IN ADDITION TO THE GENES in their nuclei, the vast majority of eukaryotic organisms also have genes in their mitochondria (so-called mtDNA). Mitochondria are cellular organelles found in the cytoplasm and responsible for oxidative phosphorylation and much of the cell’s ATP production. That is, they convert food and oxygen into units of cellular energy. This alternative location for genes derives from the fact that mitochondria are descendants of bacterial endosymbionts and still contain their own DNA. Most importantly for the topic of this book, the replication and transmission of mitochondrial genes is fundamentally different from that of nuclear genes, which translates into differences in the pattern of selection. In the typical diploid eukaryotic cell, there are 2 copies of each nuclear gene, each one is replicated exactly once during the S phase of the cell cycle, and at mitosis each daughter cell receives 1 copy of each chromosome. The consequence is that, barring mutation or gene conversion, a heterozygous cell gives rise to heterozygous daughter cells—there is no segregation of nuclear alleles at mitosis, and no change in allele frequency. Birky (2001) describes the nuclear genome as “stringent.” By contrast, mitochondria have “relaxed” genomes: the number of genomes per cell varies widely (e.g., 20 to 10,000), different mitochondrial chromosomes can be replicated different numbers of times during the cell cycle (Clayton 1982), and daughter cells typically inherit a more-or-less random partition of the chromosomes. Thus, at mitosis there can be both segregation and changes in gene frequency. Even in nondi-
viding cells, mitochondria are continually replicating and dying, and allele frequencies can change over time. These differences greatly increase the scope for within-individual selection, both among cells and within cells, and therefore for the evolution of selfish mitochondrial variants that are harmful to the host organism, but are still able to spread and persist because of a within-individual transmission advantage in the germline (Eberhard 1980).

Another major difference between mitochondrial and nuclear genes is that, in most eukaryotes, mitochondria are usually transmitted only by one sex, usually the female. This difference has the opposite effect of the first, as uniparental inheritance greatly reduces within-organism variation and thus the opportunity for within-organism selection. But it also means that mitochondrial genes are selected only to increase female fitness, whereas nuclear genes are selected to increase male and female fitness equally. Thus, the different patterns of transmission (uni- versus biparental) lead to conflicts over investment in male versus female function.

Mitochondria are not the only ancient, vertically inherited, mutually obligate endosymbionts—for example, all plants have chloroplasts, and many insects have bacteria to aid their digestion. These too have “relaxed” modes of replication and are expected to show within-individual selection, and thus the opportunity for selfish variants to arise. But we focus in this chapter on mitochondria, as the oldest, most widespread, and best-studied example. We begin with a brief survey of mitochondrial genomics, because the size and content of their genomes should in some sense define their evolutionary potential. We then review (1) examples of within-individual mitochondrial selection; (2) the idea that uniparental inheritance evolved specifically to prevent such selection; and (3) the resulting conflict between nuclear and mitochondrial genes over allocation to male and female function, particularly as manifested in widespread male sterility in flowering plants. As we will see, though nucleus and mitochondria cooperate intimately in making a functioning organism and typically cannot live without each other, all is not peace and harmony.

**Mitochondrial Genomics: A Primer**

In the time since mitochondria were free-living bacteria, the most striking change in their genome has been the extreme loss of genes, either absolutely or by transfer to the nucleus (Lang et al. 1999). The total number of pro-
teins encoded by mitochondria ranges from 97 (in the free-living flagellate *Reclinomonas*) down to 3 (in malaria-causing *Plasmodium*). In some protists the process is thought to have gone to completion: there are no mitochondria, but there are other organelles called hydrogenosomes that appear to be derived from mitochondria but do not contain any DNA at all (Embley et al. 2003). In all species, the vast majority of proteins in mitochondria are encoded in the nucleus.

Among animals, mitochondrial genomes are relatively uniform, typically encoding 12–13 proteins, all involved in electron transport and oxidative phosphorylation, as well as rRNAs and tRNAs necessary for their synthesis. Most animal mitochondrial genomes are compact: human mtDNA, for example, is only 17kb in size, of which only 7% is noncoding. Somewhat larger mtDNAs (20–42kb) have been found in some insects, molluscs, and nematodes, but they do not encode more proteins.

Choanoflagellates are the sister group to animals, and the mitochondrion of at least 1 species is somewhat less degenerate, encoding 26 proteins. It is also less compact, at 77kb, of which 53% is noncoding. The main difference in gene content involves ribosomal proteins: the choanoflagellate mtDNA has 11 and animal mtDNA has none. This suggests that some streamlining of mtDNA occurred concomitantly with the evolution of multicellularity and tissue differentiation (Burger et al. 2003).

Fungi are like animals in that their mitochondria have also lost all, or nearly all, their ribosomal protein genes. However, while some are compact (e.g., 19kb, 11% noncoding in the fission yeast *Schizosaccharomyces pombe*), others are not (e.g., 86kb, 59% noncoding in the budding yeast *Saccharomyces cerevisiae*). Among plants, the mitochondria encode 40 proteins in some species, down to 24 in others (Adams et al. 2002). Again, most of the variation is in the ribosomal proteins, with some mitochondria encoding 14 and others none. At least 2 of these ribosomal protein genes have transferred repeatedly between distantly related plant species over evolutionary time, by unknown means (Bergthorsson et al. 2003). What evidence exists suggests that most of the losses are transfers to the nucleus. In general, plant mtDNAs are large and vary greatly in size, ranging from 200–2500kb, with the vast majority of this variation thought to be in intergenic regions (Palmer 1990).

All recent movements of functioning genes from the mitochondrion to the nucleus have taken place in plants or protists and, where the mechanism is known, have usually used reverse transcription from the mitochondrial
mRNA (e.g., Covello and Gray 1992, Grohmann et al. 1992, Wischmann and Schuster 1995). The intermediary step in all transfers is, presumably, active copies in both nucleus and mitochondria. Active mitochondrial and nuclear copies of cox2 have actually been observed in some legumes (Adams et al. 1999). In this situation, why might the nuclear gene often persist and the mitochondrial one not? We can imagine many possibilities. For example, if the gene was lost by deletion, selection for the deletion might be stronger in the mitochondria than in the nucleus, either because of selection within the individual for shorter mitochondrial genomes or because of selection among organisms to reduce DNA requirements by having 2 nuclear copies rather than thousands of mitochondrial copies per cell. Or the nuclear gene might be better for the organism because of a lower mutation rate or more precise transcriptional or translational control, or because it was not clonally inherited or would be selected to work well in both sexes. It would be interesting to know whether mitochondrial genes are typically silenced by nuclear genes before they disappear, or whether they disappear “of their own volition.”

Mitochondrial Selection within the Individual

“Petite” Mutations in Yeast

Selfish mitochondrial mutations have long been known in baker’s yeast, Saccharomyces cerevisiae (Ephrussi et al. 1955, Dujon 1981, Clark-Walker 1992, Chen and Clark-Walker 2000). In this species, mtDNA is biparentally inherited: the 2 gamete types (a and α) are of equal size, and they contribute mitochondrial DNA equally to the resulting zygote. Haploid cells contain about 25–50 copies of the 80kb mitochondrial genome, organized into 10–20 DNA-protein clusters called nucleoids (MacAlpine et al. 2000). If the mitochondrial genome is defective, the strain is unable to respire, but it can still grow and replicate by fermentation. Nevertheless, the cells replicate more slowly than normal, and so the resultant colonies are “petite” (designated ρ−). Such strains arise spontaneously at a surprisingly high frequency—about 1% per cell division. At the molecular level, many types of mitochondrial mutations can disrupt respiration and give a petite phenotype, but we especially expect to observe mutants that have a within-cell replication advantage, because most of the genomes in a cell must be mutant before respiration is knocked out. A within-cell replication advantage is
most easily observed by crossing petite and normal strains, propagating the progeny until they have only one kind of mtDNA and then scoring them. Many mutants have a within-cell advantage: with so-called hypersuppressive petites, more than 95% of the progeny end up petite (Fig. 5.1). Hypersuppressive petites typically are deleted for more than half of their mitochondrial genome, with the remaining fragments tandemly repeated to make up approximately the same size genome, but enriched for so-called ori sequences. These are thought to be cis-acting sequences that promote DNA replication (possibly by acting as origins of replication, or something analogous), and this is thought to give the hypersuppressive petite genomes their replication advantage (MacAlpine et al. 2001). Many of the deletions are thought to occur by recombination between specific 20–50bp GC-rich sequences, of which there are about 200 copies dispersed around the mitochondrial genome (Clark-Walker 1992).

Despite their transmission advantage, petite mutations are not able to spread in natural yeast populations because inbreeding is frequent (Johnson et al. 2004) and the mutations impose a large cost on the organism. Nevertheless, such observations raise the question of what determines the evolutionarily stable number of ori sequences in normal mitochondrial genomes. In *S. cerevisiae*, there are 7 or 8 such sequences, all similar in organization and primary sequence (Faugeron-Fonty et al. 1984). Curiously, only 4 of them are active, the others being inactivated by short insertions. As *S. cerevisiae* is largely inbred, the mitochondria inherited from the 2 parents will usually be identical, and selection for supernumerary ori sequences may have been relaxed. Other yeasts are different: *S. castellii*, for example, does not have recognizable ori sequences; and while it spontaneously generates petite mutations at about the same rate as *S. cerevisiae*, they are much less likely to be hypersuppressive (Petersen et al. 2002). In the more distantly related *Candida glabrata*, an asexual species, there are no GC-rich sequences dispersed around the mitochondrial genome, and petites are formed much less commonly, at a frequency of about $10^{-6}$ per cell division (Clark-Walker 1992, Chen and Clark-Walker 2000).

Within-Individual Selection and the Evolution of Uniparental Inheritance

Selfish mitochondrial genomes like the petite mutations of yeast have a replication advantage over normal mitochondrial genomes in within-organism
selection, but they lose out in conventional among-organism selection. Therefore, if a population is polymorphic for 2 mitochondrial types, one more selfish than the other, a nuclear gene that somehow reduces the efficacy of the within-individual selection will tend to become associated with the less selfish type, and so can increase in frequency due to among-organism selection. That is, there will be selection for nuclear genes that modify mitochondrial behavior so as to reduce the efficacy of within-organism selection (just as there is selection on nuclear genes to suppress drive at unlinked loci). Even in yeast, the opportunity for within-individual selection is limited by the rapid segregation of parental types, which is much faster than if the genomes were well mixed and independent. Mechanistically, this is due to the clustering of mitochondrial genomes into nucleoids, and due to

**Figure 5.1** Frequency distribution of transmission rates for spontaneous petite mutations in 2 species of yeasts. Mutant mitochondria showing drive (transmission greater than 50%) are more common in *Saccharomyces cerevisiae* than in *S. castelli*, but the reason for this is not known. Data from Petersen et al. (2002).
slow mixing in the zygote (Jensen et al. 2000). The evolution of these characters may have occurred partly to prevent selection for selfish mitochondrial mutants (Hurst and Hamilton 1992).

An even better way to limit within-organism mitochondrial selection is to ensure that only one parent transmits mitochondria to the next generation—that is, to impose uniparental inheritance (Grun 1976, Hoekstra 1990, Hastings 1992, Randerson and Hurst 1999). This is the normal pattern in eukaryotes, though the underlying mechanisms responsible are quite diverse (reviewed in Birky 1976, 1995). In animals, the egg is usually much larger than the sperm and contains many more mitochondria. For example, in *Xenopus* frogs, the ratio is about a million to 1 (10⁸ versus 10²). Crayfish sperm are reported not to have any mitochondria at all. Thus, purely as a matter of dilution, one would expect an overwhelming bias toward maternal inheritance. But in many species, even this is not left purely to chance. In mammalian spermatogenesis, proteins in the mitochondrial membrane are tagged with ubiquitin, a small protein that acts as a marker for protein degradation and recycling (Sutovsky et al. 2000). All components of this system are nuclear-encoded: the ubiquitin tag, the membrane proteins that are tagged, and the proteins applying the tag. The ubiquitin is apparently masked as the sperm travels through the male reproductive tract, but then is unmasked and amplified after fertilization. This is thought to lead to the destruction of paternal mitochondria at or before the 8-cell stage of pre-implantation development. The notion that males specifically tag their mitochondria for destruction is further supported by the observation that mitochondria from spermatids are destroyed if artificially injected into embryos, but not mitochondria from liver cells (which would have no need for a tag; Shitara et al. 2000).

The existence of such a mechanism is particularly noteworthy given that paternal mitochondria are in any case outnumbered by maternal mitochondria by a factor of 10³ to 10⁵ (Ankel-Simons and Cummins 1996). One possible contributing factor is that the mitochondrial mutation rate may be higher in the male germline than in the female germline, and so male mitochondria may, on average, be of lower quality. But even if paternal mitochondria were mostly defective, their low frequency in the fertilized zygote should mean that the selection pressure for destroying them is relatively weak, were it not for the possibility of within-individual selection. Examples of such selection in mammals are reviewed later; here we simply note that
the one known instance of paternal inheritance in humans was of a defective mitochondrion. A 28-year-old man with severe lifelong “exercise intolerance” was found to have maternally derived mitochondria in most of his body, but paternal mtDNA in his muscles; and the paternal mtDNA had a novel 2bp frameshift deletion in one of the genes that obliterated downstream function (Schwartz and Vissing 2002). (There was also recombination between maternal and paternal mtDNA in his muscles; Kraytsberg et al. 2004.)

Other animals also have mechanisms for the destruction of paternally derived mitochondria (reviewed in Birky 1976, 1995, Eberhard 1980). In some sea urchins, the paternal mitochondria appear to be destroyed in the egg. In honeybees, many sperm enter a single egg and about a quarter of all mitochondrial DNA in newly laid eggs is paternally derived; but the paternal mtDNA is degraded or replicates more slowly than the maternal mtDNA and, by the time the organism hatches out as a larva, the paternal mtDNA is undetectable. In the tunicate *Ascidea*, paternal mitochondria do not even make it into the egg, being shed before the sperm passes through the chorion membrane. Analogous mechanisms are also seen outside the animals. In the single-celled alga *Chlamydomonas reinhardtii*, the fusing gametes are of equal size, but only mitochondria from the “−” mating type gamete are inherited; those from the “+” parent are destroyed after gamete fusion. In another alga, *Derbesia tenuissima*, male gametes are smaller than female ones, and while the male gamete has a large and apparently functional mitochondrion, the DNA inside it is destroyed during gametogenesis, prior to fertilization (Lee et al. 2002). The slime mold *Physarum polycephalum* has a particularly baroque system in which mitochondrial transmission is determined by a linear hierarchy of alleles at the nuclear *matA* locus, with the “losing” mtDNA being actively degraded soon after gamete fusion (Kawano and Kuroiwa 1989, Meland et al. 1991). And, in many plant species mtDNA is either stripped from mitochondria or severely degraded during pollen maturation, prior to fertilization (reviewed in Mogensen 1996). For example, in 9 of 16 species from a range of families, mtDNA disappeared from the generative cells during anther development (Miyamura et al. 1987). Finally, in redwood trees and banana trees, mitochondria are paternally inherited (Neale et al. 1989, Fauré et al. 1994), which presumably goes against the numerical trend, suggesting that there is some active mechanism for destroying maternal mitochondria.
In short, uniparental inheritance in many species is not merely a function of numerical dilution. Mechanisms have evolved for the active elimination of the mtDNA from one parent. The only explanation plausible to us is that these mechanisms have evolved to suppress selfish mtDNA—that is, uniparental mitochondrial inheritance is a system evolved by the nucleus to ensure mitochondrial quality. Especially striking are mechanisms of uniparental inheritance in which one parent or gamete type somehow cripples its own mitochondria to keep them from being transmitted. The possibility that nuclear genes might evolve to suppress selfish mtDNA by sabotaging their own mitochondria (as opposed to those being transmitted by the other gamete) has been dismissed on theoretical grounds (Hastings 1992, Randerson and Hurst 1999), but the facts suggest this dismissal may be premature. The theoretical difficulty is that the population must be polymorphic for mitochondria varying in degree of selfishness in order to select for nuclear modifiers of mitochondrial inheritance, and in simple models the spread of selfish mitochondria does not easily evolve to a stable polymorphism—either they are lost or go to fixation. But there are two counterarguments. First, uniparental inheritance can protect against selfish organelles that are too harmful to go to fixation, but nevertheless arise repeatedly. The petite mutations of yeast are like this—recall they arise at a 1% frequency. In this case a stable intermediate frequency of the selfish mutant type is reached, analogous to conventional mutation-selection balance, and uniparental inheritance can be effective in reducing the probability of inheriting a mutant organelle (and reducing the equilibrium frequency of the mutant type). Second, if the organelle is only mildly selfish, such that heteroplasmy is often maintained from one meiotic generation to the next, with only a shift in frequencies, and if fitness declines more than linearly with the frequency of the selfish type, a polymorphism can be maintained and uniparental inheritance selected for. Formal modeling of both possibilities is clearly desirable.

What role do the mitochondria play in all this? They are hardly expected to be indifferent to their own destruction in the germline and will be selected to avoid this, if possible. But there does not appear to be any good example of such avoidance behavior. At first glance, the fact that paternal leakage is more common in some interspecific mouse matings than in intraspecific ones (Kaneda et al. 1995) seems suggestive of a coevolutionary arms race between nuclei and mitochondria. But this paternal leakage appears to
come about because the ubiquitin tagging and destruction system is disrupted in some hybrid matings (Sutovsky et al. 2000) and, as we have seen, this is a wholly nuclear-encoded system. Perhaps mutations allowing mitochondria to escape destruction just do not arise. Similarly, we are not aware of mtDNA from one parent being involved in the destruction of mtDNA from the other. In a sea urchin (Paracentrotus lividus), just minutes after the first cell division of a newly fertilized egg, large paternal mitochondria are seen surrounded by small maternal ones; their membranes touch and the paternal ones begin to disintegrate (Anderson 1968). But even if the maternal mitochondria are involved in the destruction of the paternal mitochondria, most of their proteins are encoded in the nucleus, and so this gives no compelling evidence of mtDNA involvement. Nevertheless, evidence for these sorts of direct action may yet be found—an obvious place to look would be in species that have recently changed from maternal to paternal inheritance. Presumably ancient mitochondria had more influence on their own transmission, before they became so degenerate.

The idea that uniparental inheritance evolved to prevent the spread of selfish mitochondria has been extended to binary mating systems (e.g., male/female; +/−; a/α). Perhaps these also exist in order to allow uniparental transmission (i.e., for 1 mating type to be a transmitter and the other not; Hurst and Hamilton 1992, Hurst 1995). Support for this idea comes from ciliates and mushrooms, 2 groups in which it is common for mating cells not to fuse, but instead to trade nuclei. There is thus no opportunity for mitochondria from the 2 parents to mix and compete. Consistent with the hypothesis, it is common in both groups not to have a binary mating system, but instead a multipolar system with many mating types (e.g., 30 or more).

But this explanation for binary mating types cannot be universal (Hurst 1995). Both cellular and acellular slime molds also have multipolar mating systems, and each has full gamete fusion. And ascomycete fungi typically have a binary mating system, but this has nothing to do with mitochondrial transmission. For example, in Saccharomyces yeasts, there are 2 mating types, but mitochondrial inheritance is biparental. In Neurospora crassa, there are 2 mating types, each of which can act both as a male parent and as a female parent, but mitochondria are inherited from the maternal parent, regardless of mating type. Instead of organizing mitochondrial inheritance, the binary mating system of ascomycete fungi is used to organize pheromone production and reception: the a mating type produces the a pheromone and has a
receptor for the $\alpha$ pheromone, and the $\alpha$ mating type produces the $\alpha$ pheromone and has a receptor for the $a$ pheromone. Such a division of labor is presumably needed to prevent one’s receptors from being clogged up with one’s own pheromone. It may be relevant in this context that many ciliates and mushrooms do not use dispersible pheromones to attract a mate. In the case of ciliates, the pheromones of some species remain attached to the cell membrane (Görtz et al. 1999). In mushrooms, pheromones are only activated after mating and are used to distinguish self from non-self (Casselton 2002). Perhaps the abandoning of dispersible pheromones is also important in the evolution of multipolar mating systems.

It has also been suggested that selection to kill off one’s own organelles could account for the small size of sperm and, more generally, the evolution of anisogamy (reviewed in Randerson and Hurst 2001). As a mechanism for ensuring uniparental inheritance, this would be terribly crude. While it might give some slight advantage to those making smaller-than-normal gametes, surely this is trivial compared to the more general advantage of reducing the cost of a gamete, and thus being able to make more of them (Randerson and Hurst 2001, Bulmer and Parker 2002).

Within-Individual Selection under Uniparental Inheritance

Even if uniparental inheritance is absolute and there is no paternal leakage, this does not wholly eliminate the possibility of selfish mtDNA increasing in frequency due to within-individual selection. Due to the “relaxed” nature of mitochondrial replication, variant mtDNA that arise by mutation during the lifetime of an organism may spread due to within-individual selection. At least in the simplest case—in which there is a mitochondrial bottleneck in the germline and females produce mostly homoplasminic eggs—the effect of within-individual selection is to change the “effective” mutation rate: mutants with a within-individual selective advantage will behave as if they have an elevated mutation rate, while mutants with a within-individual disadvantage will behave as if they have a depressed mutation rate. For recurrent mutations that are harmful to the organism, the classic equations of mutation-selection balance will apply (e.g., Crow and Kimura 1970), and the equilibrium frequency of the mutant is $\hat{q} = \mu_e/s$ where $\mu_e$ is the effective mutation rate (taking into account within-individual selection) and $s$ is the (among-individual) selection coefficient against the mutant. This formula applies if
$u, s$. When the within-individual selection is strong, or among-individual selection is weak, it may be that $u, s$ and the mutant will go to fixation in the population.

In principle, then, within-individual selection of harmful mitochondrial variants could increase the mutational load on the population, and nuclear genes will be selected to reduce this when possible. There are two ways they may do so. First, they could reduce the actual mutation rate. And there is some evidence this has happened: in mammals, DNA polymerase $\gamma$, encoded in the nucleus, is the enzyme responsible for replicating the mitochondrial genome, and it is among the least error prone of all DNA polymerases (ca. 2 errors per million base pairs; Gillham 1994). *In vitro*, it is 2 to 4 times less likely to make a base substitution than pol-$\alpha$, the enzyme responsible for replicating nuclear DNA, and more than 10-fold less likely to make a frameshift mutation (Kunkel 1985). Perhaps because of this high accuracy, pol-$\gamma$ is also among the slowest DNA polymerases, incorporating 270 nucleotides per minute per strand, 200 times slower than the polymerase of *E. coli* (Clayton 1982). Despite the greater accuracy *in vitro*, rates of silent substitution over evolutionary time are higher in mtDNA than nuclear, perhaps because of the unavoidable proximity to oxygen radicals produced in the mitochondria, or perhaps because some repair mechanisms are absent (Gillham 1994). In plants, the silent substitution rate in mitochondria is actually an order of magnitude lower than in the nucleus, which suggests a very accurate polymerase (or low damage from oxygen radicals).

Second, nuclear genes will be selected to create a cellular environment such that within-individual selection on mitochondria is aligned as closely as possible with among-organism selection. In modern day eukaryotes, nuclear genes are largely responsible for regulating mitochondrial replication and destruction, and the manner in which they do so will determine the mitochondria’s selective environment. The results can be counterintuitive (Chinnery and Samuels 1999, Birky 2001). For example, consider the apparently sensible possibility that nuclear genes regulate mitochondrial copy number according to the local needs for oxidative phosphorylation: the more ATP required, the more mitochondria are produced. Though perhaps satisfactory in the short term, the consequence of this strategy is the selection of ever less efficient mitochondria. To understand this, note that under this scenario an abundance of ATP will inhibit mitochondrial replication. Suppose there are two types of mitochondria in the cell, one more efficient
than the other. Then an extra copy of an efficient mitochondrion will inhibit further mitochondrial replication more than an extra copy of the inefficient mitochondrion. Consequently, the cell will gradually come to be populated by the inefficient mitochondria—not necessarily what the organism wants, either for itself or to pass on to its offspring!

There is compelling evidence for within-organism selection in mice that have been artificially made to carry 2 types of mitochondria. In one set of experiments, mice were made heteroplasmic for mitochondria from 2 lab strains, NZB and BALB (Jenuth et al. 1997, Battersby and Shoubridge 2001). In hundreds of mice, in a variety of different nuclear backgrounds, the NZB mitochondria increase in frequency in the liver as the mouse ages, with a selection coefficient of about 14% per mitochondrial generation (Fig. 5.2). NZB mitochondria also increase in frequency in the kidneys. Surprisingly, selection is reversed in blood and spleen, where the BALB mitochondria come to predominate. Finally, in other tissues, there is no apparent selection. The molecular basis of the selection is unknown: there are only 15 amino acid differences in all the proteins coded by the 2 mitochondria, and there is no detectable difference in respiratory efficiency. Battersby and Shoubridge (2001) suggest that the differences are in death rates of the 2 types, but this needs confirmation.

People with mitochondrial diseases are typically heteroplasmic for defective mitochondria (homoplasmy would be lethal), and these too can show tissue-specific patterns of selection (Chinnery and Turnbull 2000, Chinnery et al. 2002). In particular, the defective mutants are typically lost from rapidly dividing tissues such as bone marrow, but may accumulate in nondividing cells such as skeletal muscles and the central nervous system (recall that, unlike nuclear DNA, mtDNA is continually degraded and replaced even in these nondividing cells). Consequently, mitochondrial diseases tend to be progressive and to most affect metabolically active, nondividing tissues. The differences observed between proliferative and nonproliferative tissues may simply be due to differences in the relative importance of within-cell selection favoring the defective mitochondria (e.g., because the cell regulates mitochondrial copy number based on ATP requirements) and among-cell selection favoring wildtype mitochondria (Taylor et al. 2002).

Studies of cultured human cells suggest that, at least under some circumstances, there can also be within-cell selection for shorter mitochondrial genomes. First, if mitochondrial copy numbers are artificially repressed 90%
by ethidium bromide treatment and then allowed to recover, mitochondrial genomes with a 7.5kb deletion (≈ 45% of the total length) recover in number more rapidly than do wildtype genomes in the same cells (Diaz et al. 2002). This difference may occur simply because in this environment mito-

Figure 5.2 Changes in the frequency of a mitochondrial genotype with age. Mice were made heteroplasmic for mtDNA from 2 lab strains, NZB and BALB, and then the frequency of the 2 types monitored by single-cell PCR of liver cells. Data are from 2 representative mice at each age. The increase in the frequency of NZB mtDNA with age indicates a selective advantage within the organism. Adapted from Battersby and Shoubridge (2001).
chondria are being replicated by the cell as fast as possible, and shorter genomes are more rapidly replicated. Second, the equilibrium or steady-state amount of mitochondrial DNA per cell was found to be identical in cultures homoplasmic for wildtype genomes, those with a 7.8kb deletion (= 47%), and those with an 8.8kb duplication (= 53%; Tang et al. 2000). These results suggest that nuclear genes sometimes regulate mitochondrial copy number based on the total amount of mtDNA (by, for example, limiting the pool of nucleotides available for mitochondrial replication). This will select for shorter, more compact genomes.

Of all tissues, selection in the female germline will be particularly important for mitochondrial evolution. In mammals, the studies published to date do not find as dramatic within-individual selection in these cells as is found in some somatic tissues (Jenuth et al. 1996, Chinnery et al. 2000, Inoue et al. 2000). It would be interesting to compare rates of mitochondrial turnover in oocytes with those in, say, skeletal muscles. One possible sign of substantial selection in the female germline is the curious pattern that in humans, mitochondrial diseases due to a point mutation may be passed on from one generation to the next (heteroplasmically), but diseases due to a deletion are not (Chinnery and Turnbull 2000). In both cases, the ATP-producing function of the mitochondria can be completely knocked out. The mechanistic basis of this difference is not known, but one possibility is some sort of differential selection in the female germline.

Another possible indication of selection in the female germline is the extreme compactness of the mitochondrial genome in mammals, and animals more generally. This is consistent with the possibility that at some point in the female germline, mitochondrial copy numbers are regulated by the total mass of mtDNA (Birky 2001). Plant mitochondria are anything but compact, and the same logic would predict that copy numbers in plants are regulated by some other means.

Finally, selection in the female germline has been experimentally demonstrated for naturally occurring mitochondrial variants of Drosophila simulans that differ in sequence by 2–3% (de Stordeur 1997). In these studies, females were made heteroplasmic by microinjection, but in some populations of D. simulans heteroplasmic females occur naturally at an appreciable frequency (1–12%; James and Ballard 2003). This suggests at least occasional paternal leakage of mtDNA, which increases the importance of germline selection in mitochondrial evolution. In other species (e.g., rabbits, Oryctolagus cunicu-
most or all females are heteroplasmic for length variation, but the relative importance of high mutation rates and different forms of selection in maintaining this polymorphism has yet to be clearly distinguished (Casane et al. 1994).

DUI: Mother-to-Daughter and Father-to-Son mtDNA Inheritance in Mussels

We conclude our review of within-individual mitochondrial selection with a description of the peculiar situation in mussels and their relatives, in which mitochondria are typically passed father-to-son and mother-to-daughter. This so-called doubly uniparental inheritance (DUI) was first discovered in species of the marine mussel *Mytilus* (Zouros et al. 1992, 1994a, 1994b, Skibinski et al. 1994). DUI was soon shown in the closely related *Gukensia demissa* (Hoeh et al. 1996), as well as freshwater mussels of the family Unionidae, separated by more than 400 million years (Hoeh et al. 1996, Liu et al. 1996). Doubly uniparental inheritance has also recently been discovered in a clam (Passamonti and Scali 2001, Passamonti et al. 2003) so the trait may be ancestral in the evolution of the bivalves. (By contrast, in *Cepea nemoralis*, a related land snail with a high degree of mtDNA variation, there is no evidence of deviation from pure maternal inheritance; Davison 2000.)

Females display the mtDNA from their mothers only, while males are heteroplasmic. In somatic tissue males show both forms, but in the testes the paternal mtDNA predominates and most males pass on exclusively (or almost exclusively) the paternal set. Thus, 2 forms of conspecific mtDNA, F (female) and M (male) mitotypes, evolve side by side in almost complete isolation and they may show extensive sequence divergence—for example, 10–20% in *Mytilus*, depending on locality and species (Rawson and Hilbish 1995, Stewart et al. 1995).

The 2 sexes are believed to start life with the same relative numbers of maternal and paternal mitochondria—in other words, 5 very large paternal mitochondria and tens of thousands of maternal ones (Cao et al. 2004). Subsequent adult distributions are known only for *M. edulis* (Garrido-Ramos et al. 1998; see also Dalziel and Stewart 2002). Most females (~70%) contain no M mitotypes and, where these are found, they are found in small amounts in some tissues, varying between individuals. A few females (~5%) appear to show small traces of M in the gonads, but it is almost impossible to study
germ cells uncontaminated by nurse cells and there exists no clear evidence of M mtDNA in unfertilized eggs. By contrast, in males M mitotypes predominate in testes, while Fs do so in all other tissues (Stewart et al. 1995, Garrido-Ramos et al. 1998). The most sensitive tests show M mitotypes in all tissues of all individuals and suggest a regular pattern of M mitotype abundance as follows: testes >> adductor muscle, digestive gland > foot, gill, and mantle. By maturity, M mitotypes so predominate in testes that Fs are virtually undetectable. On the other hand, in nature and the lab there are rare males whose maternal and paternal mitotypes both resemble the typical F form (reviewed in Cao et al. 2004).

How are these patterns achieved? And how do the 5 paternal mitochondria in males manage to overwhelm tens of thousands of maternal ones, at least in testicular tissue? Recent evidence suggests how this may happen (Cao et al. 2004). In females, the 5 paternal mitochondria are randomly dispersed in the cytoplasm and appear to go randomly to descendent cells (in early cells, without replication). By contrast, in males paternal mitochondria aggregate themselves immediately in the fertilized egg and (without replication) they pass together into the first cleavage cell leading to the germplasm. In the first 5 cell divisions, these mitochondria remain aggregated and pass preferentially to 1 daughter cell at each cleavage (Plate 3). Presumably, this is always the germinal cell. Because both germinal and adductor muscle cells are mesodermal, these findings also provide an explanation for elevated frequency of paternal mitochondria in adductor cells.

Male mitotypes evolve more rapidly than do female. That is, nonsynonymous substitutions are significantly higher in M mitotypes than in Fs, and the ratio of nonsynonymous to synonymous substitutions is more than twice as high in Ms (Stewart et al. 1996). This suggests that faster M evolution results neither from more frequent replication during male gametogenesis nor from greater damage to sperm mitochondria by free radicals, because these effects should act on synonymous and nonsynonymous sites equally (Stewart et al. 1996). A more plausible explanation is that F mitotypes are fully exposed to selection every generation in females, while M mitotypes are shielded from the direct action of selection by the overwhelming presence of F throughout the male body (Saavedra et al. 1997). That less functionally constrained organelles will show more rapid evolution is suggested by the rapid evolution of chloroplast DNA in nonphotosynthetic plant species (Wolfe et al. 1992). Male mitotypes may also be sub-
ject to greater directional selection through sperm competition, which is known in bivalves to impose strong, directional selection on nuclear DNA, primarily on sperm proteins but also on egg receptors (Yang and Bielawski 2000, Galindo et al. 2003).

F mitotypes sometimes colonize the male lineage and displace the existing Ms—a striking fact that can be seen in *Mytilus*, in which some Ms are more related to Fs than to other conspecific Ms (Hoeh et al. 1997). In one population of *M. galloprovincialis* from the Baltic Sea, the majority of males lack an M type, suggesting a very recent invasion of M types (Ladoukakis et al. 2002). (In this species, the F genome is more variable within populations and the M between populations.) Takeover of Ms by Fs may also occur between species. In European *M. trossolus*, most of the M types come from Fs of *M. edulis*. Apparently, recently introgressed F molecules first displaced the *M. trossolus* F and then were able to colonize males and displace the local M (Quesada et al. 1999, Quesada et al. 2003). Many males are heteroplasmic for 2 paternal mtDNA types and 1 maternal form, so that cotransmission by males of 2 forms must be a common occurrence. mtDNA variants often differ by length, with the short-length variants preferably transmitted by males (Zbawicka et al. 2003).

A very similar system is found in a freshwater mussel *Anodonta grandis* (Liu et al. 1996). Mitochondrial inheritance is doubly uniparental and in different locales female mitotypes hardly differ (~0.4% sequence divergence) while male mitotypes differ by 11.5%. But unlike the Mytelidae, the Unionidae show no evidence that F mitotypes ever replaced M mitotypes and the split between the 2 seems to be very ancient, at least 200mya (Curole and Kocher 2002, Hoeh et al. 2002).

Why has DUI evolved—and why in the bivalves? One possibility is provided by considering sex-antagonistic effects of mtDNA, effects that are negative in one sex and positive in the other. If such effects are large in bivalves, especially in the gonads, DUI permits mtDNA to specialize in 2 types, each adapted to 1 of the sexes. Sexual dimorphism in clams and mussels is known only from gonadal tissue. Suppose in an early bivalve there was some small amount of mitochondrial transmission from fathers to offspring (“paternal leakage”) and a mutation arose that was strongly beneficial in testes or sperm, but weakly deleterious in other male tissues and in females. The mutation could increase in frequency to some intermediate level. At that point the population would be polymorphic for 2 types of mi-
tochondria, and nuclear genes would be selected to arrange the internal cellular environments so the new mitochondria would be selected for in testes and the original mitochondria would be selected for in all other tissues. Note that morphology today is suggestive of extreme sexual dimorphism: 5 large paternal mitochondria versus thousands of small, maternal ones (Cao et al. 2004). From the mussel’s standpoint, deriving its paternal mitochondria from previous paternal ones may give a better version than having to differentiate them anew each generation from maternal mitochondria. If this argument has any merit, perhaps other species with highly dimorphic (egg/sperm) mitochondria also show DUI.

Thus we imagine that an ancestral bivalve evolved DUI in response to strong selection on male mtDNA for novel functions connected to intense sperm competition. Because there is no sexual dimorphism outside the gonads, selection on the newly emergent M mitotype may favor little or no representation within somatic tissue.

The mtDNA of *Mytilus* has a number of unique features. It is unique among animals (along with nematodes) in lacking ATPase-8, in having an extra tRNA for methionine (one using an anticodon, TAT) and in having a unique gene order that shows no homology with other coelomate metazoans (Hoffmann et al. 1992). Could these facts be related to DUI? One possibility is that DUI leads to more recombination, which increases the rate of evolution. Recombination in males has been confirmed in both *M. galloprovincialis* and *M. trossulus*, and evidence suggests a high rate in both (Ladoukakis and Zouros 2001, Burzynski et al. 2003). But for this to affect rate of F evolution, there must be at least occasional paternal leakage in females. Another possibility is that heteroplasmy in males also encourages conflict between the maternal and paternal lineages, which has the potential to greatly accelerate rates of genomic change.

How is sex determined? In principle, mtDNA itself could determine sex—for example, when male mitotypes predominate in the gonads, these become testes (Zouros 2000). But if this is how sex determination is achieved, how is the 50:50 sex ratio that the autosomes would prefer enforced? Mitotype frequencies, and thus sex, should evolve to be under autosomal control, even if they begin under mitochondrial. In any case, recent genetic evidence demonstrates that the sex ratio is under maternal, nuclear control in blue mussels (Saavedra et al. 1997, Kenchington et al. 2002). In 2 species of
Mytilus, sex ratios are highly variable from one pair to another, and the variation is associated with the mother and not the father. This variation is heritable in a manner that excludes mtDNA as the controlling element. Instead, the nuclear genome of the mother determines sex of progeny, perhaps through alleles at a single locus. A noteworthy feature of sex ratio variation is that all-female families are common but all-male families are uncommon and majority-male families are found instead. It will be most interesting to learn exactly how sex determination operates in these species, both genetically and developmentally.

Cytoplasmic Male Sterility

Uniparental Inheritance Implies Unisexual Selection

Though uniparental inheritance has the advantage (from the nuclear point of view) of suppressing within-individual mitochondrial selection, it introduces a new danger. If mitochondria are transmitted only by one sex, all that will matter for their evolution is how well they perform in that sex, with performance in the other sex being irrelevant. For example, in a species with maternal transmission, if there are recurrent deleterious mutations that cause only mild harm to the female, the mutant class can reach relatively high frequencies, even if the harm to the male is substantial (Frank and Hurst 1996). Something like this may be happening in humans (Ruiz-Pesini et al. 2000). In a survey of mitochondrial genotypes of couples at infertility clinics, one relatively common mitochondrial haplotype (“T”) was found to be associated with moderately reduced sperm motility (asthenozoospermia; Fig. 5.3), apparently because this haplotype is slightly less efficient at oxidative phosphorylation and ATP production. If, as seems plausible, sperm motility is among the most sensitive physiological parameters to ATP production, this slightly reduced efficiency may have much less negative effect on female fitness (or could even be beneficial), and as a result the haplotype may have reached higher frequencies than it otherwise would have. Among Caucasian populations, the frequency of haplotype T ranges from 4% in the Druze, to 12% in Germans, to 22% in Swedes. These are the kinds of effects that would select for nuclear genes to become ever more involved in mitochondrial functions.
Disproportionate Role of mtDNA in Plant Male Sterility

The dangers of uniparental inheritance are even more clear in flowering plants. Most plants are hermaphrodites—in other words, produce both pollen and ovules—but transmit their mitochondria only through ovules, and so mitochondrial mutants that completely abolish pollen production, causing cytoplasmic male sterility (CMS) will be positively selected if, in so doing, there is even a slight increase in ovule production (Lewis 1941). Two ways mtDNA might do this are (1) allowing some scarce resource(s) to be reallocated from pollen to ovule production, or (2) preventing self-fertilization and thus inbreeding depression. By contrast, nuclear genes are more willing to tolerate inbreeding depression because self-fertilization is associated with increased gene transmission. Because nuclear genes are transmitted equally through pollen and ovules, a nuclear gene causing male sterility will spread only if it more than doubles female fertility. Therefore, the spread of a CMS gene usually selects for nuclear suppressor genes that counteract the CMS gene and restore male fertility. Thus, the evolution of uniparental inheritance sets up a powerful conflict of interest between mito-
The result of this conflict is widespread male sterility. Even as of 1972 (the last tally of which we are aware), CMS had been described in 140 species, within 47 genera, from 20 families (Laser and Lersten 1972). It occurs in 2 different situations. First, some plant populations have 2 kinds of individuals, hermaphrodites and male-steriles (i.e., females). Such populations are said to be gynodioecious. Gynodioecy itself has been described in 350 species from 39 families (Table 10.1 in Kaul 1988) and, among European flowering plants, is the second most common gender system, after hermaphroditism (7.5% versus 72%; Richards 1986). In most gynodioecious species that have been investigated, male sterility is at least partially maternally inherited (Sun 1987 and references therein). And, even within a single species, there are often multiple CMS genotypes (Kaul 1988, Frank 1989). In maize, for example, there are 3 distinct CMS genotypes (T, S, and C), as there are in rapeseed (pol, nap, and ogu). The inheritance of male sterility usually has a nuclear component as well, indicating that nuclear restorers are also common.

The second situation in which male sterility is common is in the descendants of crosses between hermaphrodites, either from different species or from different populations of the same species. The male-sterile progeny are sometimes seen in the first generation and sometimes in subsequent generations of hybrid crosses or backcrosses. And, in hybrids too, cytoplasmic inheritance of male sterility predominates (Table 5.1). Presumably this occurs because, in at least 1 of the species, a CMS gene has arisen and its restorer has swept to fixation, reestablishing hermaphroditism, and male sterility is only uncovered when the 2 genes are dissociated (Box 5.1). That is, hermaphrodites are restored male-steriles. The data in Table 5.1 suggest that

<table>
<thead>
<tr>
<th>Type of Cross</th>
<th>Nuclear</th>
<th>Cytoplasmic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within species</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Within genus</td>
<td>13</td>
<td>41</td>
</tr>
<tr>
<td>Within family</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

From Kaul (1988, Tables 2.2, 3.2–3.4).
Coevolution of CMS Genes and Nuclear Restorers: An Example Trajectory

The figure in this box shows the fate of a CMS gene that increases ovule production by 20%, introduced at a frequency of $10^{-6}$ into a population with a selfing rate of 20%. Initially it increases in frequency. But as it becomes more common, pollen becomes less available, and male-steriles suffer more from pollen limitation than do hermaphrodites because they are unable to self. Therefore the CMS gene does not go to fixation, but instead reaches some intermediate equilibrium frequency (in this example, pollen limitation is modelled by assuming that the probability of a non-selfed ovule getting fertilized is proportional to the frequency of hermaphrodites). Then, in generation 100 a nuclear restorer gene is introduced, which spreads rapidly and goes to fixation. As it does so, the frequency of hermaphrodites, and thus the amount of pollen, increases, thus allowing the CMS gene also to increase in frequency. In the example shown here, the nuclear restorer goes to fixation before the CMS gene, with the result that the population is entirely hermaphroditic and there is a hidden mitochondrial polymorphism. The fate of the CMS gene then depends on its relative female fitness in the presence of the restorer, selection at linked loci, and drift.
If the CMS gene reaches an appreciable frequency, selection on nuclear restorers will be intense, meaning that they can spread even if they have negative side effects. But a restorer with negative side effects may not go to fixation, in which case the population will remain gynodioecious. If, at the same time, the CMS gene goes to fixation, the inheritance of male sterility will appear to be nuclear rather than maternal. Or, if both the CMS gene and the restorer have negative side effects, the population may remain polymorphic at both loci. In computer simulations, gene frequencies can show complicated dynamics over time. The coevolutionary dynamics of CMS genes and nuclear restorers has been modelled extensively (Lewis 1941, Lloyd 1976, Charlesworth and Charlesworth 1978, Charlesworth and Ganders 1979, Delannay et al. 1981, Frank 1989, Gouyon et al. 1991, McCauley and Taylor 1997, Bailey et al. 2003, Jacobs and Wade 2003).

this is not a rare event, though exactly what fraction of species pairs shows CMS on hybridization is not known.

Note that these findings do not occur because male fertility is somehow especially sensitive to mitochondrial dysfunction: induced mutations causing male sterility have turned out to be nuclear in 37 species and cytoplasmic in only 4 species (Tables 2.3 and 3.5 in Kaul 1988). As of 1994, over 20 nuclear genes that can mutate to male sterility were known in maize, compared to the 3 CMS genotypes (Gabay-Laughnan and Laughnan 1994). Note too that, as expected, male sterility is more common than female sterility. There are only 7 well-documented cases of species with mixed hermaphrodite and male individuals (“androdioecy”; Vassiliadis et al. 2002), and we are not aware that hybridization of hermaphroditic plants often leads to female sterility. (That said, it would be interesting to look for this in the few taxa with paternal transmission of mitochondria, and test for paternal transmission of mitochondria in the few species with androdioecy.)

CMS may be the tip of the iceberg in terms of cytoplasmic attempts to influence sex allocation or the mating system. A reduction in pollen pro-
BOX 5.2

The Uses of CMS in Hybrid Seed Production

Many agricultural crops are hybrids: the seeds planted in the field are derived from parents of 2 different, carefully selected lines. Most crops are self-compatible, and so it is not easy to produce hybrid seed without some selfed seed mixed in. CMS genes have been very useful in solving this problem (Wise and Pring 2002). One of the parental lines is bred to carry a CMS gene and so does not produce any pollen. It is then grown adjacent to the other line, which acts as the pollen donor, and hybrid seed is collected from the CMS line. For crops like sugar beet that are grown for their vegetative parts, it does not matter if the $F_1$ hybrid is also male-sterile. But for crops like corn from which the seed is harvested, the $F_1$ hybrid must produce pollen, and so the pollen donor line must be homozygous for a nuclear restorer. CMS is known from such crops as beet, carrot, onion, maize, sorghum, rice, rye, sunflower and wheat (Schnable and Wise 1998).

The most famous CMS genotype is the T-cytoplasm of maize, which was discovered in the 1940s. At the time, corn breeders could prevent self-fertilization only by laboriously removing tassels from the plants by hand. By the 1960s the T-cytotype came to dominate the worldwide corn seed industry. But it was susceptible to a new strain of the southern leaf blight fungus (*Cochliobolus heterostrophus*), and in 1970 an epidemic destroyed more than 700,000,000 bushels of corn and wiped out 17% of the plants in the United States alone, thus ending the use of T-cytoplasm in seed production (Tatum 1971, Ullstrup 1972). T-cytoplasm maize is also vulnerable to attack by a second fungus, *Mycosphaerella zeae-maydis*, for much the same reason. The CMS gene, T-urf13, encodes a protein that assembles in the inner mitochondrial membrane, and the fungi produce toxins that bind to the protein in a way that causes the mitochondria to leak. Levings (1990) speculates that there is some anther-specific substance that also binds to the URF13 protein, causing the mitochondria to leak, and this is how the CMS dysfunction is limited to anthers.
duction of 10% would be difficult to observe. One would like to see careful comparisons of hybrids and allotypes (individuals with organelles from one species and nuclei from another) with the parental species in terms of pollen and seed production, flower size, timing of anthesis, and so on.

Mechanisms of Mitochondrial Action and Nuclear Reaction

The mechanistic basis of CMS has been relatively well studied, particularly in crop species, in which CMS genes are especially useful in producing hybrid seed (Box 5.2). But we still do not have a clear picture for any species of exactly how it works. Morphologically, CMS manifests itself in different species in almost every conceivable way, including the complete absence of male organs, meiotic failure, the abortion of pollen at any step in its development, failure of dehiscence, and failure of pollen germination (Laser and Lersten 1972, Budar and Pelletier 2001). Even within a species, different CMS systems can produce completely different morphologies. In carrots, for example, there is a “brown anther” type of CMS, in which pollen growth ceases prior to maturation, and a “petaloid” type in which petals or petal-like structures form instead of stamens and anthers (Nakajima et al. 1999). Homeotic-like transformation of stamens into (sterile) pistils (female structures) is known in wheat, and floral malformations also occur in tobacco (Bereterbide et al. 2002, Murai et al. 2002; Plate 4; see also Fig. 5.4).

In many species the dysfunction is thought to originate in the tapetum, the specialized tissue surrounding the developing pollen grain (Schnable and Wise 1998). All nutrients used by the developing pollen grain must pass through the tapetal layer, which acts as a storage organ and also synthesizes many parts of the pollen wall (Conley and Hanson 1995). The tapetal cells, in turn, are relatively rich in mitochondria (Lee and Warmke 1979, Balk and Leaver 2001). In sunflowers, the death of tapetal cells and meiocytes associated with CMS has recently been shown to be due to the initiation of a programmed cell death pathway (Balk and Leaver 2001). This is interesting because mitochondria are well known to be involved in programmed cell death pathways (at least in mammals and yeasts), via the release of cytochrome c into the cell cytoplasm. In addition, cell death is essential for the normal development of fertile anthers: tapetal cells eventually have to lyse and release lipid compounds that coat the pollen; other cells die and shear to allow pollen release; and still others die to provide the spring mechanism.
Figure 5.4 Hermaphrodite (H) and male-sterile (MS) flowers in *Plantago lanceolata*. The 2 different male-sterile types are caused by different mutations. Development of MS1 flowers is normal up to the stage of anther differentiation, resulting in a flower in which only the stamens are affected. Filaments are short and anthers, protruding just outside the corolla tube, rapidly turn brown. Pollen sacs can be discerned, but they do not contain any pollen. Development of MS2 flowers is even more aberrant, and in the extreme form stamens are completely absent. Sometimes the style cannot leave the narrow corolla and gets coiled up inside, possibly reducing the chance of pollination. On the other hand, pistils of these flowers occasionally have 3-seeded capsules, compared to the normal 2. Adapted from Van Damme and Van Delden (1982).
for dehiscence. CMS in sunflowers may simply result from the premature activation of programmed cell death pathways necessary for normal male fertility. In some other species, CMS may result from the mitochondria failing to induce normal cell death.

The genetic basis of CMS has been uncovered in about a dozen cases. In each one, CMS is due to a novel protein-coding gene in the mitochondria that has been formed by genomic rearrangements bringing together fragments of other genes (reviewed in Schnable and Wise 1998, Budar and Pelletier 2001, Budar et al. 2003; Fig. 5.5). For example, male sterility in the T- cytoplasm of maize is due to the T-urf13 gene, which is a chimera of part of the atp6 gene, the 3′ flanking region of the 26S rRNA gene, an unidentified sequence, and part of the coding region of the 26S rRNA gene. Male sterility in Petunia is caused by the pcg gene, which is a chimera of atp9, coxII, and an unidentified sequence. CMS genes in rapeseed, sunflowers, sugar beets, and carrots all contain fragments of atp8 (= orfB; Nakajima et al. 2001). Typically, the CMS-causing gene is close to and cotranscribed with an essential mitochondrial gene, and it encodes a protein that binds to the mitochondrial membrane. In most species the gene is expressed in all tissues, but we do not know how the phenotypic effect is limited to pollen development (Budar et al. 2003). One possibility is that the CMS protein interacts with some unknown “factor X” that exists only in the male reproductive tissues to produce the harmful effects. Alternatively, the CMS genes may simply cause a slight decrease in mitochondrial efficiency, to which the male reproductive tissue is uniquely susceptible. Given that CMS mutants can only spread if they increase female fitness and many CMS types are used in crops selected for high productivity, any overall decrease in mitochondrial efficiency is probably slight.

The genetics and mode of action of nuclear restorer genes have also been investigated (reviewed in Schnable and Wise 1998, Budar et al. 2003). Most of these appear to interfere with the processing of the CMS mRNA after transcription (recall that most CMS genes are cotranscribed with a nearby essential gene). In species with multiple CMS types, each of the different types is suppressed by a different restorer. For example, in maize, cms-T is restored by Rf1 and Rf2; cms-S is restored by Rf3; cms-C is restored by Rf4; and each of these restorers is found at a different locus. Interestingly, in rapeseed (Brassica napus), the nap and pol CMS types have different restorers, but they map to the same place in the genome and may be allelic (Li et al. 1998).
Such specificity of CMS and restorer genes can help maintain polymorphism in natural populations (Frank 2000). On the other hand, there is also redundancy: more than 1 nuclear gene can suppress a particular CMS gene. For example, $Rf^8$ and $Rf^{*}$ are genes in maize that can, at least in part, substitute for $Rf^1$ to restore fertility (Dill et al. 1997). All 3 genes mediate T-$ruf$ transcript accumulation. Nuclear restorers are typically dominant, but can act either sporophytically or gametophytically—that is, if the plant is heterozygous for the restorer, either all the pollen will be functional or only those pollen grains that have inherited the restorer gene. Restorer genes for the S-cytoplasm of maize are gametophytic (Kamps et al. 1996). Restorer genes have been cloned from *Petunia* and radish plants, and in each case they contain multiple copies of a 35–amino acid pentatricopeptide repeat (PPR), a motif thought to bind RNA (Bentolila et al. 2002, Koizuka et al. 2003).

**Figure 5.5** The chimeric structure of CMS genes. Shown are 6 different CMS genes, each of which is derived from fragments of 1 or more normal mitochondrial genes and regions of unknown origin (indicated by the differential shading). These genes presumably arose by recombination. Also shown are the normal mitochondrial genes with which the CMS genes are cotranscribed. Adapted from Budar and Pelletier (2001).
CMS and Restorers in Natural Populations

The high frequency of male sterility in hybrids between populations or species (Table 5.1) suggests that CMS is often a transient phenomenon: a CMS mutant arises, spreads through a population, and selects for a nuclear restorer, which goes to fixation, leaving the population back where it began, fully hermaphroditic. The underlying tension is then only revealed when mismatched CMS and nuclear elements are thrown together in crosses between populations. Unfortunately, the phenomenon of transient sweeps has not been studied by evolutionary geneticists using modern molecular techniques, and so our information about it is largely anecdotal. Such successive sweeps happen—sometimes. This is a research opportunity crying out for study. One promising system has recently been described by Barr (2004): Nemophila menziesii (Hydrophyllaceae) populations are typically hermaphrodite, but females are found in a hybrid zone between white- and blue-flowered morphs, and experimental crosses between colors produce a higher frequency of females than those within morphs.

What has attracted more research are species with apparently stable gynodioecy, that is, coexistence of hermaphrodites and male-steriles. Three genera in particular have been well studied: Plantago (Plantaginaceae), Thymus (Labiatae), and Silene (Caryophyllaceae). In each genus, gynodioecy is widespread: in Silene, for example, there are several hundred species and gynodioecy is likely to be the ancestral gender system (Desfeux et al. 1996). Thymus also has many gynodioecious species and no fully hermaphroditic ones (Manicacci et al. 1998). Gynodioecy in these species may therefore be relatively ancient.

In each of these genera, the genes responsible have yet to be identified, but experimental crosses suggest that the underlying genetics of male sterility is often complex. In P. lanceolata, 3 different CMS types are known (de Haan et al. 1997a, 1997c) and, for the most common one, there appear to be 5 different loci segregating in natural populations for restorer alleles, 2 dominant and 3 recessive (Van Damme 1983). For a less common CMS type, there are 3 dominant restorer alleles, each at a different locus. In S. vulgaris,
there are 3 different CMS types: for one there were no restorers detected in the population; for another there were 2 dominant epistatic restorers (i.e., restorer alleles needed to be present at both loci in order to produce pollen); and for the third there were 3 dominant restorers, 2 epistatic and 1 independent (Charlesworth and Laporte 1998). In both species, all 3 CMS types can be found in a single population. In the only *T. vulgaris* CMS genotype investigated, there were 3 different restorer loci, with 1 of them (recessive) having to interact with either of the other 2 (1 dominant, 1 recessive) to produce pollen (Charlesworth and Laporte 1998). All this complexity is reminiscent of the various suppressors of *SD* and *SR* that have been found in *Drosophila* populations and species (see Chaps. 2 and 3). CMS systems have probably been highly dynamic over evolutionary time, with both mtDNA and nuclear DNA continually evolving new ploys and counterploys.

Gynodioecy is relatively stable in these genera—most populations contain both females and hermaphrodites—but it is not yet clear how stable the underlying genetics is, nor whether there is a perpetual flow of new mitochondrial and nuclear mutations. The same CMS type has been found in different populations of *Plantago*, and similarly for *Silene* (de Haan et al. 1997b, Charlesworth and Laporte 1998; see also Laporte et al. 2001 for *Beta vulgaris*). In *Thymus*, mitochondrial genotypes are highly variable (reviewed in Tarayre et al. 1997), and it is not clear whether CMS genotypes in different populations have common or independent origins. Whether or not the genes themselves are changing, their frequencies certainly are. This is most obvious in the frequency of females, which varies widely among populations: 0–34% in *P. lanceolata*, 0–75% in *S. vulgaris*, and 5–95% in *T. vulgaris* (Van Damme and Van Delden 1982, Manicacci et al. 1998; McCauley et al. 2000). Variation in the underlying gene frequencies can be even greater: for example, in *Plantago lanceolata*, 2 populations (BM and HT) with similar frequencies (7% and 9%) of a particular male-sterile morphology had almost 2-fold differences in the frequency of the underlying CMS genotype (57% and 31%)—but this difference was hidden by a corresponding difference in the frequency of restorers (de Haan et al. 1997a, 1997b, 1997c). Populations of this species also show changes in the frequency of females over time. In *T. vulgaris*, older populations (measured as time since last disturbance) show a lower frequency of females (Couvet et al. 1990).

Further progress in studying the dynamics of CMS and restorers in natural populations must await identification of the genes themselves, or markers linked to them.
CMS, Masculinization, and the Evolution of Separate Sexes

In cases like the ones we have been considering, in which a CMS mutation arises in a population and a nuclear restorer does not sweep through to fixation, there is selection for other nuclear responses as well. In particular, because reproductive investment in the population is female biased, there is selection for mutations that masculinize the hermaphrodites—in other words, cause them to allocate more to pollen production, even at the expense of reducing ovule production (Cosmides and Tooby 1981, Ashman 1999, Frank and Barr 2001, Jacobs and Wade 2003). The consequence is to increase the difference in ovule production between male-steriles and hermaphrodites, with reduced selection for restorers as one possible result. Indeed, if the difference in ovule production becomes more than 2-fold, even nuclear male sterility genes will be selected.

The extent of masculinization has been studied in 3 species of Thymus and it varies substantially (Manicacci et al. 1998). Masculinization is greatest in T. mastichina: hermaphrodites are estimated to invest some 10 times more energy into pollen production than seed and consequently produce only about a third the number of ovules as females do. This is also the species with the highest frequency of females (72%). In other gynodioecious species, the process has gone even further: in Hebe subalpina, for example, hermaphrodites produce only one-fortieth the number of ovules as do females; and in Fragaria virginiana, more than half the “hermaphrodites” never set fruit (Lloyd 1976, Ashman 1999).

The limit of this process is complete masculinization with the species consisting of separate males and females and no hermaphrodites—that is, dioecy (Maurice et al. 1993, 1994, Schultz 1994). Separate sexes have evolved many times among the flowering plants, with gynodioecy thought to be the most common ancestral state (Barrett 2002). According to the tabulations of Maurice et al. (1993), of 308 taxonomic families of flowering plants, 55% have 1 or more dioecious species. But of the 35 families in which gynodioecy is known, fully 89% of them also have dioecious species. Thus there is some evidence of comparative association (though these data need to be corrected for differences in family size). Genera with both gynodioecious and dioecious species are known from 17 families.

What happens after dioecy? The evolution of separate sexes does not remove the conflict between nuclear and mtDNA over optimal sex allocation. It merely alters the way in which this conflict manifests itself. In partic-
ular, in plants with separate sexes, mtDNA will be selected to kill male embryos, so as to free extra resources for their sisters. Killing could usefully occur at any early stage—at germination, or while the seed is still developing on the mother, or even prior to fertilization if Y-bearing pollen can be targeted. We are not aware of any such mitochondrial killers being described; but if they can evolve to target pollen, they should be able to evolve to target males. In principle, such conflicts may even contribute to the poor evolutionary prospects of plants with separate sexes. Although dioecy is widespread in flowering plants, it is also rare, occurring in only 6% of species, and dioecious clades are far less species-rich than their sister taxa (Heilbuth 2000). Another contributing factor could be increased investment in unproductive male function. Outcrossed dioecious species typically have a 50:50 sex ratio, whereas hermaphroditic ones may enjoy all the benefits of outbreeding and sexual selection, while investing as little as 5% of their resources each generation in male function (Charnov 1982). Thus, in causing a hermaphroditic species to become dioecious, CMS genes could put their species at a competitive disadvantage with overlapping hermaphroditic ones, possibly forcing early extinction.

Pollen Limitation, Frequency Dependence, and Local Extinction

Before masculinization evolves, it is an inevitable consequence of the spread of a CMS gene (at least in the short term) that less pollen will be produced. This could result in seed production being limited by the availability of pollen. If the hermaphrodites are self-compatible, they will be less affected by pollen limitation than are the females, who rely completely on pollen from other plants. Thus, the relative fitness of females may decline as they become more common. This would cause the CMS gene to come to some intermediate equilibrium frequency rather than going to fixation (Lewis 1941; see also Box 5.1). Note that this is similar to the advantage of a driving X chromosome in Drosophila populations, which declines as it increases in frequency due to the reduction in the frequency of males (Chap. 3). Exactly this sort of frequency dependence has been observed in Silene vulgaris, in both experimentally manipulated and natural populations (McCauley and Brock 1998, McCauley et al. 2000; Fig. 5.6).

In principle, such frequency dependence may not arise in self-incompatible species that show random mating, because both hermaphrodites and fe-
Figure 5.6 Frequency-dependent effects on the relative fitness of females and hermaphrodites in *Silene vulgaris*. Data are from different natural populations that vary in the relative frequency of hermaphrodites vs. females. A. Y axis is the ratio of the square root of the number of seeds per capsule for females and hermaphrodites. B. Y axis is the ratio of the arcsine square root percentage germination for hermaphrodites and females. X axis in both cases is the arcsine square root of the proportion of hermaphrodites in the population. The dashed lines indicate the proportion of hermaphrodites at which the fitness measures of the 2 sex types are equal. Note that the effect on seed production will tend to stabilize the polymorphism, whereas the effect on germination will have the opposite effect. Adapted from McCauley et al. (2000).
males rely equally on foreign pollen. In the absence of nuclear restorers, the CMS gene could go to fixation and the population would go extinct (Charlesworth and Ganders 1979). But any real population is likely to show some spatial structure and local mating, so hermaphrodites will tend to be surrounded by other hermaphrodites, which could allow them to persist (McCauley and Taylor 1997). But if a selfed seed is better than none, there may then be strong selection for self-compatible mutations.

Resource Reallocation Versus Inbreeding Avoidance

Nuclear and mitochondrial genes are in conflict over pollen production for reasons of both sex allocation and inbreeding. On the one hand, optimal allocation to female function will be higher under mitochondrial control than under nuclear control; this difference will be greatest in outcrossing species and tend to disappear with increasing inbreeding. On the other hand, mtDNA—which is transmitted through only one sex—gains no relatedness to progeny through inbreeding. It wants to avoid selfing (assuming fertilization is achieved by outcrossing) if there is any inbreeding depression; a nuclear gene wants to avoid selfing only if fitness is reduced by more than 50% (because gene transmission to selfed offspring is doubled). More generally, a cytoplasmic gene will be selected to incur a greater cost, and risk a higher probability of not being fertilized, to avoid inbreeding than will a nuclear gene.

It is not clear which of these—resource reallocation or inbreeding avoidance—is more important in the evolution of CMS. Male-sterile individuals do typically produce more seeds than do hermaphrodites; but, as we have just seen, these differences can be inflated by the masculinization of hermaphrodites subsequent to the spread of a CMS mutation. Consider Plantago lanceolata, in which compensation has been studied with unusual care (Poot 1997). The species is self-incompatible. Thus, the primary benefit from CMS is expected to come through an increase in female function in male-sterile individuals. As expected in a species with male-sterile individuals, investment in flowers in hermaphrodites is biased toward male function—as much as 65% of the total. Progeny from between-population crosses raised under nitrogen-limited conditions in a growth chamber reveal that hermaphrodites, females, and intermediate individuals consume the same amount of nitrogen, despite the fact that stamens and pollen are relatively expensive in nitrogen. Females and intermediate individuals convert
the nitrogen saved into additional reproductive and vegetative (= future reproductive) tissue, and females set about 50% more seeds than hermaphrodites. Incidentally, there is considerable variation in form and percentage of compensation depending on the particular population cross and cytotype used. This is reminiscent of the high variability found between cytotypes and their nuclear restorers within single species.

In the gynodioecious *Sidalcea oregana*, no differences are found between the sex types in total allocation to reproduction, but females allocate more biomass, nitrogen, phosphorous, and potassium to seeds (Ashman 1999). In *Hebe subalpina* and *Cucurbita foetidissima*, more biomass is allocated to seeds in females than in hermaphrodites (Delph 1990, Kohn 1989). It would be interesting to compare these (possibly) evolved differences to changes in fertility associated with new mutations, experimental emasculations, and organelle introgressions, which would give information on the advantage to a newly arisen CMS gene. Interpretation is also made difficult because “hermaphrodites” include wildtype cytoplasms without restorers, wildtype cytoplasms with restorers, and male-sterile cytoplasms with restorers, as these genotypes cannot yet be distinguished, except by laborious crosses (e.g., Van Damme 1984).

For some species—for example, those in which anthers and pollen are produced but the pollen fail to develop after reaching the stigma—inbreeding avoidance would seem to be the only possible function. The advantage to the CMS gene of not selfing, assuming no pollen limitation, is $s\delta/1-s\delta$, where $s$ is the selfing rate of the hermaphrodites and $\delta$ is the inbreeding depression suffered by selfed seeds. At least in some mixed-mating populations, this advantage can be substantial (e.g., 2-fold; Kohn and Biardi 1995, Schultz and Ganders 1996). In self-incompatible species, $s=0$, and so the benefit to the mitochondria of avoiding inbreeding depression should be much reduced (though perhaps not wholly absent because self-incompatible hermaphrodites can be pollinated by maternal half- or full siblings, but not if they carry a CMS gene). It is interesting in this context that gynodioecy appears to be severely underrepresented in self-incompatible species, though we are not aware of any formal comparative analysis (Charlesworth and Ganders 1979). This suggests that the avoidance of selfing is—from the mtDNA point of view—an important function of CMS. It would be interesting to know whether CMS is more common in hybrids of related self-compatible populations (species) than of related self-incompatible populations (species). (Note that there is an alternative explanation.)
If CMS does arise in a self-incompatible population, it may lead to extinction or to self-compatibility.

Importance of Mutational Variation

It is a truism that phenotypes can evolve only if they first arise by mutation. In every case that has been studied so far, CMS is due to mutations in mtDNA, not in chloroplast DNA. Yet chloroplasts are also usually maternally inherited, and the selective advantage of a chloroplast gene causing male sterility should be about the same as that of a mitochondrial gene. This suggests that such mutations arise less frequently in chloroplast DNA than in mtDNA, perhaps because the chloroplast genome is less recombinationally dynamic (Palmer 1990; recall that mitochondrial CMS genes typically originate from genome rearrangements). It could also be because mitochondria are more intimately involved in anther and pollen production than are chloroplasts and so may more frequently mutate to disrupt these processes. A null mutation causing mtDNA malfunction in anthers may be entirely sufficient. We noted earlier that plant species differ significantly in their mitochondrial gene content, and it would be interesting to know whether this has any effect on their propensity to generate CMS mutations.

Mitochondria in animals are like chloroplasts in plants: in principle, the selection pressures are there for CMS or its equivalent, but the appropriate mutations apparently occur rarely, if at all. Many animal taxa are hermaphroditic, but mitochondrial mutations causing testicular or spermatic failure have yet to be reported. Many arthropods have maternally inherited bacterial parasites that have evolved to feminize or kill males (Box 5.3), but none has been reported with mitochondria that do this. And in mammals, a mitochondrial variant that destroyed male embryos, thereby creating space for reproductive sisters, would be strongly selected, but has yet to be described. We attribute these failures of evolution to the relatively small size and recombinational inactivity of mitochondrial genomes in animals.

Given that plant mitochondria have somewhat sophisticated capabilities, such as the ability to convert male plant parts into (crude) female parts, it is interesting to speculate what else they may do. We have already suggested that they may increase seed production quite independently of abolishing pollen production, and that they may kill male embryos or otherwise skew
BOX 5.3

Maternally Inherited Reproductive Parasites of Arthropods

Many arthropods are host to intracellular bacteria or protozoa that, like mitochondria, are inherited through the egg, from mother to offspring (O’Neill et al. 1997). Like the mitochondria inhabiting the same cells, these maternally inherited endosymbionts are selected to skew host reproduction toward females; unlike mitochondria, many of them have successfully evolved to exploit this opportunity, and in a variety of ways (O’Neill et al. 1997, Charlat et al. 2003):

*Male killing.* In several insect and mite species, there are maternally inherited bacteria that have evolved to kill male embryos. At least for *Spiroplasma* in *Drosophila,* they do this by inducing embryo-wide apoptosis (G. Hurst, pers. comm.). Killing a male can increase a bacterium’s probability of being transmitted to the next generation if, for example, juvenile females eat their dead brothers or have more resources available in their absence, thus increasing their probability of surviving to adulthood.

*Diploid feminization.* In amphipod crustaceans such as *Gammarus,* there are microsporidian protozoans that interfere with normal sexual differentiation and cause genetic males to develop as females. In isopod crustaceans such as *Armadillidium* and some lepidopterans, there are bacteria doing the same thing. Infected females produce twice as many daughters as uninfected ones, allowing the endosymbiont to be transmitted to twice as many granddaughters.

*Haplodiploid feminization.* In some haplodiploid wasps and mites, there are bacteria that induce unfertilized eggs, which would normally develop into males, to develop instead into parthenogenetic females. In wasps the bacteria cause the chromosomes to double, and so the females are diploid, whereas in *Brevipalpus* mites the females remain haploid (Weeks et al. 2001). Again, infected females
produce twice as many daughters as uninfected ones, thereby allowing the endosymbiont to be transmitted to twice as many granddaughters.

*Cytoplasmic incompatibility.* In many insect species there are bacteria that in males somehow modify the sperm such that eggs from uninfected females that are fertilized by those sperm die; eggs from infected females are viable regardless of whether the father was infected. In this way the bacteria decrease the fitness of uninfected females, thereby increasing the (relative) fitness of infected females. Recently, one of these bacteria has swept through the California population of *D. simulans* over a 5-year period (Hoffmann and Turelli 1997).

Strikingly, there is a single genus of bacteria, *Wolbachia*, in which all 4 of these phenotypes have been observed. This rich diversity of reproductive parasitism contrasts with the apparent absence of any similar adaptations in animal mitochondria, and perhaps suggests what nuclear-mitochondrial relations may have been like in early eukaryotes, when the symbiosis was young.

...
those linked to the self-incompatibility locus), and may in some species have evolved a critical role, acting either in the style or in the pollen. But even for mitochondria in plants, constraints on the mutational spectrum should be critical. Nuclear genes have a much larger mutational spectrum than does mtDNA, because they have many more genes (and noncoding DNA), and presumably this is a major reason why the nucleus usually wins the conflict with the mitochondria—that is, why most plants are hermaphrodites, not gynodioecious, with male sterility often only uncovered in population or species hybrids.

CMS and Paternal Transmission

We have already noted that CMS can select for a diverse array of nuclear responses, including restoration of male fertility, masculinization of hermaphrodites, and self-compatibility. We end our review of CMS by noting that, in principle, it also selects for a reversal of the very thing from which it springs: maternal inheritance. In a population polymorphic for a CMS gene, mitochondria in pollen are less likely to carry the gene than mitochondria in ovules. Thus, nuclear genes may be selected to promote paternally derived mitochondria over maternally derived ones. This will be true of nuclear genes acting in the mother, the father, or the offspring. The result could be a degree of paternal “leakage” or even outright paternal inheritance. The comparative biology of mitochondrial inheritance in plants is not well understood—all we know is that occasionally there is paternal leakage, and occasionally there are species with predominantly paternal inheritance. Perhaps occasionally such reversals evolve in species troubled by CMS genes, as a way of reducing the chance of transmitting so selfish a gene.

Other Traces of Mito-Nuclear Conflict

This chapter reviews the two main forms of conflict between mitochondria and nuclei. First, mitochondria may replicate faster than is optimal for the organism, in order to swamp a competitor. This is particularly likely with biparental inheritance, because of the greater within-organism genetic variation. Second, with uniparental inheritance, mtDNA evolves to skew reproduction toward the transmitting sex. But there have undoubtedly been
other forms of conflict during the more than a billion years that mitochon-
dria have been living inside eukaryotic cells. Presumably conflicts were par-
ticularly prevalent before mitochondria became so degenerate (much as
more recent endosymbionts of arthropods are more often in conflict with
their hosts; see Box 5.3). Such ancient conflicts may have left traces in the
modern workings of mitochondria and nuclei. In this section we review sev-
eral possibilities; all of them are speculative.

Mitochondria and Apoptosis

It is a striking fact, already alluded to, that mitochondria play an active role
in apoptosis (programmed cell death; Zamzami et al. 1996, Green and Reed
though it is not clear what exactly precedes what, an early sign of death is
the opening of pores or ion channels in the mitochondrial membrane, fol-
lowed by osmotic expansion of the mitochondrion until the outer wall
bursts, releasing caspases and other chemicals into the cytoplasm (Green
and Reed 1998). At the same time, disruption of electron transport and
therefore energy metabolism is another early sign, but ATP production is af-
fected much later. Indeed ATP is itself necessary for some downstream
apoptotic events, presumably because destruction is active and requires en-
ergy. Mitochondria contain an apoptosis-inducing factor, AIF<sub>5</sub>, which is
normally confined to mitochondria but which translocates to the nucleus
when apoptosis is induced (Susin et al. 1999). There it causes chromatin
condensation and large-scale fragmentation of DNA. AIF<sub>5</sub> also induces mi-
tochondria to release the apoptogenic proteins cytochrome c and caspase-9,
which itself activates further downstream executor caspases. (The caspases
are a family of cysteine-dependent aspartate-specific proteases whose mem-
bers act as both initiators and executors of apoptosis.)

Cells lacking mtDNA are capable of apoptosis, and both AIF<sub>5</sub> and cyto-
chrome c are coded by nuclear genes, but several mitochondrial genes ap-
pear to be involved in apoptosis. In human hematopoietic myeloid cell
lines, inhibition of the mitochondrial gene NADH dehydrogenase subunit
4 decreases cell viability and generates markers of apoptosis (Mills et al.
1999). In the same kind of cell lines, a mitochondrial antisense RNA for
cytochrome c oxidase can induce large-scale DNA fragmentation within 36
hours and cell death within 3 days (Shirafuji et al. 1997). In human colonic
epithelial cells, elevation of mitochondrial gene expression appears to be important in inducing apoptosis of these cells in culture (Heerdt 1996).

How might mitochondria have come to be central players in apoptosis? Perhaps in some ancient eukaryote mitochondria were sometimes selected to kill their host cell, much as they now are in the tapetum of sunflowers. Kobayashi (1998) speculates that early mitochondria were selected to kill cells to which they had not segregated, analogous to systems now observed for some bacterial plasmids (Box 2.1). Interestingly, the one mitochondrial gene that is variably present in animals, called A8, has no detectable homology to any prokaryotic ATP synthase, but its hydropathy profile shows a significant similarity to that of hok, a gene involved in bacterial plasmid-maintenance systems (Jacobs 1991). Nuclear genes may then have evolved to co-opt the phenotype and express it when it was in their own interests to do so. The genes originally responsible for the action may no longer be in the mitochondria but instead have moved to the nucleus.

Mitochondria and Germ Cell Determination

Another organismal function in which mitochondria seem inexplicably to be involved is germ cell determination (Amikura et al. 2001). In many animal species, there is a histologically distinct region of the egg cytoplasm, called the germplasm, which is the part that will go on to form the germline, eventually producing eggs or sperm. This germplasm consists largely of germinal granules (also called polar granules) and mitochondria. The germinal granules are electron-dense structures that act as repositories for the factors required for germ cell formation. In Drosophila, they are rich in mitochondrial-type ribosomes, which are ribosomes consisting of mitochondrially encoded RNAs and some of the same nuclear-encoded proteins as used in mitochondrial ribosomes. Such ribosomes are not typically found outside of mitochondria in other tissues. Similar observations have been made for planarian, sea urchin, ascidian, and frog embryos. How might mitochondria have become involved in germ cell determination? We speculate that in the early evolution of tissue differentiation in animals (when, based on choanoflagellates, mitochondria probably had more genes than they do now, including many for ribosomal proteins; Burger et al. 2003), mitochondria would have been selected to ensure that the cell or embryonic region in which they were found ended up as part of the germline, and evolved mech-
organisms to ensure this happened. These mechanisms may then have been co-opted by the nucleus.

Mitochondria and RNA Editing

In many organisms there is a minority of genes in which the DNA is transcribed into RNA, and then enzymes act on the RNA to change the coding sequence—for example, inserting or deleting a single base or changing one base to another (Gray 2001). That is, the RNA is edited. Why might such a system have evolved—if the alteration is beneficial, why would it not simply occur at the DNA level directly? Gray (2001) suggests that perhaps the editing machinery originally evolved neutrally, by genetic drift, but now is maintained by selection because the genome has acclimatized to it. But this seems like a lot to ask of random drift unaided by selection. An alternative scenario is suggested by the observation that editing is disproportionately common for mtRNA, while the enzymes responsible are encoded in the nucleus. We speculate that in some cases RNA editing originally evolved as a way for nuclear genes to rewrite or override the instructions of mitochondrial genes that were antagonistic to nuclear interests. In other cases, partial editing may have evolved so as to make 2 proteins from a single gene. In either case, once the machinery has evolved, the genome may then “degenerate” to the point at which editing is essential for life (Gray 2001).