent: between 1/2 and 1 on average, depending on factors such as variance in replication between mitochondria and inbreeding in the host population. This horizontal transmission allows for conflict between the mitochondria, even at the cost of lowering the fitness of the organism or the nuclear genes. Thus a conflict with the other genetic elements in the cell ensues.

Possible differences in replication rate between mitochondria within a cell provide one mechanism for such a conflict to take shape. A mitochondrion that replicates faster within a cell will have a higher chance to transmit to all offspring.

Energy allocation in the eukaryotic cell depends on the adenine nucleotide translocator (ANT), a common protein on the mitochondrial inner membrane which exchanges adenosine diphosphate (ADP) from the cytosol with ATP from the matrix. If ANT genes were mitochondrial, loss-of-function mutations would produce variants of mitochondria that could allocate all their ATP into their own

replication. Consequently, it is not surprising that ANT genes are always found in the nucleus.

Mitochondria that destroy other mitochondria which do not carry a certain marker could also invade the population. To our knowledge, this has not been observed in mitochondria but has been in chloroplasts (Chiang 1976; Sears et. al. 1977).

Uniparental Transmission

Uniparental inheritance with developmental bottlenecks reduces heteroplasmy, so that the mitochondria within a cell are often clonally related and differ only by recent mutations. Therefore within a lineage, the above-mentioned conflicts will be restricted.

Since uniparental transmission creates an asymmetry in transmission between the eggs (ovules) and sperm (pollen), mitochondria that enhance their own transmission via eggs can invade a population, even if this transmission advantage is achieved at the cost of an even greater reduction of the fitness of offspring produced with the sperm. Cytoplasmic male sterility (CMS) caused by the mitochondria has evolved many times in flowering plants and provides the paradigmatic example of conflict between mitochondria and the nucleus. Mitochondria gain benefits through female fitness by causing the failure of pollen development. The effects of CMS genes in mitochondria are often countered by those of "restorer" genes in the nucleus. (See also below the discussion on parent–offspring interaction.) Thus, mitochondria can increase their own fitness in the female line even while decreasing the fitness of autosomal genes in the male and female line. Such mitochondria can increase in frequency or fix in the female population. Mitochondria could increase their fitness in female lineages by killing male offspring, by feminizing males, and by biasing the sex ratio toward females. These scenarios have been extensively analyzed by Cosmides and Tooby (1981). Mitochondria that increase their own replication rate at the cost of the female lineage they are in will be selected against at the population level. Such mitochondria should then be present in the population at a mutation-selection balance and should usually be the result of recent mutations.

During regular mitosis, no bottlenecks exist for mitochondria, since the cell division is symmetric. In the developmental process of multicellular organisms, however, such bottlenecks and asymmetries might exist. These would then select for selfish mitochondria within the soma of the organism, which again would be selected against at the population level. (See also below the discussion of conflicts between mitochondria in multicellular orginisms.)

Mitochondrial lineages in the mother go through a bottleneck before reaching the egg. These bottlenecks have been hypothesized to reduce the effect of Müller's ratchet, which can present a problem in mitochondria when they have limited amounts of recombination (cf. Kawano et al. 1995) and especially outcrossing in species with uniparental transmission (Bergstrom and Pritchard

1998; Krakauer and Mira 1999). When such bottlenecks exist, they cause mitochondria within an egg to compete to be the ones that survive the bottleneck.

In all the above-mentioned cases of conflict between mitochondria, a conflict between nuclear genes and the mitochondria would also arise, since in these cases the fitness of the organism that the mitochondria are in is lowered. Thus, mutations that could reduce the possibility of the conflicts occurring or reduce their effect would invade the chromosomal genes. Because of the asymmetry in the mutational space between mitochondria and the nucleus, the "parliament of genes" concept claims that such conflicts will usually be won by the nucleus.

In summary, both in biparental and uniparental inheritance, conflicts exist that are associated with the mitochondria. In biparental inheritance, strong conflict between mitochondria in the cell can exist; in uniparental inheritance, mitochondria are in conflict with the nucleus because of the different transmission patterns of nucleus and mitochondria. Mitochondria can outcompete other mitochondria in the population by increasing their own replication rate, by killing unmarked mitochondria or mitochondria that came from the different sex, and by increasing their own chance to go through bottlenecks. In biparental inheritance, a mitochondrion can lower the total fitness of the organism it is in but increase its own chance vs. the chance of other mitochondria to propagate to the offspring, thus increasing its own total fitness in the population. In uniparental inheritance, if a conflict between the mitochondria in the cell reduces the organism's fitness, they can be weeded out at the population level. However, this will not eliminate all effects of conflict between mitochondria in the cell. Imagine that the mitochondria in a population of organisms replicate at a slightly faster rate than would be optimal for these organisms. Now a mutation appears in one of the mitochondria that reduces its reproduction rate, so that the total fitness of the organism is higher. That mitochondrion will be outcompeted by other mitochondria in the cell, and thus there will be a low chance that the offspring of the organism will also carry a mitochondrion with the beneficial mutation. Of course, if this unlikely event happened and an organism appeared that has only mitochondria with the beneficial low rate of replication, then that organism will have a higher fitness and will most likely fix in the population. We see that the internal selection mechanism in the mitochondria will thus create a biased transmission profile: mutations that decrease the fitness of the mitochondrion they are in have a lower chance to be inherited to the next generation than ones that increase the fitness of the mitochondrion within the cell.

Conflict Mediation

Mechanisms that reduce conflict can minimize the causes or the means of conflict. Uniparental transmission of mitochondria reduces (but does not eliminate) conflict between mitochondria in the cell. Bottlenecks and segregation will do this as well. As we have seen above, these mechanisms also create opportunities

for new conflict. Uniparental inheritance increases the asymmetry of transmission between nucleus and mitochondria. Transmission creates an asymmetry of transmission between the mitochondria themselves. Transfer of genes from the mitochondria to the nucleus can be a major contributor to the reduction of conflict. Out of the hundreds to thousands of protein-coding genes that existed in the original proteobacterium, only a very small number are present in the mitochondria of metazoans and only 13 are present in the mitochondria of mammals (Scheffer 1999). Many of the original genes have transferred to the nucleus; others might have simply been lost because they were no longer needed by the mitochondria inside a host. The fact that such a small number of genes are present in mitochondria probably reduces the frequency with which such conflicts arise, and this increases the ease with which the "parliament of genes" overcomes them. This does not necessarily mean that the genes have been transferred to the nucleus for that reason; mechanisms in which a direct selective pressure for such a transfer based on a reduction of conflict, are hard to envision. The transfer of the genes could have been beneficial in itself.

As mentioned above, the transition to becoming an organelle seems to have occurred only a handful of times. To explain why acquiring an organelle is so hard, Cavalier-Smith (2000) proposed that some membranes need pre-existing machinery in a membrane in order to target proteins into it. Therefore some of the membranes of cells can only be formed by splitting pre-existing membranes and are therefore called "genetic membranes." A "naked" membrane, one without the proteins necessary to incorporate proteins specific to a certain membrane type, can never become a membrane of this type. The cell membranes of bacteria belong to the category of genetic membranes, including the thylakoid membranes of cyanobacteria. In eukaryotes the endoplasmic reticulum-nuclear membrane complex belongs to this category, along with the double membranes of plastids and mitochondria. Nuclear genes code for most proteins of these organelles today, and the respective proteins are synthesized in the cytoplasm of the eukaryotic cell. For genes to transfer from the endosymbiont to the nucleus, a special mechanism for the gene products needs to evolve to target the gene products from the nucleus to the endosymbiont membrane. No nuclear-targeting gene targets that membrane, and no endosymbiont gene targets the outside of its membrane. Cavalier-Smith argues that because this targeting is hard to evolve, the evolutionary transition from endosymbiont to organelle is rare (see also Szathmáry 2000).

Why have not all mitochondrial genes been transferred to the nucleus? Changes in external electron sources and sinks (e.g., the food supply) perturb the redox state of electron carriers; if this perturbation can be transduced into gene activity, an adaptive response can ensue. Allen (1993) suggests that for an efficient functioning of this mechanism, the involved genes must reside spatially close to their gene products inside the mitochondrion (for a review, see Race et al. 1999). Others suggest that a generalized retargeting difficulty, because of size or hydrophobicity, is the cause (von Heijne 1986; Cavalier-Smith 2000). Notice

that a eukaryotic organelle — the hydrogenosome — seems to have originated from mitochondria and subsequently lost all its genes (see Palmer 1997).

CLASSIFICATION OF MULTICELLULARITY

We define multicellularity as the spatial association between cells that occurs under genetic control. Unicellular organisms often appear in aggregations. We distinguish multicellularity as those cases in which the aggregation is under evolved genetic control of the individual cells. This type of control evolved many times over life's history (see Bonner 2000; Szathmáry and Wolpert, this volume). Recent discovery of a previously unknown multicellular fruiting body in such a well-studied organism, *Bacillus subtilis*, suggests that multicellular stages in the life cycle of bacteria may be more common than previously suspected (Branda et al. 2001; see also Table 18.1).

Since these associations occur at different levels of cooperation, we start by listing possible features of multicellular organisms that are relevant for cooperation, conflict, and conflict mediation within the multicellular organism.

1. Different cell types within the organism: Specialization and differentiation
   a. Reproductive division of labor: Do all cells in the organism reproduce, or do only some of the cells in the organism produce a new generation?
   b. Spatial differentiation

Table 18.1 Classification of multicellularity.

	Blue-green algae	Cellular slime molds	Plants	*Gonium*	*Porifera*
Different cell types and specialization					
• Reproductive division of labor	Yes	Yes	Yes	No	Yes
• Spatial differentiation	Yes	Yes	Yes	No	No
• Fate commitment	Yes	Yes	No	No	No
Aggregation vs. clonality	clonal, through splitting	aggregation	sexual/ asexual clonal	sexual/ asexual clonal	sexual/ asexual clonal
Size of propagules (How many cells from the original multicellular entity disperse together in the spore/ seed/embryo?)	splitting, i.e., 1/2 the organism	1 per spore	more than 1 cell	1 cell	1 egg for sexual reproduction; for asexual reproduction by fragmentation or gemmules.

c. Fate commitment: Do cells commit to their destiny so that their differentiated state cannot be reversed anymore?
2. Aggregation vs. clonality: Do the cells that form a new individual come from different parents, or from the same parent?
3. Size of propagules: How many cells from the multicellular parent(s) have offspring cells in the multicellular offspring?

Benefits and Detriments of Multicellularity

1. Reduced effective population size. For the same level of nutrients, a population of multicellular organisms will have a reduced population size, since multiple cells comprise a single organism and only the germ cells contribute to the effective population size.
2. Increase in generation time and reduction of mutation rate. Since multicellulars have many cells per organism, more cell divisions are required between generations. This results in an increase in generation time as defined by dispersal events. On the other hand, by controlling the rate of cell division of somatic vs. germ cells, multicellular organisms can control the number of cell divisions between generations, and thus reduce the effective mutation rate (relative to the total number of cell divisions in the organism).
3. Multicellular organisms are often larger than unicellular ones, or at least larger than the single cells that comprise them. This has several effects:
   - Dispersal: Larger size enables better dispersal in some multicellular organisms, e.g., through the creation of fruiting bodies, as occurs in myxobacteria.
   - Reduced ratio of surface area to volume: This has some drawbacks in transport of nutrients and disposal of waste, since there is less surface for exchange with the environment. The reduced ratio can also be advantageous when a slower exchange with the environment is desirable, e.g., for protection from heat loss or maintaining a high osmotic pressure. Furthermore, some cells may be internal and lose their contact with the external environment. This means that they have to rely on transport by other cells for nutrients, but it also means that they reside in a more protected environment.
   - Predation: Larger size provides an advantage in protection from predation as well as the ability to be a better predator, especially for engulfing larger prey.
   - Evading constraints of Reynolds number: Larger organisms can have a less random motion in watery solutions.
   - Survival advantages: Each multicellular organism can have a higher survival rate than a unicellular organism, since only the germ cells need to survive to produce progeny. The organism can increase the survival of the germ cells while reducing the survival chance for other

cells. Thus, initially the continuity of the lineage might have been assured through a strategy in which some cells "eat" other cells in time of nutrient deprivation (see Szathmáry and Wolpert, this volume).

4. Possibility of enclosing spaces within the multicellular organism: Three-dimensional topology provides an easier way of engulfing intercellular spaces for multicellulars. Thus, *Volvox* has a large space enclosed within the ball of cells in which it can store nutrients (Kirk 1998). An enclosing space is conducive to homeostasis through the regulation of the milieu interieur sensu Claude Bernard.
5. Division of labor: Specialization enables the different cells in the multicellular organism to invest only in the production of certain resources and cellular structures. It also reduces the potential for interference from the simultaneous execution of several tasks. For example, division of labor is thought to provide movement during cell division in *Volvox* (Kirk 1998).
6. Information sharing: Information about the environment gathered by the cells of the multicellular organism can be shared among them for zero or very low cost to enable better response to the environment (Zahavi 1971; Lachmann et al. 2000).

ANALYSIS OF COOPERATION IN A MULTICELLULAR ORGANISM

Interchangeable vs. non-interchangeable units: Cells are interchangeable units.

Partner association: We distinguish four cases.

1. The adult originates from a single cell, and this cell's components come from only one parent. *Volvox*, during their asexual life cycle, provide one example. Here, cells within an organism are permanently associated for the organism's lifetime, but this association is not transmitted to the next generation. Thus there is no partner change. Since all cells come from a common ancestor cell, and this comes from only one parent, kinship is high.
2. The adult originates from a single cell, and this cell's components come from multiple parents. This is the case, for example, in animal sexual reproduction. Here, the association between cells in the multicellular stage is permanent within the organism's lifetime, but there is a potential change of partners between generations. Kinship between cells depends on the number of parents. There is a potential for partner choice.
3. The adult originates from multiple cells, and these cells come from one parent. This is the case in asexual budding in plants. Here, the cells are in association for several generations if other types of reproduction (e.g., sexual reproduction) occur occasionally or in permanent association, if this is the only mode of reproduction. In this case there is the potential possibility that

the last common ancestor of the cells in the organism occurred many generations ago, since multiple parallel lineages of cells could exist within a single lineage of multicellulars. Thus kinship can be quite low.

4. The adult originates from multiple cells, and these come from multiple parents. This is the case in slime molds, since the organism is formed by aggregation of cells that potentially come from different parents. Here, cells are associated for the lifetime of the organism and change partners between generations. Kinship between cells in the organism depends on the kinship between the parents and the number of parents from which the cells originate. There is a potential for partner choice.

Asymmetry of transmission: Asymmetry of transmission occurs in several cases. First, an obvious asymmetry of transmission occurs in organisms with a germline–soma distinction. More generally, asymmetry will occur if some tissues in the multicellular organism have a higher chance to produce the next generation than other tissues. Second, in some organisms, different types of offspring can be produced, e.g., sperm and eggs, or flowers that are produced by different parts of the organism, or seeds with different dispersion strategies. All these will also produce an asymmetry.

Difference in replication rate: If replication of cells is not coordinated, then some cells could potentially reproduce faster than others.

Mutational space: In general, the mutational space available to all cells is identical. Some tissues might have an elevated mutation or epi-mutation rate.

Type of benefit function: Many different types exist.

Conflicts of Multicellularity

Cells in a multicellular organism are interchangeable units, i.e., in direct competition. This competition can arise within a single such organism, within the lineage of multicellular organisms, or at the population level. Below we will explain each of these levels further. The association of cells in a multicellular organism can also cause conflict between the interchangeable and non-interchangeable units that make up each of the cells.

Population Level

Conflicts at the population level can arise when there is vertical transmission between lineages. This happens when the organism is formed by aggregation or the cells that form the organism are formed by aggregation, e.g., in sexual reproduction. In this case, selfish elements that reduce the fitness of the organism they reside in, but increase their own fitness by increasing vertical transmission, can spread through the population. For a conflict to exist between cells, there needs to be a genetic variance between cells in the organism. Two examples follow.

Cellular slime molds form by aggregation. Usually, kinship between the cells is high because of the dispersal patterns. A mutant cell that decreases its own ability to become stalk (soma) and increases its own probability to become spore forming (germ) would decrease the total fitness of the organism (since the stalk is somewhat smaller the more stalk cells are in an organism), but would increase its own spore production. Some of these spores would then spread to aggregations of other genotypes, and the mutation could spread through the population (see Strassmann et al. 2000).

In organisms that originate from a single cell formed by fertilization (i.e., aggregation) and in which the mitochondria are inherited biparentally, the mitochondria in each cell in the organism are an aggregation of the mitochondria in the parents. Since the replication of mitochondria is not coordinated so that exactly one copy of each mitochondrion enters each of the daughter cells, there exist genetic differences between the mitochondria in different cells in the organism. A mitochondrion could invade that reduces the total fitness of the organism, but increases the chance of the cells that it resides in to become germ line. If one parent of the organism has such mitochondria, these will be overrepresented among the organism's offspring and thus increase their frequency in the population. In this case the conflict between mitochondria in different cells also creates a conflict between mitochondria and the nucleus, or other genetic elements. If the nucleus is genetically identical between cells and is highly related to the nuclei in other cells, there exists a selection pressure to negate the effects of the mutated mitochondria, increasing the organism's total fitness. Such conflicts can be lessened by increasing the relatedness of cells within the organism or forming new organisms by cloning instead of aggregation.

Lineage Level

Here a conflict can occur when there is genetic variance between cells in the organism, and this variance can be inherited between generations. In this case, cells that increase the representation of their offspring among the offspring of the organism will increase in frequency within the lineage, even if this comes at a cost of reduced fitness of the lineage as a whole. This lineage will then be selected against at the population level. The main difference between this type of conflict and the previous type is that when a conflict is limited to a conflict within a lineage, then population-level selection between lineages will select against lineages with selfish cell types. In the case of vertical transmission, population-level selection favors selfish cell types, and only higher-level structure selects against them. Conflicts within a lineage can arise through differences in replication rate or asymmetry of transmission. Since a lineage creates multiple sublineages, the population-level selection will also affect the frequency of the selfish individuals within a lineage. Below, three examples are given for these types of conflicts.

- In a multicellular organism that replicates by splitting, but in which the replication of cells and their distribution among the organism's offspring is not coordinated, a cell type that increases its own replication rate would increase in frequency within the lineage.
- In a multicellular organism that reproduces sexually and develops from a single egg and in which mitochondria are transmitted uniparentally, a mutant mitochondrion that increases the chance of the cell it is in to become the germ cell will increase its frequency within a lineage.
- In a multicellular organism that replicates by budding, a mutant cell type that increases its frequency in the buds would increase in frequency within the lineage. Thus in plants that undergo asexual reproduction through budding, a mutant cell type that reproduces faster or has a higher chance of producing new buds will spread through the lineage.

Organism Level

Conflict within an organism occurs when genetic variation within the organism is not transmitted between generations; the competition between cells is restricted to the organism's lifetime. At the population level, individuals with selfish cell types will be selected against. Since competition occurs within the individual, no conflict between cells to "take over" the germ cells will take place. On the contrary, a mutation in cells that invests less in producing germ and more in reproducing within the individual, will have a benefit within the organism for that cell type.

Conflict Mediation

A major hurdle in the evolution of multicellularity is the appropriate down-regulation of cell division at the right time and place. Multicellular organisms are made of cells. A proper functioning of the multicellular organism usually entails that not every cell that has enough resources to replicate will do so. Many cells in the organism have to give up their reproductive capability in the organism.

Linked with the three types of conflicts outlined above, we delineate three main areas of conflict mediation: (a) reduction of horizontal transfer and increase of kinship between cells in the multicellular organism, (b) reduction of the number of cell lineages within the organism that will produce offspring, (c) reduction of replication potential and detection of aberrant cells within the organism. Not all of these conflict-reducing mechanisms necessarily were selected for; in some cases, the life history of the organism results in fewer conflicts between the cells that compose the organism.

Germ Line

The first area of conflict mediation is the evolution of a germ line, where we distinguish three stages:

1. Propagule size: A smaller number of cells within the propagule increases kinship within the organism and thus reduces conflict. A larger number of cells in the propagule decreases kinship, and thus creates a conflict—a selection process that selects for cells that have a better ability to enter the propagule at a fitness cost to the organism. Such mutations can spread through the population if the organism is produced by aggregation but not if it is produced by clonality. Nevertheless, in clonality they reduce the overall fitness of the multicellular organism in which they occur.
2. Reproductive division of labor: Who proceeds to the next generation? A multicellular organism can evolve a reproductive division of labor, in which only some of the cells will produce the next generation of the multicellular. In a multicellular organism with a single-celled propagule, there is no selective process that selects for somatic cells that invade the propagule at a cost to the organism. A mutation like that could occur and could reduce the fitness of the organism it occurs in, but there would be no selective advantage to the mutation in the population.
3. Early sequestration: When is the germ line sequestered? Early sequestered germ line can reduce the number of cell divisions in a generation, and thus the mutational load that the organism experiences.

Soma

The second mechanism to reduce the conflict is the evolution of a soma. When some of the cells in the organism are forced to give up their ability to replicate, or replicate indefinitely within the organism, the potential for conflict between cells in the organism is reduced. Note that this is not the same as reproductive division of labor, in which some cells give up their ability to produce the next generation of the organism.

Other than reduction of conflict, there are several other benefits to the evolution of soma and germ. As mentioned above, an early sequestered germ can reduce the number of cell divisions between generations and thus the effective mutation rate. A disposable soma can have lower maintenance cost. It provides an efficient division of labor, since some cells can put all their resources into reproduction, whereas others do not need to maintain any reproductive ability.

It is important to note that soma and a full, early sequestered germ line are not present in all multicellular organisms. Even in metazoans it seems to be a relatively late evolutionary event. Plants have no real germ line. In sexual reproduction the propagule size of the fertilized seed can be one or more cells, and in vegetative growth, the propagule size is more than one cell. A terminally differentiated cell type does occur early in evolution.

Programmed Cell Death

A third mechanism of conflict mediation is programmed cell death (PCD). This is a slightly stronger mechanism of control of cell growth than simply

preventing cell division. PCD is triggered by extracellular signals, following an orderly, stereotyped cascade of events regulated by an intrinsic pathway. There are two major types of such pathways: (a) extrinsic Fas pathways and (b) pathways in which mitochondria are central. Interestingly, there does not seem to be an equivalent pathway in which another organelle, the chloroplast, is in control. PCD has a couple of further functions. It is used in development for the formation of structures and for neuronal selection. It is used in infection control to eradicate infected cells and in oocyte selection. Finally, it is used in preventing uninhibited growth in the control of cancer.

PCD does occur in unicellular organisms, though we would expect such cases to occur only in kin groups or under similar selective scenarios.

BREAKDOWN OF CONFLICT MEDIATORS IN MULTICELLULARITY

When a conflict mediator exists, we can predict a pathological condition under which it will break down. In the absence of a mediator, such conditions will be even more common, as we see from the following examples.

In metazoans some somatic cells are unable to replicate indefinitely, which is one of the mechanisms to prevent conflict between cells within the organism. When this mechanism breaks down, we expect that a cell will start to divide indefinitely; PCD can then prevent further growth. When the mechanism of PCD breaks down, we expect cancer to occur. Cancer is somewhat more likely in cell types that have not lost the ability to replicate indefinitely.

The conflict between mitochondria in the same cell, as described earlier for diploid cells, is still present in the multicellular organism. This conflict is mediated in part by the small size of the mitochondrial genome and the reduced number of functions that it still controls. Although these mechanisms reduce the chance of conflict to occur, they do not eliminate it. A mutant mitochondrion could invade, resulting in cells that have a large number of mitochondria, each of which is not very functional for the cell. Only few cells like this should be observed, since there is no benefit for the cell. This phenomenon would mainly be expected in cells in which there is high turnover of mitochondria. Conflict between mitochondria in different cells is analogous to conflict between cells since there is no horizontal transfer of mitochondria inside the multicellular organism.

ANALYSIS OF COOPERATION AND CONFLICT IN THE ASSOCIATION BETWEEN PARENT AND FETUS

In some multicellular organisms a tight association between a parent (often, but not necessarily, the mother) and sexually produced offspring exists. This association provides increased survival chance for the offspring. Because of the highly asymmetric association between interchangeable units (see below), this association awakens many conflicts from lower levels of association in the organisms.

Interchangeable vs. non-interchangeable units: Parents and offspring are taken from the same gene pool and thus are, by our definition, interchangeable units. If a type invades the offspring population and takes over, it will also have wiped out all the genetic variation in the parent population. Because of the highly asymmetric nature of the association, this classification seems very nonintuitive. We can think of the association as a cooperation between pairs of individuals from the population, as might occur at early stages of the evolution of multicellularity: one cell divides to produce two daughter cells, which stay attached and cooperate for some time.

If we break down the association into the lower-level elements that make up each of the units, we encounter both interchangeable and non-interchangeable units. Thus the association between genes at different loci in these organisms are non-interchangeable units.

Partner association: Parent and offspring remain in association only for part of one generation, and thus the association between units in the parent vs. those in the offspring is 0.

Type of formation: Offspring are produced by an aggregation of genes from the parents, since they are produced sexually. Other elements in offspring are passed only through one parent. Kinship between different fetuses within the mother is usually 1/2, but can be lower for cases in which they are the product of multiple matings.

Asymmetry in transmission: Parent and offspring have different functions and futures in this association. The expected fitness of mother and offspring can differ, which might cause a preference for elements to stay with the mother or continue to the offspring. If more than one offspring is carried, then those have usually a similar projected future.

Difference in replication rate: Since neither parent nor offspring reproduce during the association, this difference does not exist.

Mutational space or available strategies: Mutational space is identical, although cells in the fetus undergo more replications. Difference in available strategies can be created, e.g., if the genes of the fetus are not expressed up to a certain age, or if a tight control is kept over which gene products can be transferred from mother to fetus.

Type of benefit function: The parent provides all the benefit to the offspring. The loss of fitness to the parent from terminating the association is usually smaller than the loss to the offspring. In this case many conflicts at lower levels of association are reawakened, each of which are discussed separately below.

Conflicts between Cells

Mother and fetus are both multicellular. In a multicellular organism, the fact that the offspring is produced by only one cell prevents a conflict between cells in which a selection pressure exists favoring cells that become the germ cells. When the fetus is associated with the mother for a longer time, this restriction might be overcome by a cell in the mother that would also enter the fetus, so that more than one cell from the mother produces cells in the fetus.

A mutation in cells that would cause them to be transferred from parent to offspring, or from offspring to offspring could invade a lineage. If only one parent carries offspring, then such a mutant could not spread through the population (since, e.g., it is transmitted only by daughters), and would be selected out at the population level. Thus such mutants would be kept at a mutation-selection balance within lineages. This conflict is very similar to the conflict between mitochondria that can arise at the evolution of multicellular organisms; here, the fact that only one sex is carrying the fetus is equivalent to uniparental inheritance. In hermaphrodites such a mutation could spread through the population. One therefore expects fewer cases in which hermaphrodites carry fetuses internally, and in those cases this type of "parasitic cancer" could be present.

Conflicts between Autosomal Genes

Since the genomes of sexually produced offspring are produced by aggregation, there is a basis for conflict between alleles at the population level. Of the two alleles present in a diploid parent, only one is transmitted to each offspring. The randomizing process of meiosis provides protection against this conflict because the two alleles present in the parent cannot easily detect which one of them was transmitted to the offspring. In the absence of such information, the best they can do is to maximize the total number of surviving offspring and take their chances with the flip of the meiotic coin. This mechanism breaks down in some cases. Postsegregation distorters act by reducing the frequency of noncarrier individuals after fertilization. The distorter benefits if this increases the fitness of carriers (e.g., by reduced competition). Examples include spore killers in fungi and the Medea locus in flour beetles.

Similarly, alleles in the offspring are uninformed about whether they came from the mother or father. However, when such information is available, as is the case in genes that are imprinted (Haig 2000), then a conflict can result because alleles that come from the father could demand more investment from the mother, who does not carry those alleles. Alleles that come from the mother, on the other hand, have a higher interest in ensuring that the mother will have further offspring. If genes carry epigenetic marking that differs according to their sex of origin, then they can evolve to have a different behavior when marked or unmarked, and thus when transmitted through mother or father (Haig 2000).

Conflicts between Genes on Sex Chromosomes

The chromosome that is unique to the heterogametic sex is never present in the homogametic sex. Thus there will always be a conflict on parental investments between offspring of different sexes. Thus a gene on the Y chromosome that decreases parental investment in females but increases survival of male offspring will invade the population.

For parent-offspring conflict we should differentiate whether the heterogametic or homogametic sex carries the pregnancy. Many other modes of sex determination can exist and, in each, one could carry out the analysis of conflict as follows:

Pregnancy carried by homogametic sex (XX females): Alleles on the Y chromosome in a male fetus "know" that they did not originate from the parent carrying the pregnancy. Thus these alleles would increase investment in themselves even at a high cost to the mother (see Hurst 1994).

Pregnancy carried by heterogametic sex (ZW females): Alleles on the mother's W chromosome "know" that they are not present in any homogametic offspring, and thus W-linked alleles would invade the population if they increase the fitness of heterogametic offspring even at a high cost to the homogametic offspring. Thus pregnancy in a ZW sex determination system has more potential for conflict. A potential mechanism for conflict mediation would be a reduction of the size of the W chromosome and a mirroring of all genetic expression of the W in female fetuses by the Z and autosomal chromosomes in male fetuses.

All are similar to the conflict produced by segregation distorters, where genes at the other chromosomes would be in conflict with the genes causing the distorted investment, since they, on average, have a 1/2 chance of having an identical allele in the parent/offspring.

Conflicts between Cytoplasmic Elements

In uniparental inheritance, conflicts between parent and offspring created by elements of the cytoplasm are similar to those created by sex chromosomes. Obvious examples are for those elements that are transmitted in the cytoplasm of eggs but not via sperm (such as mitochondria and chloroplasts). There are many cases where such elements distort the sex ratio toward females by various mechanisms, including male killing, feminization of genetic males, and induction of asexuality (dispensing of males).

FURTHER REMARKS ON MULTICELLULAR ORGANISMS

Many further subjects arose in our discussions which did not fit into this chapter. We include a few of the interesting questions that came up in these discussions.

A major hurdle in the evolution of multicellularity is the appropriate down-regulation of cell division at the right time and place. This hurdle was

overcome to different degrees by various separate evolutionary events in the evolution of multicellularity. Complex multicellularity, on the other hand, seems to have evolved only a very few times, which hints that there might be other "hurdles." One might be the evolvability of new cell types in the organism and linking them to the developmental plan of the organism. To address this question it would be instructive to expand our knowledge of the evolution of cell types. Can cell types be well defined? How often do new cell types evolve? How many cell types are there in multicellular organisms? In regard to these questions, a greater synthesis between evolutionary theory and molecular cell biology will further illuminate both fields.

Why are all complex multicellular organisms primitively sexual and eukaryotic? This question is linked to the question of why complex multicellularity evolved so late. What is the relationship between propagule size and evolvability of differentiation, under various assumptions about the type of control of differentiation that exists (i.e., differentiation through environmental signals or signals from other cells)?

Mitochondria and chloroplasts of most flowering plants are uniparentally inherited via ovules. Therefore, chloroplast genes could presumably benefit in the same manner as mitochondrial genes by causing male sterility. All known systems of CMS, however, involve mitochondrial genes; none involve the chloroplast. Why is this so? Do systems of chloroplast CMS exist but simply have not been recognized? Or does the chloroplast, unlike the mitochondrion, have little power to assert its interest during pollen formation?

Are there measurable costs to policing, e.g., for mechanisms that delete every gene duplication and prevent transposable elements? What are the measurable fitness costs of intragenomic conflict in real organisms? What is the balance sheet for meiosis in terms of increasing or reducing cooperation? How is fair meiosis maintained? Which features of eukaryotes evolved before the mitochondria became an endosymbiont of the eukaryotic cell?

What are the preadaptations of multicellularity? (See Szathmáry and Wolpert, this volume.) Under the right definitions, it should be far more widespread than currently thought. To understand the general principles of the evolution of multicellularity, we should seek and study those cases. It should be more widespread in prokaryotes — what are its distinctive features and advantages?

From our discussion on mitochondria, deleterious mutations, bottlenecks, and conflicts, we predict that germ cells should be subject to selection pressure that selects for least-loaded mitochondria in terms of mutational load that affects mitochondria performance.

SUMMARY

Every entity that we call "individual" in biology is made of many separate units. These units, if identical or different, will not strive toward (i.e., be selected for) the same goals. When we examine a certain biological system, we can classify

the type of cooperation that exists according to the classification scheme we constructed. This will then point to the possible conflicts in the system. These conflicts should exist, or there should be mechanisms of conflict mediation that reduce the conflict. We could also make predictions of what is expected to happen when one of these mechanisms of conflict mediation breaks down.

It should not be expected that conflicts in the organisms will disappear. In some cases, mechanisms of conflict mediation reduce the conflicts; however, even if the conflict causes a fitness cost to the organism, and mechanisms for conflict mediation are directly selected for, conflict would still exist at some kind of mutation-selection balance (and in some cases a biased mutation-selection balance, as we pointed out in the discussion on conflicts between the mitochondria and the nucleus). In other cases a mechanism that reduces some of the conflicts will create others. Thus uniparental inheritance of mitochondria reduces the conflict between the mitochondria in the cell but increases the conflict between the mitochondria and the nucleus, since it causes the mitochondria to be transmitted on different lineages than the autosomal genes. Recombination reduces the mutational space available to the meiotic drive gene but makes it possible for transposons to spread through the population.

ACKNOWLEDGMENTS

We thank those conference participants who contributed to our interesting discussions, in particular Matthias Nöllenburg. We wish to thank Steve Frank, Franjo Weissing, and John Tooby, who contribued to this chapter, S. Ptak for reading and helping in its rewriting, and E. Jablonka for comments on the manuscript.

REFERENCES

Allen, J.F. 1993. Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol*. **165**:609–631.

Axelrod, R. 1984. The Evolution of Cooperation. New York: Basic.

Bergstrom, C.T., and J. Pritchard. 1998. Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. *Genetics* **149**:2135–2146.

Bonner, J.T. 2000. First Signals: The Evolution of Multicellular Development. Princeton, NJ: Princeton Univ. Press.

Branda, S.S., J.E. González-Pastor, S. Ben-Yehuda et al. 2001. Fruiting body formation by *Bacillus subtilis*. *Proc. Natl. Acad. Sci. USA* **98**:11,621–11,626.

Cavalier-Smith, T. 1981. The origin and early evolution of the eukaryotic cell. *Symp. Soc. Gen. Microbiol*. **32**:33–84.

Cavalier-Smith, T. 2000. Membrane heredity and early chloroplast evolution. *Trends Plant Sci.* **5**:174–182.

Chiang, K.S. 1976. On the search for a molecular mechanism of cytoplasmic inheritance: Past controversy, present progress and future outlook. In: Genetics and Biogenesis of Chloroplasts and Mitochondria, ed. T. Bucher, W. Neupert, W. Sebald, and S. Werner, pp. 305–312. Amsterdam: North Holland.

Cosmides, L.M., and J. Tooby. 1981. Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol*. **89**:83–129.

Gimble, F.S., and J. Thorner. 1992. Homing of a DNA endonuclease gene by meiotic conversion in *Saccharomyces cerevisiae*. *Nature* **357**:301–305.
Haig, D. 2000. The kinship theory of genomic imprinting. *Ann. Rev. Ecol. Syst.* **31**:9–32.
Hamilton, W.D. 1964. The genetical evolution of social behavior. *J. Theor. Biol.* **7**:1–52.
Hurst, G.D.D., and J.H. Werren. 2001. The role of selfish genetic elements in eukaryotic evolution. *Nature Rev. Gen.* **2**:597–606.
Hurst, L.D. 1994. Embryonic growth and the evolution of the mammalian Y chromosome. I. The Y as an attractor for selfish growth factors. *Heredity* **73**:223–232.
Jaenike, J. 1996. Sex-ratio meiotic drive in the *Drosophila quinaria* group. *Am. Nat.* **148**:237–254.
Kawano, S., H. Takano, and T. Kuroiwa. 1995. Sexuality in mitochondria: Fusion recombination, and plasmids. *Intl. Rev. Cytol*. **161**:48–110.
Kirk, D.L. 1998. Volvox: Molecular-Genetic Origins of Multicellularity and Cellular Differentiation. Cambridge: Cambridge Univ Press.
Krakauer, D., and A. Mira. 1999. Mitochondria and germ-cell death. *Nature* **400**:125–126.
Lachmann, M., G. Sella, and E. Jablonka. 2000. On the advantages of information sharing. *Proc. Roy. Soc. Lond. B* **267**:1287–1293.
Leigh, E.G. 1977. How does selection reconcile individual advantage with the good of the group? *Proc. Natl. Acad. Sci. USA* **74**:4542–4546.
Margulis, L. 1981. Symbiosis in Cell Evolution. New York: W.H. Freeman.
Maynard Smith, J., and E. Szathmáry. 1995. The Major Transitions in Evolution. Oxford: W.H. Freeman.
Palmer, J.D. 1997. Organelle genomes: Going, going, gone! *Science* **275**:790–791.
Partridge, L., and L.D. Hurst. 1998. Sex and conflict. *Science* **281**:2003–2008.
Pesole, G., C. Gissi, A. De Chirico, and C. Saccone. 1999. Nucleotide substitution rate of mammalian mitochondrial genomes. *J. Mol. Evol.* **48**:427–434.
Race, H.L., R.G. Herrmann, and W. Martin. 1999. Why have organelles retained genomes? *Trends Gen.* **15**:364–370.
Rand, D. 2001. Units of selection on mitochondrial DNA. *Ann. R. Ecol. Syst.* **32**:415–448.
Sagan, L. 1967. On the origin of mitosing cells. *J. Theor. Biol.* **14**:225–276.
Scheffler, I.E. 1999. Mitochondria. New York: Wiley.
Sears, B., J.E. Boynton, and N.W. Gillham. 1977. Effect of increasing zygote age on transmission of chloroplast genes in green-alga *Chlamydomonas-reinhardtii*. *Genetics* **86**:S56–S57.
Selker, E.U. 1999. Gene silencing: Repeats that count. *Cell* **97**:157–160.
Strassmann, J.E., Z. Young, and D.C. Queller. 2000. Altruism and social cheating in the social amoeba *Dictyostelium discoideum. Nature* **408**:965–967.
Szathmáry, E. 2000. Evolution of replicators. *Phil. Trans. Roy. Soc. Lond. B* **355**:1669–1676.
von Heijne, G. 1986. Why mitochondria need a genome. *FEBS Lett.* **198**:1–4.
Whatley, J.M., P. John, and F.R. Whatley. 1979. From extracellular to intracellular: Establishment of mitochondria and chloroplasts. *Proc. Roy. Soc. Lond. B* **204**: 165–187.
Wolfe, K.H., W.-H. Li, and P.M. Sharp. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proc. Natl. Acad. Sci. USA* **84**:9054–9058.
Zahavi, A. 1971. The function of pre-roost gatherings and communal roosts. *Ibis* **113**:106–109.