## CHAPTER 17

# Cooperation and conflict during the unicellular–multicellular and prokaryotic–eukaryotic transitions

**Richard E. Michod and Aurora M. Nedelcu**

Individuals often associate in groups that, under certain conditions, may evolve into higher-level individuals. It is these conditions and this process of individuation of groups that we wish to understand. These groups may involve members of the same species or different species. For example, under certain conditions bacteria associate to form a fruiting body, amoebae associate to form a slug-like slime mold, solitary cells form a colonial group, normally solitary wasps breed cooperatively, birds associate to form a colony, and mammals form societies. Likewise, individuals of different species associate and form symbiotic associations; about 2000 million years ago, such an association evolved into the first mitochondriate eukaryotic cell. The basic problem in an evolutionary transition in individuality is to understand why and how a group of individuals becomes a new kind of individual, possessing heritable variation in **fitness** at a new level of organization.

Certain alliances and associations of individuals are more stable than others, yet not all associations qualify as groups. In groups, interactions occur that affect the fitnesses of both the individuals and the group. Groups are often defined by a group property, usually the group frequency of a phenotype (or some other property reflecting group composition). Groups exist when the fitness of individuals within the group is not a frequency dependent function of the membership of other such groups (Uyenoyama and Feldman 1984). Within a group, member fitness usually is a function of the composition of the

group. Initially, group fitness is taken to be the average of the lower-level fitnesses of its members, but, as the evolutionary transition proceeds, group fitness becomes decoupled from the fitness of lower-level components. Witness, for example, colonies of eusocial insects or the cell groups that form organisms; in these cases, some group members have no individual fitness (sterile castes, somatic cells) yet this does not detract from the fitness of the group, indeed it is presumed to enhance it.

The essence of an evolutionary transition in individuality is that the lower-level individuals must as it were "relinquish" their "claim" to fitness, that is to flourish and multiply, in favor of the new higher-level unit. This transfer of fitness from lower to higher-levels occurs through the evolution of cooperation and mediators of conflict that restrict the opportunity for within-group change and enhance the opportunity for between-group change. Until, eventually, the group becomes a new evolutionary individual in the sense of generating heritable variation in fitness (at its level of organization) and being protected from the ravages of within-group change by **adaptations** that restrict the opportunity for defection (Michod 1999). Of course, no individual ever rids itself from the threat of change within, as evidenced by the numerous examples of conflict among different units of selection remaining in evolutionary individuals.

Cooperative interactions are a source of novelty and new functionality for the group. During evolutionary transitions, new higher-level evolutionary
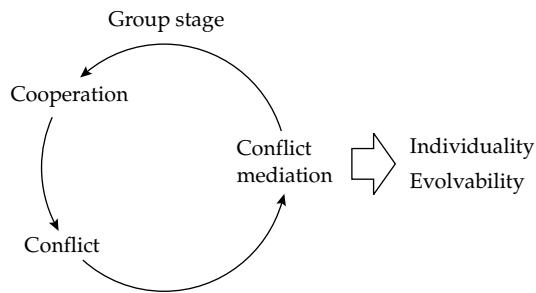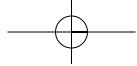
**Figure 17.1** Cooperation and conflict in evolutionary transitions. Stability of the group requires the mediation of conflict resulting from cooperative and conflictual interactions. Conflict may arise directly in response to cooperation as defection spreads within the group. Conflict mediation leads to further increases in cooperation and individuality at the group level. Continued evolvability of the new higher-level unit is fueled by new modes of cooperation and new ways to mediate conflict among component entities leading to new adaptations at the higher level.

units (e.g. multicellular organisms, mitochondriate eukaryotic cells) gain their emergent properties by virtue of the *interactions* among lower-level units (e.g. cells). Cooperation is fundamental to the origin of a new higher-level unit of fitness because cooperation trades fitness from a lower-level (the costs of cooperation) to the higher-level (the benefits for the group) (Michod 1999).

Although eventually lower-level units must cooperate in the formation of a new higher-level unit, initially the fitness interactions within the group may be based on any form of ecological interaction ranging from beneficial interactions, such as **mutualism**, to antagonistic forms such as competition and exploitation (predation, parasitism, pathogenism, slavery) as discussed later (Fig. 17.4; see van Ham *et al.*, Chapter 9). Nevertheless, both mutualism and exploitation involve conflict; exploitation because of its very nature, mutualism because, as do all cooperative types of interaction, it creates the opportunity for defection (Fig. 17.1). Fundamental to the emergence of a new higher-level unit is the mediation of this conflict among lower-level units in favor of the higher-level unit resulting in enhanced cooperation among the lower-level units.

Before the group becomes an individual, cooperation creates conflict and the temptation for defection.

This quarrel among units of selection reduces the scope for cooperative interactions and higher-level functions. **Evolvability** (used here to mean the capacity to evolve into more complex forms) of the emerging higher-level unit depends on the invention of new and more intricate forms of cooperation which provide the basis for new adaptations at the higher-level. Conflict mediation leads to enhanced individuality and **heritability** of fitness at the new level. Continued evolvability requires the resolution of this conflict in favor of the higher level so that the continued cooperation so necessary for adaptation is not constantly threatened by conflict within. In the case of multicellular groups, conflict mediation may involve the spread of conflict modifiers producing self-policing, germ line sequestration, or apoptotic responses (see below). In the case of organelle (i.e. mitochondria and chloroplasts) containing eukaryotic cells, conflict mediation may involve the uniparental transmission of organelles.

Until the emergence of the new level is complete (say with the evolution of a structure to "house" the new higher-level unit), interactions among lower-level units are likely to be density and/or frequency-dependent; therefore, there will be problems with rarity, advantages to commonness, and, the constant threat of defection. One of the most basic consequences of frequency-dependent natural selection is that there need not be any benefit for the individuals or the group. In the language of population genetics, the average fitness of the population need not increase under frequency-dependent selection (Wright 1969; Michod 1999). The well-known **Prisoner's Dilemma** game illustrates well the inherent limits of frequency-dependent selection in terms of maintaining the well-being and evolvability of evolutionary units (Michod *et al*. 2003). Natural selection not only fails to maximize the fitness of individuals in the Prisoner's Dilemma game, it minimizes it.

The dilemma of frequency-dependent selection is that while frequency-dependent interactions among members of the group are the basis of higher-level group functions, frequency-dependent selection does not necessarily increase group fitness (Michod 1999). How can frequency-dependent interactions be the basis of higher-level units but

not lead to the increase of fitness of those units? This paradox of frequency dependence is the basic problem that must be solved by multilevel selection, both during evolution within a species and during the transition to a new higher-level unit of organization.

## Cooperation

We see the formation of cooperative interactions among lower-level units as the *sine qua non* of evolutionary transitions, even if the groups initially form for exploitative reasons (as may have likely been the case with the origin of the first mitochondriate eukaryotic cell as we discuss below). For this reason we have paid special attention to the evolution of cooperation within groups. As Lewontin (1970) pointed out, a levels-of-selection perspective follows naturally from Darwin's theory of natural selection; however, the role of cooperation in the history of life has been less well appreciated. Thirty years ago, the study of cooperation received far less attention than the other forms of ecological interaction (competition, predation, and parasitism). Scholars generally viewed cooperation to be of limited interest, of special relevance to certain groups of organisms to be sure—the social insects, birds, our own species, and our primate relatives—but not of general significance to life on earth. All that has changed with the study of evolutionary transitions and the appreciation of the importance of population structure in evolution and that selection is usually a multilevel process. What began as the study of animal social behavior some 30 years ago, has now embraced the study of interactions at all biological levels. Instead of being seen as a special characteristic clustered in certain groups of social animals, cooperation is now seen as the primary creative force behind ever greater levels of complexity and organization in all of biology.

As already mentioned, cooperation is special as a form of interaction because it trades fitness from lower- to higher-levels. Cooperation creates new levels of fitness by increasing the fitness of the group, and, if costly at the lower level, by trading fitness from a lower level to a higher level. As cooperation creates new levels of fitness, it creates the opportunity for conflict between levels as deleterious mutants arise and spread. As discussed further, adaptations that restrict the opportunity for conflict between higher and lower levels (what we term **conflict modifiers**) are instrumental in the conversion of the group into a new evolutionary individual. Here, we consider the conversion of two different kinds of cell-groups. In the case of the origin of the first mitochondriate eukaryotic cell (as an endosymbiotic unit), the cell-group was composed of cells from different species; in the case of the origin of multicellular organisms, the cell-groups are composed of cells belonging to the same species.

Cooperation may be additive (in terms of the cost and benefit as is often assumed in models of altruism) or synergistic. Synergistic forms of cooperation benefit both the cell and the cell-group. In the case of synergistic cooperation, there is no obvious conflict between levels (at least in terms of how cooperation is defined), but if the loss of cooperation harms the higher level more than the lower level, modifiers still evolve that increase the heritability of fitness and evolvability of the group (see fig. 6 of Michod and Roze 2001).

The benefits of cooperative interactions usually depend upon the frequency with which they occur, while the costs of performing a cooperative behavior are usually an inherent property of the behavior itself, not depending on its frequency in the population or group. To the extent that cooperators are frequent in the population, it may pay a particular individual to forgo providing benefits, thereby reaping the benefits bestowed by others while not paying the cost. For these reasons, so long as selection is frequency-dependent, there is always a "temptation" to defect, that is, not help others, and so gain an advantage within the population relative to cooperators.

While it may be easy to agree on the basic role played by cooperation in the diversification of life, cooperation remains a difficult interaction to understand and to model especially when considering the different settings involved in the origin of multicellularity and the origin of first mitochondriate eukaryotic cell. In Tables 17.1 and 17.2, we further discuss the issues introduced previously (Michod and Roze 2001). In Table 17.1, we consider the
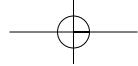
**Table 17.1**  Issues and contrasts in understanding cooperation

|  | Cooperators | | |
|---|---|---|---|
|  | One kind of cooperator | More than one kind | |
| Level of Interaction | Within Species Behavioral structure. Kin selection models. Cooperate/defect payoff matrix. | Within species | Behavioral structure. Models: hypercycle, stochastic corrector |
|  |  | Between species | Behavioral structure. Mutualisms. |
| Competition | Is the population viscous so that cooperators compete? Competition reduces advantage of cooperation in viscous populations | Is competitive exclusion possible? Competitive exclusion is usually not possible in the between species case (due to some prior niche displacement), but is possible in within species case (e.g. hypercycle) | |

References: behavioral structure (Michod and Sanderson 1985), viscous populations (Hamilton 1971; Goodnight 1992; Taylor 1992; Wilson et al. 1992; Queller 1994; van Baalen and Rand 1998), hypercycle model (Eigen and Schuster 1977, 1978a, 1978b, 1979; Michod 1983, 1999; Frank 1995, 1997), stochastic corrector model (Maynard Smith and Szathmáry 1995; Grey et al. 1995).

**Table 17.2**  Three contrasts in understanding the benefits of cooperation: additive versus synergistic, exchangeable versus nonexchangeable, and immediate versus delayed

|  | Benefits | |
|---|---|---|
| Additive | Synergistic | |
| Cheating possible | Cheating may not be possible | |
| Exchangeable | Nonexchangeable | |
| Sculling games | Rowing games | |
| Sharing | Functional differentiation, reproductive | |
| Economics of scale | versus nonreproductive specialization | |
| Immediate | Delayed | |
|  | Reciprocation, reciprocal altruism | |

Synergistic benefits may be exchangeable or not, immediate or delayed, reproductive or nonreproductive. Likewise with the additive case. References: sculling and rowing games (Maynard Smith and Szathmáry 1995); economics of scale (Queller 1997); reciprocation (Trivers 1971, 1985; Brown et al. 1982).

number of different kinds of cooperators, the potential for competition among cooperators and whether the cooperation occurs within or between species. When there is just one kind of cooperator (a single cooperative genotype), the cooperators must belong to the same species; when there are more than one kind of cooperator, the cooperators may belong to the same or different species. The latter situation applies to the origin of the mitochondria-containing eukaryotic cell, while the former situation of a single kind of cooperator is more applicable to the origin of multicellularity. The study of cooperation is often divided by the issue of whether the interactions occur within or between species, because kin selection is possible in the former but not the latter. However, both within and

between species cooperation requires spatial and or temporal correlations in the behavior of cooperating individuals. That is to say, there must be **behavioral structure**, that is, structure in the distribution of behaviors (Michod and Sanderson 1985). In the case of within-species interactions, genetic structure may facilitate behavioral structure and this is the basis of kin selection. Because of the need for behavioral structure, competition may also occur among members of cooperative groups and this may reduce the advantages of cooperation and/or lead to the loss of cooperative types. The **hypercycle** is a cooperative group of interacting replicators in which cooperation dynamically stabilizes the densities of the different replicators, thereby resisting competitive exclusion of any of the members (Eigen 1971; Eigen and Schuster 1977, 1978a,b, 1979).

The number of different kinds of cooperators also affects how cooperation is modeled. When there is just a single kind of cooperator, game theoretic payoff matrices are often used to conceptualize the interaction, as in the well studied Prisoner's Dilemma game. The payoff matrix approach can be extended to interactions between two species (Law 1991; Maynard Smith and Szathmáry 1995). When there are multiple members involved in the interaction, different approaches are used such as the

hypercycle or stochastic corrector models. The **stochastic corrector** model considers a population of groups of hypercycles containing different numbers of members in a multi-level selection framework (references given in the legend to Table 17.1).

Table 17.2 discusses another major issue in the study of cooperation, the nature of the benefits bestowed by cooperators. A fundamental question is whether cheating (obtaining the benefits of cooperation without paying the costs) is possible. **Synergism** occurs when benefits received from cooperation require the benefactor to participate in the interaction. In other words, it is not possible for an individual to receive the (synergistic) benefits of cooperative acts of others without itself cooperating; defection or cheating is either disadvantageous or not possible. Some of the scenarios for the origin of the eukaryotic cell assume that cooperation is synergistic (López-García and Moreira 1999) as does the explanation for the evolution of cooperation among unrelated ant foundresses (Strassmann and Bernasconi 1999).

Synergism requires nonlinearities in the contribution to fitness of each partner's behavior. If we were to let variables $X$ and $Y$ be the cooperative propensity of each partner, under an additive model of cooperation, fitness of each partner would be a linear function of these propensities. Cheating is possible for linear models, because one individual could have zero propensity to cooperate but still benefit from the cooperative acts of its partner. If we wanted there to be no benefits unless both partners cooperated, we might let each partner gain proportional to the *product* of their cooperative propensities, giving a nonlinear fitness function. If one partner did not cooperate, neither would receive any benefits. Of course, other more realistic functions are possible, our main point is that synergism requires nonlinear models of the fitness effects of the interaction.

A problem with synergism alone as a scenario for the origin of cooperation is that it has difficulty explaining how cooperation gets started in a population of noncooperators. If there is one kind of cooperator, say $C$, interacting with defectors, $D$, we may model the interaction in terms of the familiar

payoff matrix

|   | $C$ | $D$ |
|---|---|---|
| $C$ | $a$ | $b$ |
| $D$ | $c$ | $d$ |

with the understanding that the elements $a$, $b$, $c$, $d$ give the fitness of the strategy on the left when interacting with the strategy on the top. If $a > c$, we say there is synergism (Maynard Smith 1998), cooperation is stable, and cheating is not possible when cooperation is established in the population. However, even in this case ($a > c$), if cooperators pay a cost when their partner is not cooperating, $b < d$, cooperation cannot invade when rare, because most of their interactions are with defectors. One way around this problem is to assume that cooperation is neutral when associated with defection, or $b = d$. Explaining the origin of cooperation is a special virtue of kin selection. Kinship among individuals provides the requisite behavioral structure locally (say, within families), and cooperation can increase (because cooperators tend to be concentrated in certain families), even though cooperators are rare in the global population.

Another important issue in understanding cooperation is whether the benefits contributed by different cooperators are similar or different in kind (Queller 1997). This relates to the issue in Table 17.1 concerning the kinds of cooperation. Sharing food is an example where the cooperating members provide similar benefits that are exchangeable. In contrast, role specialization in the castes of a termite colony, or cell and tissue specialization in a multicellular organism, are both situations where the cooperators provide different kinds of benefits, and, hence, one kind of benefit cannot be exchanged for another. The separation of reproductive functions between germ and soma is another example of non-exchangeable benefits. The distinction made by Maynard Smith and Szathmáry (Maynard Smith and Szathmáry 1995) between rowing and sculling games expresses a similar issue. In **rowing games**, the oarsmen row on different sides of the boat (and so provide different and nonexchangeable functions). In **sculling games**,
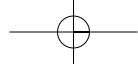
each oarsmen rows on both sides simultaneously (and so provide similar and exchangeable functions). The distinction is important, because cheating is much more costly in rowing games than in sculling games. In both kinds of games, the cooperators are in the same boat, which is another way of saying that there must be spatial and temporal correlations, that is, behavioral structure.

Synergism may occur between functionally similar (sharing food, sculling games) or dissimilar members (rowing games, interspecies mutualisms). Synergism among functionally similar members must come from the "economics of scale" (Queller 1997). Alliances of similar members must draw their (synergistic) benefits from the numbers of these members, that is, scale. For example, larger things are less likely to be eaten than smaller things (everything else being equal) (Stanley 1973; Shikano *et al.* 1990; Gillott *et al.* 1993; Boraas *et al.* 1998), and this may be one of the advantages to forming groups early during the evolution of multicellularity.

Kin selection operates between genetically similar members. Whether genetically similar individuals are functionally similar depends upon development and differentiation. Indeed, developmental differentiation of cells derived from a single zygote or spore attains the best of both worlds, it achieves the benefits of functional complementation (which will lead to synergism) along with the security of kin selection (security with regard to the spread of selfish mutants). Cooperation is one possible consequence of genetic relatedness among interactants; in addition heightened resource competition and inbreeding may occur. Because genetically related individuals are phenotypically similar, their ecological requirements overlap and competition increases, as in **viscous populations** in which the interactions occur nonrandomly and locally. The benefits of cooperation must overcome these costs of increased competition if cooperation is to spread in viscous populations (Hamilton 1971; Goodnight 1992; Taylor 1992; Wilson *et al.* 1992; Queller 1994; van Baalen and Rand 1998). The theory of sib-competition for the advantage of sex is based on the idea that sexually created variation among offspring helps create dissimilarity and avoid competition. As a result of this variation more offspring survive to

reproduce. Depending on the mating system and population structure, genetic relatedness among interactants may also imply genetic relatedness among mates, that is inbreeding (Hamilton 1972; Michod 1979, 1991).

## Origin of multicellular organisms

We illustrate our approach of cooperation, conflict, and conflict mediation, by considering first the origin of multicellular organisms and, in the next section, the origin of the first mitochondriate eukaryotic cell. A multilevel selection approach to evolutionary transitions in individuality begins by partitioning the total change in frequency of phenotypes of lower-level units (and their underlying genes) into within and between-group components.

### Model framework

During the transition from single cells to multicellular organisms, we assume that cells belonging to the same species form groups composed of $N$ cells as in Fig. 17.2 (Michod 1999; Michod and Roze 1999,
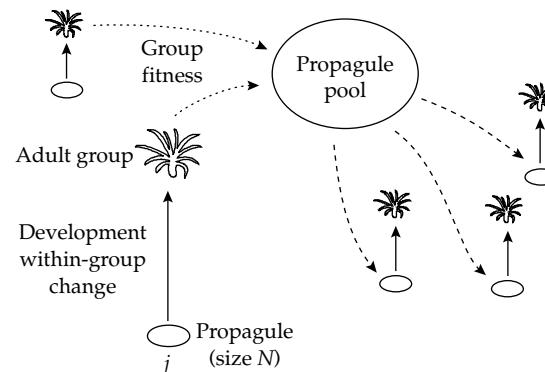


**Figure 17.2** Multilevel framework for the origin of multicellular organisms. The subscript $j$ refers to the number of *cooperating* cells in a propagule; $j = 0, 1, 2, \ldots, N$, where $N$ is the total number of cells in the offspring propagule group, assumed constant for simplicity. The variable $k_j$ refers to the total number of cells at the adult stage of propagules that start out with $j$ cooperating cells. The variable $W_j$ is the fitness of group $j$, defined as the expected number of propagules produced by the group, assumed to depend both on size of the adult group after development and its functionality (or level of cooperation among its component cells) represented by parameter $\beta$ in the model discussed in the text.

2000). The level of kinship among **propagule** cells is determined by the size of the founding group, $N$, and the way in which the group is formed, whether by fragmentation or aggregation and whether sex is involved. During development, cells replicate and die (possibly at different rates depending on cell behavior) to create the adult cell-group. Deleterious mutation may occur during cell division leading to the loss of cooperative cell functions and a decrease in fitness of the adult group. The adult group produces offspring groups of the next generation. We have considered several different modes of reproduction according to how the propagule offspring group is produced: single cell (or spore reproduction), fragmentation, and aggregation (Michod and Roze 2000; Roze and Michod 2001).

Because there are two levels of selection, the cell and the cell-group, there is the opportunity for both *within* and *between-group* selection. Fitness at the cell level involves the rates of cell division and cell death, these in turn depend upon cell behavior. We consider two kinds of cell behavior, cooperation, and defection. Most of our previous work has assumed genetic control of cell behavior, specifically we have assumed that cell behavior is controlled by a single genetic locus with two alleles, $C$ and $D$, for cooperation and defection, respectively. Alternatively, we may assume parental (or spore) control of cell behavior, in this case cell behavior is determined by the genotype of the mother cell. The latter assumption is made in parental manipulation models for the evolution of altruism. As is well known in the theory of kin selection, it is easier for costly forms of cooperation to spread under parental manipulation than under sibling control of the altruistic behavior (Michod 1982).

In the case of genetic control of cell behavior, cell behavior depends upon the cell's genotype, and mutations during development may disrupt cooperative cell functions and harm the group. Uniformly deleterious mutations are assumed to be disadvantageous at both the cell and group levels, while selfish mutations are assumed to be advantageous for the cell and disadvantageous for group. Fitness at cell-group or organism level depends upon the number of propagules produced, which, in turn, depends upon adult size and the level of cooperation among cells. The basic parameters of the model include development time, $t$, the within organism mutation rate per cell division, $\mu$, the effect of mutation on the cell replication rate, $b$ ($b < 1$ or $> 1$ means uniformly deleterious or selfish mutations, respectively), the benefit of cooperation for the group or organism, $\beta$ assumed $> 1$, and the propagule size, $N$. In addition, there is a parameter that tunes the relative effect of group size on fitness. Using this model we have studied the levels of cooperation maintained in populations and the partitioning of fitness among the cell and group levels (Michod 1997, 1999).

Mutation occurs during development and leads to the loss of cooperative group functions (loss of the cooperative benefit $\beta$ at the group level with effect $b$ at the cell level). In our studies of genetic mutations we use a genome wide mutation rate per cell division similar to that in extant microbes of $\mu = 3 \times 10^{-3}$ (Drake 1974, 1991), even though the more relevant rate is that of the primitive single celled ancestors of multicellular organisms. It is likely that the mutation rate has been lowered in modern microbes as a result of the very forces under study in our models. By this, we mean that under most conditions, the model predicts that it is advantageous to lower the mutation rate so as to reduce the scope for selection within-groups and increase the heritability of fitness at the group level. We use this genome wide mutation rate for the single $C/D$ locus, as we imagine this locus to represent all the cooperative functions in the genome. Of course, this is not realistic and we have extended our treatment of mutation using more realistic models based on a random infinite alleles model and the Luria Delbrück distribution (Michod and Roze 2000; Roze and Michod 2001). **Epigenetic mutations** are also likely to be frequent and important in the origin of multicellularity but we have not yet studied them.

## Conflict mediation and programmed cell death

To study how evolution may shape development and the opportunity for selection at the two levels of organization, the cell and cell-group, we assume a second modifier locus that affects the parameters
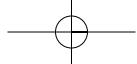
of development and/or selection at the primary cooperate/defect (*C*/*D*) locus. The evolution of these conflict mediators are the first emergent functions that serve to turn the group into a new higher-level individual. The modifier locus has two alleles (*M* and *m*) and may affect virtually any aspect of the model, such as propagule size, *N* (Michod and Roze 1999, 2000, 2001), and adult size (whether it is determinate or indeterminate). In the case of the evolution of a differentiated germ line (Michod 1996, 1999; Michod and Roze 1997), the development time and or the mutation rate may be lowered in the germ line relative to the soma. In the case of the evolution of self-policing (e.g. the immune system), the modifier affects the parameters of selection at both levels, *b* and *β*, reducing the temptation to defect at a cost to the group (Michod 1996, 1999; Michod and Roze 1997). In the case of the evolution of programmed cell death discussed below, we assume the modifier lowers the replication rate of mutant cells directly to $b - \delta$.

As an illustration of conflict mediation in the case of multicellularity, we consider the evolution of **programmed cell death** (PCD). PCD, sometimes termed **apoptosis**, is an evolutionarily conserved form of cell suicide that enables metazoans to regulate cell numbers and control the spread of cancerous cells that threaten the organism. It is best studied in *Caenorhabditis elegans* and mammals, but similar traits have also been described in unicellular organisms such as slime molds (Ameisen 1996), trypanosomatids (Moreira *et al.* 1996; Barcinski 1998; Welburn *et al.* 1999), and yeast (Madeo *et al.* 1997, 1999; Ligr *et al.* 1998). Presumably, in unicellular organisms, PCD is a form of altruism (Frohlich and Madeo 2000), although there is little direct evidence on this point. We illustrate briefly how it may be viewed as a conflict mediator using our theory. We model the evolution of PCD by using the same two locus modifier methods we have used previously (Michod 1999; Michod and Roze 1999) to study the conditions under which germ line or self-policing modifiers spread and tilt the balance in the units of selection conflict in favor of the cell-group, or organism, thereby enhancing its individuality (Michod 1996, 1999; Michod and Roze 1997, 1999). A PCD modifier lowers the rate of division (or survival) of

the mutated cell (parameter *pcd*). We assume this occurs at some cost, $\delta$, to the cell-group, or organism. If there were no costs for the modifier, the modifier would always increase so long as it was introduced in a population in which cooperation was present (the role of cooperation is discussed below).

In Fig. 17.3 we report results for the evolution of PCD modifier alleles, assuming sexual reproduction
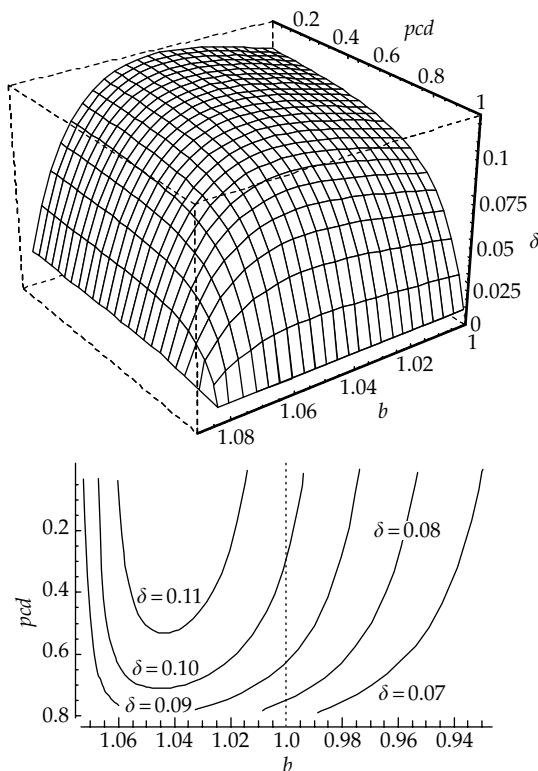


**Figure 17.3** Evolution of apoptosis. Bottom panel gives 2D slices through the 3D surface in the top panel. PCD modifiers evolve for parameter values below the 3D surface and above the 2D curves. The parameter *b* is the replication rate of non-regulated mutants (relative to non-mutants) and PCD is the factor decrease in replication rate of PCD modified cells; modified cells replicate at rate *pcd* × *b*. In the bottom panel five curves are plotted for different values of the cost to organisms of the PCD phenotype. Parameter values in the model (for specification of the model details see Michod 1999): offspring group size *N* = 1, time for development *t* = 20, benefit of cooperation *β* = 3, recombination rate between mutated locus and modifier locus *r* = 0.2, survival is incorporated in replication so $s_C = s_D = 1$, and mutation rate $\mu$ = 0.003. In the bottom panel selfish mutations lie to the left of the vertical dotted line and uniformly deleterious mutations lie to the right. See text for explanation.

($N = 1$ with sex) and a single class of mutant cells $D$ with fixed effect $b$ (the replication rate of mutant cells without the PCD modifier allele; the replication rate of nonmutant cells is unity). Cells with the modifier allele express the PCD phenotype: mutant cells replicate at rate PCD $\times$ $b$, instead of at rate $b$ in nonmodified cells. A perfect PCD phenotype would mean that all proliferating mutant cells die; in this case we would set $pcd = 0$. Of course, it is unlikely that the first PCD response was perfect, so we consider the entire range of possible values for the PCD phenotype ($0 \leq pcd < 1$). The cost of the PCD phenotype at the organism level is assumed to be $\delta$—the benefit of cooperation is reduced in PCD cells to $\beta - \delta$, instead of $\beta$ in non-PCD cells ($\beta = 3$ in Fig. 17.3).

An interesting feature of the results shown in Fig. 17.3 is that uniformly deleterious mutations (ones that disrupt the functioning of the group and proliferate more slowly than normal cells, $b < 1$), may also select for PCD modifiers, but, to invade, the modifier requires lower costs of the PCD phenotype to the organisms. It is common in the literature on PCD to assume that the risk of selfish mutations has lead to the evolution of the PCD phenotype. However, we see in Fig. 17.3 that both uniformly deleterious and selfish mutations can select for PCD. We have also observed that both kinds of mutations select for the other kinds of modifiers that we have studied, such as germ line and self-policing modifiers.

Why do the curves in Fig. 17.3 fall off rapidly as $b$ increases up towards a value of approximately 1.07? As the proliferation advantage of mutants, $b$, increases, the equilibrium frequency of nonmutant cooperating cells decreases, eventually reaching zero at about 1.07 (when within-group change overpowers between-group selection for cooperation). Without variation at the cell interaction $C/D$ locus the PCD modifier, $M$, is disadvantageous, because when the modifier is introduced the only genotypes are $MD$ and $mD$ (assuming haploidy for explanation purposes; where $D$ is the mutant and $M$ and $m$ are the PCD and non-PCD modifier allele, respectively). Cell-groups initiated by PCD cells ($MD$) end up being smaller than groups initiated by non-PCD cells ($mD$), because of the lower replication rate (or higher death rate) of PCD cells. However, when cooperating cells are maintained in the population before the PCD modifier is introduced, the significant competition is between groups initiated by $CM$ and $Cm$ cells. The cooperating groups carrying PCD modifiers (initiated by $CM$) end up being more functional and having fewer mutant cells in the adult stage and the associated fitness advantage can make up for the cost of PCD, $\delta$ in the regions under the curves shown in the figure). The dependence of the evolution of PCD on the maintenance of cooperation reflects the need for a higher-level unit of selection (the cell-group, or organism). The PCD modifier increases by virtue of tilting the balance in favor of the cell-group, by enhancing its individuality and heritable fitness (Michod 1999).

## Origin of the eukaryotic cell

One of the most significant events in the diversification of biological life is the transition from the prokaryotic to the more complex eukaryotic type of cellular organization. Although the symbiotic monophyletic origin of the eukaryotic cell is now widely accepted, there are many questions yet unanswered. Why did only one particular type of symbiotic association become stable and selected? Initially, what type of partners were involved in this "lucky" association and what was the nature of their interaction? What were the selective pressures that triggered this interaction and its subsequent evolution? Most importantly, how did individuality at the higher level emerge? That is, how did heritability of fitness—the defining characteristic of an evolutionary individual—arise at a new higher level, out of the coevolution of partners who were initially evolutionary individuals in their own right?

Most of the current evolutionary scenarios to explain the origin of the eukaryotic cell considered below (Table 17.3) are based on molecular, cellular, or biochemical data (Cavalier-Smith 1987; Rudel et al. 1996; Martin and Müller 1998). Whether eukaryotic features (such as a membrane-surrounded nucleus and a cytoskeleton) evolved before (Cavalier-Smith 1987) or during (Martin and Müller 1998) the acquisition of the mitochondria, is still debatable; nevertheless, this event is recognized as
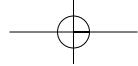
**Table 17.3** Conflict and conflict mediation in the origin of the eukaryotic cell. nctb: nucleus-cytosol-to-be; mtb: mitochondria-to-be. References to hypotheses: 1: Martin and Müller (1998); 2: Blackstone (1995); Blackstone and Green (1999); 3: Moreira and López-García (1998); López-García and Moreira (1999); 4: Sagan 1967; Cavalier-Smith (1987); Guerrero (1991); 5: Rudel *et al.* (1996); Kroemer (1997); Frade and Michaelidis (1997).

| Hypothesis | Initial interaction | Conflictual stage | Mediation stage |
|---|---|---|---|
| Hydrogen (1) | Commensalistic: nctb fed on mtb's respiration waste ($H_2$) | nctb limits mtb's ability to import organic substrates required for its growth and reproduction | nctb 'fed' the mtb in exchange for $H_2$; mtb's genes transferred to nctb |
| Units-of-selection (2) | Commensalistic/mutualistic: mtb fed on nctb's excreted carbon; nctb benefits from the re-oxidation of NADH | In aerobic conditions, mtb increased its growth rates and ROS production that damaged the nctb | nctb increased its rate of growth, division and recombination |
| Syntrophic (3) | Mutualistic: mtb and nctb fed on each other's waste | Disagreement on efficiency of production of waste | Aerobic metabolism replaced methanogenesis; gene transfers |
| Predatory–prey (4) | Exploitation: nctb fed on living mtb or vice versa | One partner escapes the other partner's digestion | mtb provided energy in exchange for its transmission |
| Pathogen–host (5) | Exploitation: mtb fed on nctb's organic substrates | mtb digests nctb when nctb's ATP concentration drops | nctb 'stole' ATP from mtb and avoided mtb's lytic mechanisms; gene transfers |

the fundamental step in the prokaryotic–eukaryotic transition, and is the focus of our analysis below. We have approached understanding this major evolutionary transition by translating the different scenarios for the origin of the first mitochondriate eukaryotic cell into the language of multilevel selection theory as a prerequisite to careful population modeling. This approach is intended to help identify the critical factors and thresholds involved in the transition from prokaryotic to mitochondria-containing eukaryotic cells. Our longer-term goal is to evaluate and compare in a common framework the proposed theoretical scenarios for the origin of the first eukaryotic cell.

Our framework is based on understanding the selective and population processes acting during initiation, establishment, and integration of the association so as to understand the emergence of a new unit of evolution with heritable variation in fitness. More specifically, we have considered selective pressures acting on the free-living partners-to-be, which in turn affect the initiation of the association, the initial benefits and costs for the partners versus the free-living relatives, the coevolutionary responses of the partners to each other (and of their association to the environment), the selective forces acting on the group (in relation to the free-living relatives as well as other groups based on different phenotypic associations), and ways of maintaining and integrating the group.

Here, we (i) summarize our multi-level selection approach to investigating the various scenarios regarding the origin of the eukaryotic cell, (ii) pinpoint the key steps in this evolutionary transition and the emergence of individuality at a higher level, and (iii) present the multilevel selection framework that we use in our mathematical modeling (R. E. Michod and A. M. Nedelcu, unpublished). Due to the diversity of scenarios, and especially the multitude of interspecific relations proposed in the

current hypotheses, the generic terms "host" and "endosymbiont" as the ancestors of the nucleo-cytosolic compartment and mitochondrion, respectively, can sometime be misleading (i.e. depending on the scenario the "host" is either the "victim", the "predator", or the "prey"). Therefore, we are using the ecologically neutral terms **nucleo-cytosol-to-be** (nctb) and **mitochondria-to-be** (mtb) for the two types of partners, according not to the role they played in the initial interaction but rather to what they are suggested to have evolved into.

There are a variety of verbal scenarios for the origin of the first mitochondriate eukaryotic cell involving almost every form of ecological inter-action. Based upon the type of initial interaction between partners, we grouped the current hypo-theses in three classes. Commensalistic interactions are invoked in the symbiotic theory (Margulis 1970, 1981), the hydrogen hypothesis (Martin and Müller 1998), and the units-of-evolution hypothesis (Blackstone 1995; Blackstone and Green 1999); mutualistic interactions are suggested in the syn-trophic hypothesis (Moreira and López-García 1998); and exploitative interactions are implied in the predator–prey hypotheses (Sagan 1967; Margulis 1981; Cavalier-Smith 1987; Guerrero 1991), as well as pathogen–host hypotheses (Rudel *et al*. 1996; Frade and Michaelidis 1997). This classifica-tion reflects only the type of initial interspecific interaction between the nctb and mtb; in fact, some hypotheses involve a succession of commensalistic, exploitative and mutualistic interspecific interac-tions, for example, the hydrogen and the units-of-evolution hypotheses (Frade and Michaelidis 1997).

To provide a general framework that applies to all the current scenarios, we have identified four stages in the evolution of the association towards the new higher-level unit, the mitochondriate eukaryotic cell. These stages are **initiation** (what type of ecological interactions were present ini-tially), **establishment** (how did these interactions become stabilized in space and over time), **integra-tion** (how did the symbiotic association evolve into an obligate functional unit), and **emergence** of the higher-level individuality (how did the functional unit evolve into an evolutionary individual with fitness heritability).
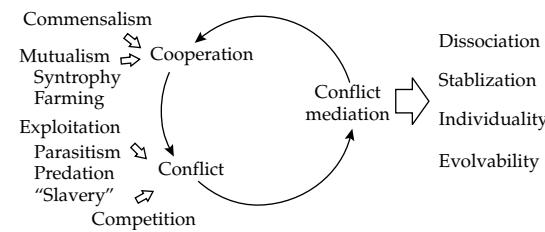
**Figure 17.4** Cooperation and conflict in the origin of the eukaryotic cell. The relation between the various ecological interspecific interactions proposed in various scenarios for the origin of the eukaryotic cell and the stages that characterize the dynamics of behavioral interactions in a group (see Fig. 17.1). Potential outcomes of the conflict mediation stage are also indicated.

We then viewed the scenarios from the point of view of the behaviors associated with the inter-actions in each stage, namely, cooperation, conflict, and conflict mediation, and mapped them to the evolutionary stages presented above. Interestingly, we found that the partners can enter the cycle in Fig. 17.1 (cooperation, conflict, conflict mediation), through either a cooperative (i.e. mutualism, com-mensalisms) or conflictual type of interaction (i.e. parasitism, predation, slavery) (Fig. 17.4).

Furthermore, regardless of the initial ecological interaction in each scenario, the association had to face at least one conflictual stage on its way towards integration and higher-level individuality (Table 17.3). This is true even for those scenarios that start out assuming the association was mutualistic or commensalistic to begin with (Margulis 1981; Martin and Müller 1998; López-García and Moreira 1999). Conflict is not associated with a particular stage in the evolution of the association, for it occurs in different stages depending on the scenario ana-lyzed. However, it appears that a conflictual stage is a *sine qua non* condition for the integration of the association and its evolution towards a higher-level individual, and the evolution of the group into a new evolutionary unit depends crucially on the outcome of the conflictual stage. The conflict could result in the dissolution of the association by revert-ing to free-living state. Alternatively, conflict media-tion could stabilize the association by promoting or enhancing cooperation among partners; further-more, if the cycle is repeated, conflict mediation
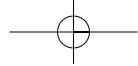
could later on contribute/result in the integration of the association and the emergence of individuality at the higher level. Last, the way in which the conflict is mediated in each round through the cycle of cooperation, conflict, and conflict mediation, can affect the potential for further evolution (i.e. the evolvability) of the newly emerged evolutionary individual. Below we exemplify our findings with a succinct analysis of three of the scenarios proposed to explain the origin of the first mitochondriate eukaryotic cell.

In the **hydrogen hypothesis** (Martin and Müller 1998), conflict arises during the establishment phase. The selective pressure, that is, the decrease in the level of atmospheric free hydrogen, which initiated a commensalistic interaction between nctbs and mtbs, gained new dimensions as the concentration of free hydrogen continued to drop. To benefit more from the hydrogen released by the mtbs, nctbs surrounded the mtbs to the point where the interaction became detrimental for the latter (by limiting their cell surface, and thus their ability to import the organic substrates required for their growth and reproduction). Consequently, the initially commensalistic interaction changed into an exploitative interaction, and the growth and reproduction of the mtbs were negatively affected. The conflict has likely resulted in the dissolution of many such associations. However, with the decrease in the concentration of free hydrogen, the nctbs became more and more dependent on the mtb-produced hydrogen; therefore, there must have been strong selection for keeping the mtbs alive and functional. Associations in which the nctb found ways to ensure both the survival of the mtb and the mtb's transmission to the nctb offspring were favored. Ways to provide the by now intracellular mtb with organic substrates required (i) the evolution of importers of reduced carbon in the nctb, or (ii) the transfer of the mtb's genes for such importers as well as those for carbohydrate metabolism to the nctb's chromosome. The gene transfer not only resolved the conflict but also resulted in the metabolic and genetic integration of the symbiotic association.

In the **units-of-evolution hypothesis** (Blackstone 1995), conflict arises during the integration phase.

The initial association was established through commensalistic interactions; mtbs were mutants with damaged glycolitic mechanisms and, thus, feeding on intermediary metabolites excreted by nctbs. Later, the interaction might have become mutualistic; nctbs benefited from mtbs' oxidation of the reduced cofactor NADH, which allowed the former to produce more ATP and use some of the pyruvate for biosynthesis. The association became and remained stable (but not obligate for both partners) as long as the environmental conditions remained unchanged. However, once the oxygen increased in the environment, the interaction between the nctb and mtb changed dramatically. Due to its aerobic capabilities, the mtb produced a lot more ATP than its host (and, thus, enjoyed a higher rate of growth and reproduction), as well as an increased level of endogenous oxidants for which the nctb did not have the tolerance and the ability to deal with. Consequently, the nctb's fitness decreased and the association became highly unstable. Blackstone (1995) pointed out that a "successful endosymbiosis would depend on successful resolution of units-of-evolution conflicts". By "leaking" ATP, some mtbs contributed to the increase of nctb's growth and division rate, which was in turn beneficial for the mtbs. In addition, some nctbs responded to the oxidative damage inflicted by the mtbs with increased rates of recombination; this has allowed deleterious mutations to be eliminated from the nctb population, and benefited back the mtb by providing novel better-fit genetic **clones** to infect. Because the mtbs became more and more dependent on the nctb, and the nctb benefited from the extra ATP as well as higher rates of growth, division, and recombination, there must have been strong selection for maintaining such associations. In this way, not only did both partners benefit from the resolution of the conflict, but they also became dependent on each other (i.e. the association became an integrated symbiotic unit).

In **pathogen–host scenarios** (Rudel *et al*. 1996; Frade and Michaelidis 1997), conflict arises during the initiation phase. Accidentally engulfed pathogenic mtbs became surrounded by vacuolar membranes produced by the nctb; to ensure their release when the nctb's physiological state deteriorated,

the mtbs translocated porin-type membrane channels into the vacuolar membrane and secreted inactive caspase-type proteases into the nctb's cytosol (both porines and caspases are components of the eukaryotic apoptotic machinery). This exploitative association was maintained as long as enough catabolites were present in the nctb and available for the mtbs. When the concentration of catabolites/ATP dropped, the porins opened; this event triggered a series of changes culminating with the death of the nctb and the activation of proteases that eventually digested the nctb. This outcome allowed the mtbs to both leave a weakened/dying host as well as to take advantage of the nutrients released following the caspase activation. This resolution of the conflict was lethal for the nctb, and resulted in the dissolution of the association. The conflict became mediated through the evolution of proteins (such as adenine nucleotide translocators) that the nctb inserted into the mtb's membrane to "steal" ATP from them. As a consequence, the ATP level in the nctb stayed above the threshold that triggered the lytic mechanisms inflicted by the mtbs. Because the mtbs were "leaking" ATP, the association became obligate for such mtbs. Although the direct effects of the new interaction were detrimental for both partners (the nctb looses catabolites to the mtbs, and the mtbs loose ATP to the nctb), they did favor the association (i.e. exchange of catabolites for ATP and vice versa); ultimately the net effect became beneficial for the association relative to other free-living relatives. With the transfer of some genes (including the ones coding for caspases) from the mtb genome to the nctb counterpart, conflict mediation resulted in the metabolic and genetic integration of the two partners into a symbiotic unit.

Table 17.3 succinctly summarizes and compares several of the hypotheses for the origin of the first mitochondriate cell, with respect to the initial interaction, the conflictual stage and its mediation. Our main point is that regardless of the scenario, all these associations between the mtb and the nctb had to pass through a conflictual stage that needed to be mediated, much as we have observed and studied in the unicellular–multicellular transition (Michod 1996, 1997, 1999; Michod and Roze 1997,
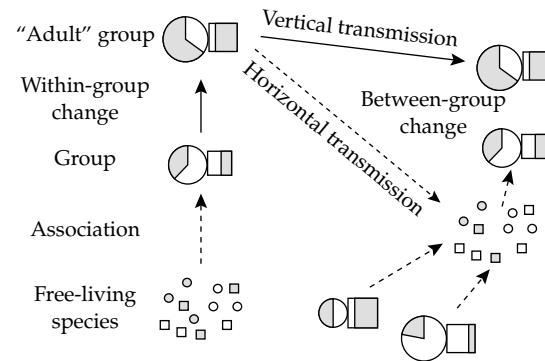


**Figure 17.5** Multilevel selection framework for mixed species groups. Two species are indicated by squares and circles. Within each species there are two phenotypes indicated by open and shaded shapes. A two species group is indicated by touching shapes; the size of each touching shape indicating the relative proportions of each species within the group and the shading of each shape indicating the relative proportion of the two phenotypes within each species in the group.

1999, 2000). Because conflict mediation is so central to these scenarios, and because the eukaryotic cell is a group of organelles (once independent cells themselves), we plan to extend our multilevel selection methods (Michod 1996), previously used to understand the origin of multicellularity and the origin of life, to study the transition from prokaryotic to complex eukaryotic organizations.

In Fig. 17.5 we extend the framework of Fig. 17.2 to the origin of the eukaryotic cell. During the transition from bacteria cells to eukaryotic cells, we assume that cells belonging to different species form groups as in Fig. 17.5. Kinship may exist among cells belonging to the same species but not between cells from different species. Initially the interactions between the two species need not be cooperative. The two different species (host and symbiont, predator and prey, two different syntrophic feeders, etc.), are indicated by squares and circles. Within each species there are two phenotypes (cooperate or defect, cultivate or exploit, etc.) indicated by open and shaded regions. A two species group formed by association of members from different species is indicated by the touching shapes; the size of each shape indicating the relative proportions of each species within the group
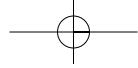
and the shading of each shape indicating the relative proportion of the two phenotypes within each species in the group. After group formation, members may reproduce or survive at different rates leading to within-group change. An additional factor leading to within group change is mutation leading to loss of cooperative cell phenotypes. The total change in frequency of different phenotypes within species and the relative proportions of different species is also affected by the between group change that occurs during horizontal and/or vertical transmission of group properties. Groups may break up and reform as in the case of horizontal transmission, or reproduce as groups in the case of vertical transmission.

## Conclusions

Recall that the basic problem in an evolutionary transition in individuality is to understand why and how a group of individuals becomes a new kind of individual, possessing heritable variation in fitness at a new level of organization. This transfer of fitness from lower to higher levels occurs through the evolution of cooperation and mediators of conflict that restrict the opportunity for within-group change and enhance the opportunity for between-group change. We have illustrated these principles with two major transitions, the origin of multicellular organisms and the origin of the first mitochondriate eukaryotic cell. Although the occurrence of mixed species groups in the case of the eukaryotic cell creates more opportunity for conflict (both ecological interactions and defection), the basic multilevel selection model of cooperation and conflict appears to provide an appropriate conceptual framework for understanding both these evolutionary transitions.