One can summarize this chapter by considering the origins and functions of the subystems of the chemoton. The origin of the membrane has not been dealt with here in terms of chemistry (see Maynard Smith and Szathmáry 1995), but we have seen that it provides the most stringent version of group selection by acting as a physical barrier to gene flow and forcing local interactions on the constituent genes. Local interactions were important before cellularization as well. Molecular cooperation of naked, unlinked replicators is inconceivable without such an effect.

The first replicators are likely to have emerged on mineral (probably pyrite) surfaces. The templates in the chemoton are assumed to gain ribozymic function aiding metabolism of the compartment. Obviously, evolution must have started before enzymes and templates. Simple autocatalysts, replicating in a holistic manner and having limited heredity (and thus limited evolutionary potential) are likely to have been the first replicators. Replicators carrying information in digital form appeared when replication became modular, rather than holistic. We know from experiments that such replicators can form a growing population in the absence of enzymes, but we do not know the evolutionary pathway to the appearance of the first RNA molecules. Nucleic acids are important because they are replicators with unlimited heredity. There can be so many types (sequences) that evolution may go on indefinitely.

Metabolism of autotrophic protocells hinges on the presence of an autocatalytic network of small molecules, modeled by the central cycle of the chemoton. At some point in evolution, nucleic acid replication became grafted onto such a metabolic network. Systems in which nucleic acids acted as ribozymes had an advantage: Metabolism and replication proceeded faster. The enzymatic function of RNAs in the has-been RNA world probably led to the fixation of the size of the genetic alphabet; accuracy decreases too fast if the number of base pair types is increased. The advantage from an increase in catalytic potential cannot compensate for this adverse effect.

Many experiments along the lines suggested by the current theories have not been carried out yet. This is an important task for the future. One would like to see a sensible scenario for the chemical origin of nucleic acids. Then one would like to see the spontaneous formation of protocells (chemotons) in the lab. Once we have achieved this, we shall have understood how life originated.

## Acknowledgments

This work was supported in part by the Hungarian Scientific Research Fund (OTKA) and by a grant from the Ministry of Culture for research in higher education.

# 4 Individuality, Immortality, and Sex

Richard E. Michod

The emergence of new and higher levels of organization during evolution provides a compelling context for understanding the relations among certain fundamental properties of life, such as individuality, immortality, and sex. By *immortality*, I mean following Weismann (1890) the never-ending cycle of life; by *individuality*, I refer to the familiar levels in the hierarchy of life and their capacity to function as units of selection during evolution (genes, cells, organisms, societies, species); and by *sex*, I mean breakage and reunion of DNA molecules from different individuals.

The evolution of multicellular organisms is the premier example of the integration of lower evolutionary levels into a new, higher-level individual. Explaining the transition from single cells to multicellular organisms is a challenge for evolutionary theory. Sex and individuality are in constant tension as new units emerge, because sex mixes elements from different individuals and naturally threatens the integrity of evolutionary units. Yet, sex is fundamental to the continued well-being of evolutionary units and the immortality of life (Michod 1995). Although sex by creating mixis would seem to undermine individuality, history shows that sex is reinvented as each new level of individuality emerges in the evolutionary process.

## Cooperation and Conflict

The benefits of cooperation provide the imperative for forming new, more inclusive evolutionary units. Increments in fitness are traded among levels of selection through the evolution of behaviors that are costly to individuals yet beneficial to groups. Cooperation is necessary for the emergence of new units of selection, precisely because it trades fitness from the lower level (the costs of cooperation) for increased fitness at the group level (its benefits). In this way, cooperation can create new levels of fitness and individuality (see table 4.1). This trade, if sustained through group selection, kin selection, and conflict mediation, results in an increase in the heritability of fitness and individuality at the new higher level. In this way, new higher levels of selection may emerge in the evolutionary process.

Although fueling the passage to higher levels, cooperation provides the opportunity for its own undoing through the frequency-dependent advantage of defection. Selfish interactions (defection) reap the benefits of cooperation while avoiding the costs and, for this reason, can be expected to spread

TABLE 4.1
Effect of Cooperation on Defection on Fitness at the Cell and Organismal Level

| | *Level of Selection* | |
|---|---|---|
| *Cell Behavior* | *Single Cell* | *Cell Group (organism)* |
| Defection | +, replicate faster | −, less functional |
| Cooperation | −, replicate slowly | +, more functional |

within the cooperating group. Selfish individuals typically stand more to gain than their selfishness costs any other individual, especially when rare. The "tragedy of the commons" (Hardin 1968) leads to conflict among lower-level units, which may sabotage the viability of cooperation and the creation of new higher levels of selection. By "conflict," I mean competition among lower-level units of selection leading to defection and a disruption of the functioning of the group.

## Immortality, Mortality, and the Cycle of Life

From parent to offspring, from parent cell to daughter cell, from DNA strand to daughter DNA strand, the cycle of life continues. Life does not begin anew each generation, but is passed on through time like a family heirloom. Most evidence supports the view that life began once around 4 billion years ago and has been passed down through the eons. Each of us can, in principle, trace our ancestry back in time to this ancient founder. This immortal ancestry or cell lineage Weismann referred to as the germ line. (Used in this way, the term *germ line* refers to the cell lineage that can be traced backward in time from any living thing, whether or not it is a multicellular organism and possesses a germ line, in the sense of a sequestered and differentiated cell type specialized in forming gametes, and a somatic line with terminal differentiation.) In 1890, Weismann first defined the immortal in biological terms (Weismann 1890, p. 318). Weismann contrasted immortality with eternity; immortality is a state of activity and change in which the cycle of life continues indefinitely through time.

> And what is it, then, which is immortal? Clearly not the substance, but only a definite form of activity . . . the cycle of material which constitutes life returns even to the same point and can always begin anew, so long as the necessary external conditions are forthcoming . . . the cycle of life, i.e., of division, growth by assimilation and repeated division, should [n]ever end; and this characteristic it is which I have termed immortality. It is the only true immortality to be found in Nature—a pure biological conception, and one to be carefully distinguished from the eternity of dead, that is to say, unorganized, matter.



The continued well-being of life resides in the information encoded in genes, and life's immortal potential requires that genes be passed on in good repair. Sex functions to repair damaged genes and otherwise cope with genetic errors such as mutations (Michod 1995). In so doing, sex is an integral part of the well-being and immortality of life, both in unicellular and multicellular organisms.

In multicellular organisms, immortality requires totipotency. Sex, totipotency, and immortality became special characteristics of certain differentiated cells termed germ cells, in contrast to the somatic cells, which replicate by mitosis and are terminally differentiated into the various cell types that make up the tissues and organs of multicellular organisms. Why did immortality and reproduction become the function of one group of cells, the germ cells, with the other somatic cells becoming terminal? Again it was Weismann who first spoke convincingly on this subject.

According to Weismann, the germ-soma differentiation was invented in multicellular organisms because of the advantage to the fitness of the organism of specialization and division of labor among its cells. Somatic cells specialize in making bodies adapted to the contingencies of existence, and germ cells specialize in making good gametes. Furthermore, once somatic cells began specializing in making bodies, they naturally would lose their immortality and capacity to divide forever. Why? Because, unnecessary but costly structures or activities should be lost in evolution. If the germ cells specialize in immortality, there is no longer any need—from the point of view of the whole organism—for the soma to maintain this capacity. Sounds reasonable, at least when you think in terms of the needs of the whole organism, and this was how Weismann approached the matter.

The problem with Weismann's approach is that multicellular organisms do not always exist as evolutionary units and consequently organismal needs are not always recognizable to selection. Before and during the transition from solitary cells to multicellular organisms, cells cannot be counted on to behave in the interests of the organism. After all, cells have been evolutionary individuals in their own right for billions of years before the first multicellular organism emerged. Even now, with our individuality well protected by such marvelous adaptations as a germ line, immune system, and programmed cell death, humans are threatened by the evolutionary potential of extant microbes (witness the recent antibiotic-resistant forms of bacteria and other microbes).

Why would cells relinquish their evolutionary rights in the interests of the organism? Although there can be no question that division of labor among cells is important to the functioning of an organism, evolution must first

settle the question of individuality. Upon which evolutionary unit will selection focus: the cell, the multicellular organism, or some mixture of the two? We have clearly gotten ahead of ourselves by depending on the needs of the organism to explain the ancient differentiation of the germ and the soma.

According to our studies on the evolution of individuality reviewed below, the evolution of segregated and differentiated germ cells may be an example of conflict mediation. Conflict mediators may arise during the transition between single cells and multicellular organisms, and resolve, in favor of the organism, the multilevel selection process that must have been responsible for the origin of cell groups in the first place. Conflict mediation probably played a role in the origin of the ancient differentiation between the immortal (germ) and the mortal (soma) cells. By preserving the fitness gains at the level of the cell group, or organism (these fitness gains resulting from cooperation among cells), the germ line served to increase the heritability of fitness at the level of the multicellular organism and allowed it to emerge as an evolutionary individual (Michod 1996, 1997a,b, 1999; Michod and Roze 1997). To understand the basis for this claim, let me consider the question of organisms more systematically. Where did multicellular organisms come from? To get our bearings on this question, let us go back to the very beginning and sketch out a plausible scenario of the first 3 or 4 billion years of life on earth.

## The First Individuals

### In the Beginning

As far as we know, life first originated as simple replicating molecules, probably similar to extant single stranded RNAs. These ancient replicators could both encode information as a sequence of nucleotides and fold up upon themselves to act like proteins. Replication was a little sloppy at first because the copying of information from parent strand to daughter strand relied heavily on the free energies of base-pair formation between complementary nucleotides. Proteins that aid in this process and make it more faithful in extant life forms had not been invented yet. For thermodynamic reasons, in DNA, the nucleotide A pairs with T and G with C. This complementarity provides the basis for replication of an RNA or DNA strand and also provides the basis for reproduction at higher levels (cell, organism, etc.).

### Cooperative Gene Networks

Genes began cooperating because two genes can do more than a single gene alone. Perhaps one sequence could serve as a kind of catalytic surface that facilitated the replication of another sequence. These cooperative interactions



led to cooperative networks of genes, termed *hypercycles* by Eigen and his colleagues (Eigen and Schuster 1979), in which each gene contributed to the replication of other genes and also shared in the beneficial effects of the products produced by its neighbors. In time, proteins would be produced as a way of mediating these cooperative interactions. Although beneficial to others, proteins are produced at some cost, if only for the time and energy put into their production. Costly acts that are beneficial to the group provide a (short-term) advantage to cheating: There is a "temptation" (read immediate selective advantage) to use the benefits produced by others and not contribute to the group. Before considering the consequences of cheating for group living, I would like to consider another aspect of these early gene networks: their lack of individuality and how mixing of genes from neighboring networks could serve the function of recovery from genetic damage.

### Sex and Promiscuity among the Naked Genes

These early networks of genes were a poorly defined lot, with few barriers to the flow of genes between gene groups. The lack of individuality may have had at least one advantage (Michod 1995). It would be very easy for genes to become damaged, exposed as they were without a cell membrane to the ultraviolet radiation and damaging reactions that must have been frequent on the primitive earth. Breaks, loss of nucleotides, and loss of methyl groups are just a few of the kinds of damage that occur to DNA in modern organisms and probably threatened the existence of these early gene networks. Damaged genes would just fail to replicate. As long as there was at least one undamaged copy of the same gene in the vicinity, this good copy could replicate and replace its damaged brethren. The result would be a kind of mixing of genes, or sex, with undamaged genes replicating to take the place of damaged neighbors. Sex (mixing for purpose of recovery from genetic error) came easily to these early replicators. There was much promiscuity and little individuality at this early stage. However "mixed up" we imagine the early gene networks to be, without any cell membrane to trap the errors, a kind of repair (or recovery from genetic error; Bernstein et al. 1984) could occur spontaneously with little "effort" or design on the part of the gene network.

### Tragedy of the Commons

Now let us return to the costs of cooperation and the immediate selective advantage of defection. Hardin identified the tragedy of the commons as the fundamental problem of group living (Hardin 1968). The tragedy of the commons occurs when an individual stands to gain more by behaving selfishly than his selfish behavior costs each member of the group. For this

reason, in cooperative groups there is always a temptation for individuals to defect. What keeps defection from arising and taking over the group, ruining the network of cooperation on which its very advantage depends? Human societies have laws and police forces to reduce this temptation. What happens during evolution? How can behavior beneficial to the group ever evolve? In short, the group must become an individual, but how does this happen? Conflict mediation underlies the transition to new levels of individuality.

### Conflict Mediation and Individuality

In time, the cooperating networks of genes would become encased in a cell. The cell was a wonderful invention, protecting the genes from the damaging effects of the environment and allowing resources and proteins to be kept close at hand, instead of diffusing away to be used by others. By encapsulating gene networks into a cell-like structure, one's proteins would be available only to nearest neighbors, those sharing the same cell. If one of these neighbors turned selfish—say, it used its neighbor's proteins but did not take time to make its own—all the genes in the same cell would be threatened, including the selfish gene. By putting everybody in the same boat, so to speak, everybody's self interest becomes more closely aligned with the interest of the group.

The cell is an example of a device that reduces conflict because it more closely aligns the interests of the genes with the interests of the group. For all the genes to replicate, the cell must replicate. Therefore, each gene has an interest in promoting the replication and well-being of the cell and in policing any selfish renegade genes. To understand the evolution of individuality and new levels of organization, we need to identify and explain evolutionarily those mechanisms and structures that serve to align the interests of the lower-level units with the interests of the group. More inclusive individuals must regulate the selfish tendencies of their components—genes in the case of gene networks; component cells in the case of multicellular organisms. How is individuality created at a new level (cell or organism) so that the lower-level units may be regulated, especially when no controlling organizer sits outside of the system? Conflict mediation is necessary; otherwise, new adaptations at the new level cannot evolve.

## Sex and Individuality

Individuality has costs. Once the cell was invented, genetic errors were trapped on the inside. Sex had come easy to the free-living molecular replicators because genetic redundancy was always available in the form of gene copies in neighboring groups. With the gene group now encapsulated in a



cell, new forms of sex had to be invented between cells as a means to obtain backup copies of genes for genetic repair.

Sex between cells involved mating (fusion) of two cells followed by mutual repair (recombination) and then splitting into daughter cells again. Diploidy (just stay fused and carry a set of genes in tow) was another possible strategy for coping with genetic error, but it has costs in terms of the extra resources and time needed to replicate additional genes. The costs of diploidy can make sex the preferred strategy under certain conditions (Michod 1998).

Sex and individuality are in constant tension, because sex involves fusion and mixis of genetic elements and thus naturally threatens the integrity of evolutionary units. Yet, sex is fundamental to the continued well-being of evolutionary units too. Sex and its antithesis in the evolution of reproductive systems, parthenogenesis, provide different options for the reproduction of evolutionary units. Although sex seems to undermine individuality, sex has been rediscovered as each new level of individuality emerges in the evolutionary process. Sex holds the promise of a better future and a more whole and undamaged individual.

Theories discussing the benefit of sex are discussed in three collections of papers on the topic (Stearns 1987; Michod and Levin 1988; American Genetics Society Symposium for the Evolution of Sex 1993). According to the repair hypothesis, genetic redundancy and repair occur during the sexual cycle and are the key to greater wholeness and well-being for the individual (Michod 1995). Cloning, on the other hand, offers ease and efficiency of reproduction at the expense of future generations and the well-being of the individual.

But with the successful cloning of a sheep recently announced in Scotland, has biological science found a more direct means to perpetuate what makes us the individuals we are (Michod 1997c)? The possibility, however faint, that a person might create offspring without the benefit of a partner has brought that question and others about sexual reproduction into unusual prominence. After all, sex extracts high costs in energy, time, and resources. Would it not be more efficient to make copies of ourselves asexually, as some think? Does generating one new person by combining the genes of two aging parents make any more sense than a one-for-one exchange? Would begetting a clone bring about a closer approximation of immortality than procreating in the usual fashion?

For those who have fantasized—and the fantasy seems all too common—that cloning could lead to the endless renewal of individual lives, the biological evidence suggests otherwise. In fact, it turns out that sex leads to a kind of immortality by repairing the genes of the egg and sperm cells so essential for the continuation of life (Michod 1997c). Far from being rejuvenating, cloning, on the contrary, could threaten the continuing evolutionary well-

being of genes, cells, organisms, and even the very nature of species (see Michod 1991; 1995, chap. 9).

## Major Transitions in Levels of Selection

The major transitions in evolutionary units are from individual genes to networks of genes, from gene networks to bacterialike cells, from bacterialike cells to eukaryotic cells with organelles, from cells to multicellular organisms, and from solitary organisms to societies (Buss 1987; Maynard Smith 1988, 1990, 1991a; Maynard Smith and Szathmáry 1995). These transitions in the units of selection share two common themes: (1) the emergence of cooperation among the lower level units in the functioning of the new higher level unit, and (2) regulation of conflict among the lower-level units.

Eigen and Schuster proposed the hypercycle as a way to keep individual genes from competing with one another so that cooperating gene networks could emerge (Eigen and Schuster 1979; Eigen 1992). Localizing genes in the cell keeps selfish parasitic genes from destroying the cooperative nature of the genome (Michod 1983; Eigen 1992; Maynard Smith and Szathmáry 1995). Chromosomes reduce the conflict among individual genes (Maynard Smith and Szathmáry 1993, 1995). Meiosis serves to police the selfish tendencies of genes and usually insures that each of the alleles at every diploid locus has an equal chance of ending up in a gamete. As a result of the fairness of meiosis, genes can increase their representation in the next generation only by cooperating with other genes to help make a better organism. Uniparental inheritance of cytoplasm may serve as a means of reducing conflict among organelles either through the expression of nuclear genes (Hoekstra 1990; Hurst 1990; Hastings 1992), or organelle genes (Godelle and Reboud 1995), or both. Finally, concerning the final transition—from organisms to societies of cooperating organisms—the theories of kin selection, reciprocation, and group selection provide three related mechanisms for the regulation of conflict among organisms: genetic relatedness, repeated encounters, and group structure. These are just a few of the ways in which the selfish tendencies of lower-level units are regulated during the emergence of a new higher-level unit.

As initially conceived, the field of sociobiology focused on the transition from solitary organisms to groups of organisms, or societies, and the emergence of cooperative functions at the social level, the level of the colony, say, in the case of eusocial behavior in insects (Wilson 1975). However, the set of tools and concepts used in studying conflict and cooperation during the transition from organisms to societies has proved useful for studying the other major transitions.

What happened during the transition between solitary cells and multicellular organisms? Organisms can be thought of as groups of cooperating cells.



Selection among cells could destroy this harmony and threaten the individual integrity of the organism. For the organism to emerge as an individual, or unit of selection, ways must be found of regulating the selfish tendencies of cells while at the same time promoting their cooperative interactions. The purpose of our work is to use multilevel selection theory to study the transition from cells to cell groups to multicellular organisms. More generally our goal is to develop a theoretical framework to study the emergence of individuality and new levels of fitness.

## Evolution of the Organism

### Individuality Reconsidered

Natural selection requires heritable variations in fitness. Levels in the biological hierarchy—genes, chromosomes, cells, organisms, kin groups, groups, societies—possess these properties to varying degrees, according to which they may function as units of selection in the evolutionary process (Lewontin 1970). Beginning with Wilson (1975) and the transition from solitary animals to societies, then Buss (1987) with the transition from unicellular to multicellular organisms, and more recently Maynard Smith and Szathmáry (Szathmáry and Maynard Smith 1995; Maynard Smith and Szathmáry 1995), attention has focused on understanding transitions between different levels of selection.

To understand the origin of individuality, therefore, we must understand how the properties of heritability and fitness variation emerge at a new and higher level from the organization of lower-level units, these lower-level units being units of selection in their own right initially. As already mentioned, unicellular organisms enjoyed a long evolutionary history before they merged to form multicellular organisms. In so doing, single cells relinquished their evolutionary heritage in favor of the organism. Why and how did this occur?

### A Scenario

To help fix ideas, let us consider a scenario for the initial stages of the transition from unicellular to multicellular life. We may assume that reproduction and motility are two basic characteristics of the early single-celled ancestors to multicellular life, and these single cells were likely able to differentiate into reproductive and motile states (Margulis 1981, 1993; Buss 1987). Cell development was probably constrained by a single microtubule organizing center per cell, and, consequently, there would have been a tradeoff between reproduction and motility, with reproductive cells being unable to develop flagella for motility, and motile cells being unable to develop

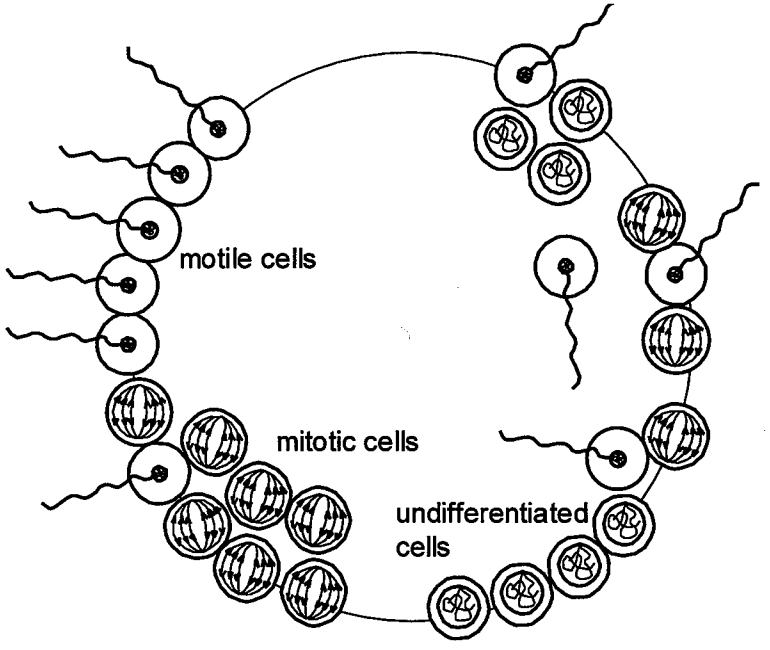


FIG. 4.1. Scenario for the first organisms (groups of cells). Adapted from Michod and Roze (1999).

mitotic spindles for cell division. Single cells would switch between these two states according to environmental conditions. Finally, the many advantages of large size might favor single cells coming together to form cell groups. At this point, our investigations begin. Figure 4.1 shows three kinds of cells: motile cells with a flagella; nonmotile mitotically dividing cells; and cells that have yet to differentiate into either motile or mitotic states. Because of the constraint of a single microtubule organizing center per cell, cells cannot be motile and divide at the same time. Motile cells are an example of cooperating cells, and mitotically reproducing cells are an example of defecting cells.

If and when single cells began forming groups, the capacity to respond to the appropriate environmental inducer and differentiate into a motile state would be costly to the cell but beneficial for the group (assuming it was advantageous for groups to be able to move). Because having motile cells is beneficial for the group, but motile cells cannot themselves divide, or divide at a lower rate within the group, the capacity for a cell to become motile is a costly form of cooperation, or altruism. Loss of this capacity is then a form of defection, as staying reproductive all the time would be advantageous at the cell level (favored by within-group selection), but disadvantageous at the group level (disfavored by between cell-group selection). We are led, according to this scenario (and many others), to consider the fate of cooperation and defection in a multilevel selection setting during the initial phases of the transition from unicellular life to multicellular organisms.



## A Model for the Origin of Multicellular Organisms

During the past several years, we have developed mathematical models of the evolutionary transition between single cells and multicellular organisms using the methods of population genetics and multilevel selection theory for the purpose of evaluating the levels of variation created within cell groups and studying the effect of this variation on the levels of cooperation and individuality attained.

Mathematical models show what is possible, based on assumptions about how the world works. By themselves, they cannot prove a hypothesis is true. They can, however, rule out poorly formulated or illogical hypotheses as well as suggest new hypotheses and fruitful lines of inquiry. By guiding experiment and observation, models are an integral part of scientific discovery. I primarily use simple population genetics models because they have great predictive and heuristic value in the understanding of complex evolutionary dynamics (Provine 1971, 1977, 1986; Ruse 1973; Wimsatt 1980; Michod 1981, 1986).

To understand the origin of organisms, it is helpful to think about them as groups of cooperating cells related by common descent (often from a single cell, the zygote). Selection among cells—below the level of the organism—could destroy the harmony within the organism and threaten its individual integrity. Competition among cells might favor cancerlike defecting cells that pursue their own interests at the expense of the organism. For the organism to emerge as an individual, or evolutionary unit, ways must have been found of regulating the selfish tendencies of cells, while at the same time promoting their cooperative interactions for the benefit of the organism. In addition, ways must have been found to ensure the heritability of the properties of these ensembles of cells so that the organism could continue to evolve as an evolutionary unit.

Consider a multicellular organism without a well-developed germ line, like a tree, coral, or hydra. An overview of the model life cycle is given in figure 4.2. The subscript $j$ indexes types of zygotes. After zygote formation, organisms grow by replication of cells during development. This proliferation of cells during development is indicated by the solid vertical arrows in figure 4.2. During this proliferation, deleterious mutation may lead to the loss of cell function. Cell function is represented by a single cooperative strategy, and mutation leads to loss of cell function and thus is assumed to produce defecting (selfish) cells from cooperative cells. Mutant cells use less time and resources to cooperate and, as a result, may survive better or repli-

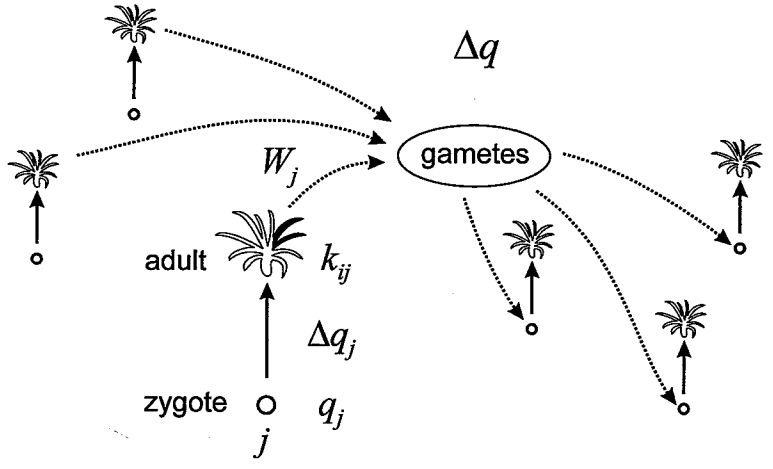


FIG. 4.2. Model life cycle of organisms. Adapted from Michod (1997b).

cate faster than cooperating cells (as occurs in the development of human cancers). Deleterious mutation will also produce uniformly deleterious cells that are impaired at both the cell level and the level of the group. Both kinds of mutations (selfish and uniformly deleterious) select for the modifiers of within-organism change discussed in subsequent sections (see Michod and Roze 1999). We assume no back mutation from defection to cooperation, because it is much easier to lose a complex function like cooperation and synergism between cells than it is to gain it. Because of mutation and different rates of replication of different cell types, gene frequencies change within the organism during its development. This is within-cell group or within-organism change, represented by the solid vertical arrow and $\Delta q_j$ in figure 4.2 (mutant cells in the adult are represented by the solid black color).

After development, the adult organism contributes gametes to start the next generation, and the genetic makeup of these gametes will reflect the new gene frequencies in the adult form (after within-organism change). In the case of sexual reproduction, the gametes produced by the cell group fuse randomly to form a diploid zygote. In the case of asexual reproduction, the gametes become zygotes and develop directly into the adults of the next generation. Gene and genotype frequencies also change in the total population of organisms because of differences in fitness (gamete output) among the adult organisms. Adult fitness is a function both of adult size (number of cells in the adult stage) and functionality, represented here by the level of cooperation in the adult stage. Differences in adult fitness lead to between-cell group, or between-organism, change, and is represented by the dashed arrows in figure 4.2. The two components of frequency change—within organisms and between organisms—give rise to the total change in gene frequency in the population, $\Delta q$.



Cooperation trades fitness between levels in a selection hierarchy (table 4.1). In the case of cells within organisms, cooperation benefits the organism while detracting from the fitness of cells. Because it takes time and energy to help the group, cooperating cells may replicate more slowly or survive worse than mutant defecting cells. I assume that adult size is indeterminate and that organism fitness is a function of both adult size as well as the level of cooperativity among the organism's cells.

In modern multicellular organisms, there is a dual inheritance system: genetic and epigenetic (Maynard Smith 1990; Maynard Smith and Szathmáry 1995). During development, differentiated cell types are generated by turning on and off different genes in different cells. This epigenetic state is passed on during cell reproduction so that, say, liver cells (once differentiated) give rise to liver cells. Deleterious mutation during development may then involve, not just mistakes at the loci-determining cell type (as I have considered), but also errors in the epigenetic state, that is, turning on or off the wrong genes. The situation we have considered to date only involves genetic inheritance. Because we assume that only a single locus determines cell type, our model cannot allow for different genes to be turned on and off in different cells. A more complicated multilocus model determining cell type with epigenetic inheritance is under development. Presumably, allowing for epigenetic effects will increase the levels of within-organism variation, but this variation may or may not be heritable, depending on the epigenetic inheritance systems in place in the single cells (Jablonka and Lamb 1995).

## KIN SELECTION REDUCES CONFLICT AMONG CELLS

In the models described here, all cells are clonally derived from a single cell zygote and related genetically (for consideration of propagule reproduction, see Michod and Roze 1999). This provides a kind of worst case for the study of the evolutionary effects of within-organism variation and conflict, because the zygote should restrict the opportunity for within-organism variation. If the organism began as a mixture of cells of different ancestries, as is probably the case for a migrating slug in the cellular slime mold *Dictyostelium discoideum*, the levels of within-organism change and conflict would likely be greater. By often reproducing through a single cell stage—the zygote—organisms insure close genetic relatedness among their component cells. Maynard Smith and Szathmáry argued that close kinship among cells should be sufficient to regulate the selfish tendencies of cells in an organism (Maynard Smith and Szathmáry 1995). In the limit, if cells are absolutely genetically identical, then their interests are one and the same. Is the kin structure created by the zygote stage sufficient to regulate the selfish tendencies of cells?

Levels of cooperation can be low in organisms even when reproduction

occurs through a single-cell zygote stage (Michod 1997a,b). Depending on the level of mutation, intensity of selection, and time for development, mutation and selection can create sufficient within-organism change and, hence, genetic variation within the cell group. This suggests that there is a problem in coping with within-organism variation and selection that the zygote stage (and the resulting kinship among cells) does not adequately deal with. We have used two-locus modifier theory to show that this variation and conflict can select for "conflict modifiers," genes that restrict the opportunity for within-organism change, for example, modifiers that create a germ line or police the selfish tendencies of cells (Michod 1996; Michod and Roze 1997).

## Conflict Modifiers

For the organism to emerge as a new unit of selection, within-organism change and interaction must be controlled so that heritability of fitness may increase at the organismal level. We model this by considering a second modifier locus that modifies the parameters of within-organism change at the first cooperate/defect locus possibly at some cost to the organism. In our model, a modifier allele is introduced at an equilibrium that is polymorphic for cooperation and defection (cooperation can never reach fixation because of recurrent mutation leading to defection). At this equilibrium, there exist two kinds of groups, those stemmimg from cooperating zygotes and those stemming from defecting zygotes. By definition, cooperative genotypes bias selection toward the group or organismal level (because cooperation takes fitness away from the cells and gives it to the group), while defecting genotypes do the opposite and bias selection toward the cell level. To maintain cooperation at the equilibrium before the modifier is introduced, cooperative groups must be more fit (produce more gametes) than defecting groups, because the fitness benefits of cooperation at the group level must compensate for mutation at the cellular level toward defection. The modifier allele increases by virtue of being associated with the more fit cooperating genotype. As a result of increase of the conflict modifier, both the level of cooperation within the organism and the heritability of fitness at the organism level increase (see figs. 4.3–4.5 below).

How might evolution modify the parameters of within-organism change so as to increase the fitness of the organism? According to Buss (1987), the individual integrity of complex animal organisms is made possible by the germ line, the sequestered cell lineage set aside early in development for the production of gametes. By sequestering a group of cells early in development, the opportunity for variation and selection is limited. As a consequence, evolution depends on the fitness of organisms, and the covariance of adult fitness with zygote genotype, and not the fitnesses of the cells that comprise the organism. The heritability of organismal traits encoded in the zygote is thereby protected. The trait of interest here concerns the level of specialization and differentiation among cells within organisms, which is represented here by the level of cooperativity among the cells.



The essential feature of a germ line is that gamete-producing cells are sequestered from somatic cells early in development. Consequently, gametes have a different developmental history from cells in the adult form (the soma) in the sense that they are derived from a cell lineage that has divided for a fewer number of cell divisions with, perhaps, a lower mutation rate per cell replication. The main parameters affected by germ line modifiers are the developmental time and mutation rate per cell division in the germ line relative to the soma.

Such germ line modifiers that lower the developmental time or mutation rate may be selected in our studies. Maynard Smith and Szathmáry (1995) suggested that germ line cells may enjoy a lower mutation rate but do not offer a reason why. Bell interpreted the evolution of germ cells in the Volvacale as an outcome of specialization in metabolism and gamete production to maintain high intrinsic rates of increase while algae colonies got larger in size (Bell 1985; see also Maynard Smith and Szathmáry 1995, pp. 211–213). I think there may be a connection between these two views.

As metabolic rates increase, so do levels of DNA damage. Metabolism produces oxidative products that damage DNA and lead to mutation. It is well known that the highly reactive oxidative by-products of metabolism (e.g., the superoxide radical $O_2^-$, and the hydroxyl radical ·OH produced from hydrogen peroxide $H_2O_2$) damage DNA by chemically modifying the nucleotide bases or by inserting physical cross-links between the two strands of a double helix, or by breaking both strands of the DNA duplex altogether. The deleterious effects of DNA damage make it advantageous to protect a group of cells from the effects of metabolism, thereby lowering the mutation rate within the protected cell lineage.

This protected cell lineage, the germ line, may then specialize in passing on the organism's genes to the next generation in a relatively error-free state. Other features of life can be understood as adaptations to protect DNA from the deleterious effects of metabolism and genetic error (Michod 1995): Keeping DNA in the nucleus protects the DNA from the energy-intensive interactions in the cytoplasm; nurse cells provision the egg so as to protect the DNA in the egg; and sex serves to repair genetic damage effectively while masking the deleterious effects of mutation. The germ line may serve a similar function of avoiding damage and mutation—by sequestering the next generation's genes in a specialized cell lineage, these genes are protected from the damaging effects of metabolism in the soma.

As just mentioned, according to Bell (1985), the differentiation between the germ and the soma in the Volvocales is correlated with increasing colony size, with true germ soma differentiation occurring only when colonies reach about $10^3$ cells as in the *Volvox* section *Merillosphaera*. Although Bell inter-

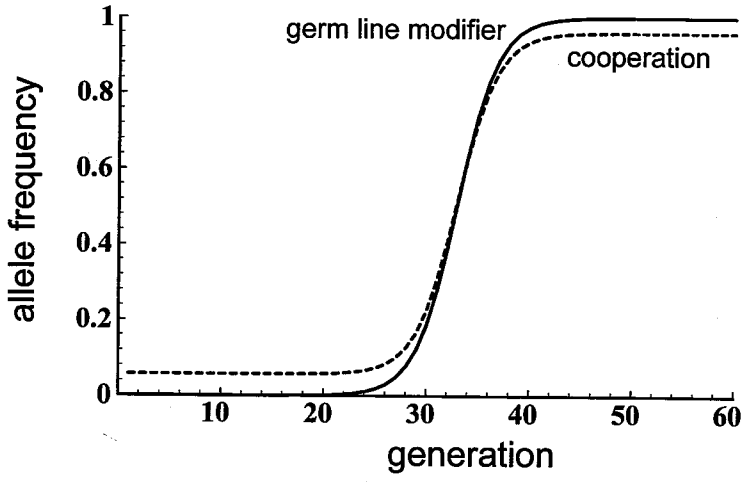


FIG. 4.3. Allele frequencies of cooperation and germ line modifier during an evolutionary transition. Adapted from Michod and Roze (1997) in which the underlying model is explained. See also Michod (1996).

preted the dependence of the evolution of the germ line on colony size as an outcome of reproductive specialization driven by resource and energy considerations (as did Weismann over a century ago), this relation is also explained by the need for regulation of within-colony change (see Michod and Roze 1999).

Another way to reduce conflict among cells is for the organism to actively police and regulate the benefits of defection (Boyd and Richerson 1992). How might organisms police the selfish tendencies of cells? The immune system and programmed cell death are two possible examples. For an introduction to the large and rapidly developing area of programmed cell death, or apoptosis, see Carson and Ribeiro (1993), Ameisen (1996), and Anderson (1997). This hypothesis is studied more in Michod and Roze (1999) and Michod (1999).

## Effect of Transition on the Level of Cooperation

Mediation of conflict among lower-level units is an essential feature of transitions to new higher levels of organization. I now consider the consequences of the evolution of conflict modifiers for the level of cooperation among cells and the heritability of fitness at the cell group, or organismal level. For reasons of space, I only consider the evolution of the germ line modifiers and our results for asexual reproduction, but we have obtained



qualitatively similar results for sexual reproduction and the other forms of conflict mediation such as self-policing modifiers and modifiers that regulate group size.

In figure 4.3, the evolution of a germ line modifier dramatically increases the level of cooperation in the organism. The level of cooperation always increases during the evolution of conflict modifiers. To understand the forces that lead to the evolution of the modifier, we have used covariance methods.

## Increase of Fitness at Organismal Level

An especially useful and illuminating method for representing selection in hierarchically structured populations is Price's covariance approach (Price 1970, 1972, 1995). Price's approach posits a hierarchical structure in which there are two selection levels—in our case, (1) between cells within organisms, viewed as a group of cells, and (2) between organisms within populations. Both levels of selection can be described by the single equation 4.1, Price's equation for organisms:

$$\Delta q = \frac{\mathrm{Cov}[W_i, q_i]}{\overline{W}} + \mathrm{E}[\Delta q_i]. \qquad (4.1)$$

Variables $q$ and $q_i$ are the frequencies of a gene of interest in the total population and within zygotes; Cov[$x$,$y$] and E [$x$] indicate the weighted covariance and expected value functions, respectively. The first term of the Price equation 4.1, Cov[$W_i$,$q_i$], is the covariance between fitness and genotype and reflects the heritable aspects of fitness. The second term of equation 4.1, E[$\Delta q_i$], is the average of the within-organism change resulting from mutation and selection among cells.

In figure 4.4, the two components of the Price covariance equation 4.1 are plotted during the increase in frequency of the germ line allele given in figure 4.3. These components partition the total change in gene frequency into heritable fitness effects at the organism level (solid line) and within-organism change (dashed line). In the model studied here, within-organism change is always negative, because defecting cells replicate faster than cooperating cells, and there is no back mutation from defection to cooperation. At equilibrium, before and after the transition, the two components of the Price equation must equal one another, or the population could not be in equilibrium (fig. 4.4). During the transition, however, we see that the covariance of fitness with genotype at the emerging organismal level (fig. 4.4, solid curve) is greater than the average change at the cell level (fig. 4.4, dashed curve). This greater heritable covariance in fitness at the higher level forces the modifier into the population.

In figure 4.4, we see that modifiers of within-organism change evolve by making the covariance between fitness at the organismal level and zygote

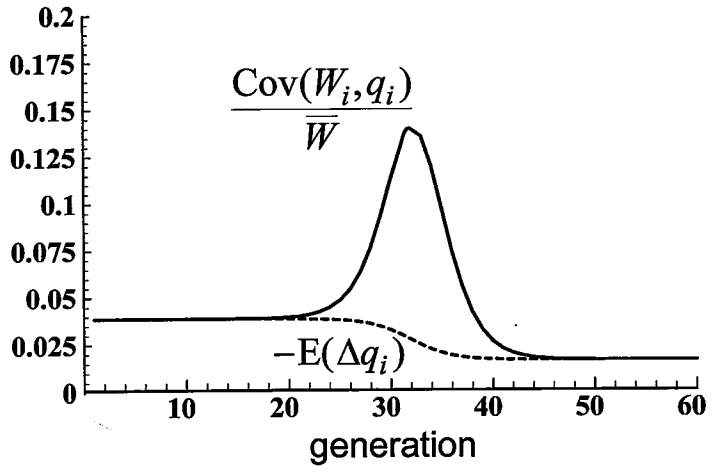


FIG. 4.4. Study of evolutionary transition by Price equation. Same model and parameter values as figure 4.3. The x-axis is the same as in figure 4.3. Adapted from Michod and Roze (1997). See text for explanation.

genotype more important than the average within-organism change. This implies that modifiers increase the heritability of fitness at the level of the new organism.

## HERITABILITY OF FITNESS AND THE EVOLUTION OF INDIVIDUALITY

Before the evolution of cooperation among cells, the population is assumed to be genetically homogeneous (all cells defect, and there is no modifier allele). In such a population, the heritability of fitness equals unity. When the cooperation allele appears in the population, evolution (directed primarily by kin and group selection) may increase its frequency, leading to greater levels of cooperation. However, within-organism change increases along with cooperation. Deleterious mutation produces nonfunctional defecting cells that have an advantage over cooperating cells at the cellular level. As a consequence of within-organism change, the heritability of fitness must decrease as soon as cooperation evolves.

The basic problem for the evolution of a new unit of selection—in this case, the multicellular organism—is that the organism cannot evolve new adaptations, such as the traits enhancing cooperation, if these adaptations are costly to cells, without increasing the opportunity for conflict within and thereby decreasing the heritability of fitness. Deleterious mutation is always a threat to new adaptations because it produces cells that go their own way. By regulating within-organism change, there is less penalty for cells to help the organism. Without a means of regulating within-organism change, the "organism" is merely a group of cooperating cells related by common descent. Such groups are not individuals, because they have no functions that exist at the new organism or group level.



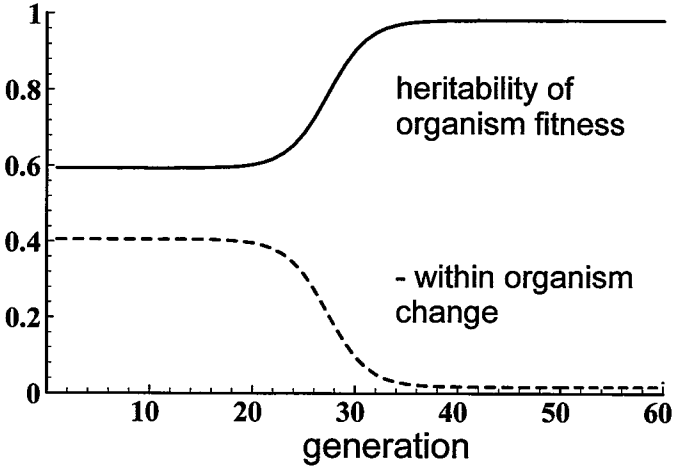


FIG. 4.5. Heritability of organism fitness and within organism change during evolutionary transition. Same parameter values as figure 4.3. The x-axis is the same as in figure 4.3. Adapted from Michod and Roze (1997).

Conflict modification is the first, uniquely organismal level function. Does heritability of fitness, the defining characteristic of an evolutionary individual, increase during the evolutionary transition mediated by conflict modifiers? Heritability of fitness may be defined as the regression of the fitness of offspring on fitness of the parents. It can be shown that the evolutionary transition mediated by the evolution of conflict modifiers always leads to an increase in the heritability of fitness. In addition, the eigenvalues of the different equilibria involve ratios between products of fitnesses and heritabilities (Michod 1999; Michod and Roze 1999). This illustrates clearly that what determines whether a new characteristic can increase in frequency in the population is the heritability of fitness of the new evolutionary individuals with this feature.

Before the evolution of modifiers restricting within-organism change, the "organism" is just a group of cooperating cells related by common descent from the zygote. Because of high kinship, heritability is initially significant at the group (organism) level ($h_w^2 \approx 0.6$ for the particular model studied in fig. 4.5), but this value is still low for asexual haploidy. (Heritability at the organismal level should equal unity in the case of asexual organisms when there is no environmental variance.) Low heritability of fitness at the new level resulting from within-organism change poses a threat to the continued evolution of the organism. In the case considered here, developmental time, and hence organism size, could not increase without the evolution of conflict modifiers. Indeed, the continued existence of cell groups at all is

highly unlikely before the evolution of the conflict modifier, because the cooperation allele is at such a low frequency (fig. 4.3), and stochastic events would probably lead to its extinction. As the modifier begins increasing, the level of within-organism change drops (fig. 4.5, dashed curve), and the level of cooperation among cells increases dramatically (fig. 4.3, dashed curve), as does the heritability of organism fitness (fig. 4.5, solid curve).

## Transitions in Individuality

### Effect of Sex and Diploidy on Evolutionary Transitions

Sex and diploidy have profound effects on the evolutionary transitions in our models (see Michod 1999). With diploidy, there are two dominance relations to consider at the cellular and organismal levels. Diploidy may facilitate the initial increase of cooperation through the masking of the advantage of defection in heterozygous zygotes. Diploids may also reach larger organism sizes than haploids, although at much reduced levels of cellular cooperation. If adult size is held constant, however, these advantages of diploidy no longer pertain. The buffering effect of increasing the heritability of fitness at the organismal level, whereby the level of harmony and cooperation within the organism is maintained in the face of increasing within-organism change, still pertains under diploidy, although it is affected by dominance.

There are quantitative effects of recombination in breaking up the association between the modifier and the cooperate/defect locus. In the case of germ line modifiers, sex requires larger decreases in developmental time in the germ line (for the effect of recombination on evolution of the germ line modifier, see Michod 1996, fig. 2). With sex, it also takes longer for the transition to occur (results not shown here for reasons of space). The modifier increases by virtue of being more often associated with cooperative alleles in gametes, and recombination breaks apart this association. Although sex can retard the transitions modeled here, because of the effects of recombination in breaking up the genetic associations needed for the modifier to increase, I do not see these quantitative differences as presenting any real barriers to the evolution of conflict modification and evolutionary transitions in sexual progenitors. More important, I think, is the way in which sex organizes variability and heritability of the traits and capacities that affect the fitness of the new emerging unit.

Sex helps diploids maintain a higher heritability of fitness under more challenging conditions, especially when there is great opportunity for within-organism variation and selection. With sex, as the mutation rate, and concomitantly the amount of within organism-change, increases more and more of the variance in fitness is heritable, regardless of dominance. Sex allows



the integration of the genotypic covariances in a way not possible in asexual populations.

The genome-wide mutation rates are vastly different in modern multicellular organisms ($\approx 0.5$ on haploid genome basis) and in modern microbes ($\approx 0.003$). Once multicellularity evolved, the continued evolution of multicellular organisms required new gene functions with a corresponding increase in genome size. With increasing genome size came the problem of increased rates of deleterious mutation. It is often noticed that diploidy helps multicellular organisms tolerate this increase in mutation rate by the masking of recessive or nearly recessive deleterious mutations (see, e.g., Michod 1995, fig. 1 and associated discussion). Once the diploid species reaches its own mutation selection balance equilibrium, however, the mutation load in diploid species actually increases beyond what it was under haploidy (Haldane 1937; Hopf et al. 1988). There must be another factor that allows complex multicellular diploids to tolerate a high mutation rate.

We have found that, as the mutation rate increases in sexual diploid organisms, the regression of fitness on zygote gene frequency actually increases (Michod 1999). In other words, as the mutation rate increases, and along with it the amount of within-organism change, more of the variance in fitness in sexual diploids is heritable, that is, explained by the alleles carried in the zygote. How can this be? The greater mutation rate must result in greater levels of within-organism change. At equilibrium, this within-organism change must be balanced by a larger covariance of fitness with zygote frequency. This is what Price equation (4.1) says. In haploid and asexual diploid populations, this is accomplished by a greater variance in zygote gene frequency, whereas in sexual populations, this can be accomplished by a greater regression of organism fitness on zygote frequency. Sex allows a greater precision of mapping heritable propensities of the zygote onto adult fitness under more challenging conditions.

These conclusions are based on equilibrium statistics before and after the evolutionary transition, and it is unclear whether these conclusions can be extended into the nonequilibrium realm of the transition and if the results will hold up under more realistic genetic models. If so, the greater precision in the mapping of cooperative propensity onto organism fitness should allow sexuals to make the transition from cells to multicellular organisms more easily under more challenging circumstances. This result is consistent with the view that the protist ancestor of multicellular life was probably sexual (Maynard Smith and Szathmáry 1995).

### Components of Evolutionary Transitions

Our results suggest that, even in the presence of high kinship among cells, within-organism change can be significant enough to lead to the evolution of

a means to regulate it. Examples of such conflict mediators may be the segregation of a germ line during the development and the evolution of a means of policing cells, such as the immune system or programmed cell death. The germ line functions to reduce the opportunity for conflict among cells and promote their mutual cooperation both by limiting the opportunity for cell replication (Buss 1987) and by lowering the mutation rate (Maynard Smith and Szathmáry 1995). Mutual policing (Boyd and Richerson 1992) is also expected to evolve as a means of maintaining the integrity of the organisms once they reach a critical size. Any factors that directly reduce the within-organism mutation rate are also favored.

Once within-organism change is controlled, high heritability of fitness at the new organismal level is assured. Individuality at the organismal level depends on the emergence of functions allowing for the regulation of conflict among cells. Once this regulation is acquired, the organism can continue to evolve new adaptations at the new level, without increasing the conflict among cells, as happened when cooperation initially evolved.

Development evolves so as to restrict the opportunity for conflict among cells. The evolution of modifiers of within-organism change lead to increased levels of cooperation within the organism and increased heritability of fitness at the organismal level. The evolution of these conflict mediators are the first new functions at the organismal level. An organism is more than a group of cells related by common descent; to exist, organisms require adaptations that regulate conflict within. Otherwise, continued improvement of the organism is frustrated by the creation of within-organism variation and conflict. The evolution of modifiers of within-organism change are a necessary prerequisite to the emergence of individuality and the continued well-being of the organism.

In summary, what happens during an evolutionary transition to a new higher-level unit of individuality, in this case, the multicellular organism? While taking fitness away from lower-level units, cooperation increases the fitness of the new higher-level unit (cell to organism). In this way, cooperation may create new higher-levels of selection. However, the evolution of cooperation sets the stage for conflict, represented here by the increase of defecting mutants within the emerging organism. The evolution of modifiers restricting within-organism change are the first higher-level functions at the organismal level. Before the evolution of a means to reduce conflict among cells, the evolution of new adaptations (such as the underlying traits leading to increased cooperation among cells) is frustrated by defecting mutants. Individuality requires more than just cooperation among a group of genetically related cells, it also depends on the emergence of higher-level functions that restrict the opportunity for conflict within and ensure the continued cooperation of the lower-level units. Conflict leads—through the evolution of adaptations that reduce it—to greater individuality and harmony for the organism.

# 5 Sexual Conflict in Animals

Catherine M. Lessells

Whatever the reasons for the evolution of sexual reproduction, it leads to a situation in which there are two individuals who may have no genetic interest in each other's future, the parents, but who nevertheless have a joint genetic interest in another individual or group of individuals, their offspring. If investment to improve the prospects of the current offspring reduces the parent's own prospects (i.e., there is a "cost of reproduction"; Williams 1966b; Lessells 1991), then there will be a conflict between the two parents (Trivers 1972). This conflict arises because each parent's fitness is generally maximized if it invests less and the other parent invests more than would maximize the other parent's fitness. Although this conflict has been referred to as "the battle of the sexes" (Dawkins 1989), selection for each parent to exploit the efforts of the other parent relies only on sexual reproduction and not the existence of separate sexes.

## The Evolution of Sexes

Conflict between parents may even be responsible for the evolution of sexes. Theoretical models show that conflict between initially sexually undifferentiated parents over gamete size can lead to the evolution of gamete dimorphism, and subsequently to disassortative mating between large and small gametes (Parker et al. 1972; Parker 1978). A parent with a fixed quantity of resource for the production of gametes can only produce more gametes at the expense of gamete size. When the fitness of zygotes depends strongly on zygote size, there may be disruptive selection for gamete size: Parents producing large gametes have the advantage of zygotes with high fitness, and parents producing small gametes have the advantage of numerous zygotes. Once gametes are dimorphic, there is conflict between the two sizes of gamete over fusion partners. Small gametes are selected to fuse disassortatively with large gametes, but large gametes are selected to fuse assortatively among themselves. Small gametes appear to have won this evolutionary conflict. Possible reasons include the stronger selection on small gametes to mate disassortatively; the larger number of mutations occurring each generation in the more numerous small gametes (Parker et al. 1972); the lack of suitable fusion partners for mutant ova that could fuse only with other ova (all wild-type ova having been rapidly removed from the gamete pool by the vastly more numerous sperm; Parker 1978, 1982); and the cost for larger