

Animal mitochondrial DNA recombination revisited

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Exchange of homologous sequences between mitochondrial DNA (mtDNA) molecules is thought to be absent in animals, primarily because of a failure to observe clear cases of recombinant haplotypes in natural populations. However, whether mtDNA recombination occurs is a different issue from whether it produces new haplotypes. A requirement for the latter is heteroplasmy – the presence of more than one type of mtDNA in an individual, which is rare in animals. In male mussels, in which heteroplasmy is the rule, recombination is common, arguing against an innate impediment to mtDNA recombination in animals. In addition, recent biochemical studies suggest that recombination is an indispensable part of the mtDNA replication and repair machinery and that most animal genomes have the necessary enzymes for mtDNA recombination. When strict maternal mtDNA transmission is compromised, recombinant haplotypes can be generated and eventually become fixed. Although the pervasiveness of mtDNA recombination in animals is unknown, its presence could have important consequences for phylogenetic studies of closely related taxa (e.g. leading to incorrect phylogenetic inferences and incorrect rejection of the molecular clock) and human mtDNA-associated diseases.

Animal mitochondrial DNA (mtDNA) is a small, usually circular, DNA molecule that occurs in mitochondria and contains genes that support aerobic respiration. Mitochondrial genes and genomes are important tools in a variety of fields related to the study of animal evolution, such as phylogeography [1], population genetics [2] and phylogenetics [3]. Central to the success of mtDNA as the marker of choice are several key characteristics, such as its strict maternal transmission and its high(er) mutation rate (compared with nuclear DNA). Another important, and commonly accepted, animal mtDNA feature is its clonal inheritance and lack of recombination. By contrast, mtDNA recombination is the norm in most plant, fungal and protist species [4]. However, in recent years, several studies have questioned whether the lack of recombination holds across the animal kingdom [5–8].

mtDNA inheritance and genetics

A typical somatic cell contains 500–1000 mitochondria, whereas an oocyte contains some 10^4 – 10^5 mitochondria, each with a few DNA molecules [9]. These mtDNA molecules are likely to be immobile inside the mitochondrion because they are attached to the mitochondrial inner membrane [10], clustering into NUCLEOIDS [9] (see Glossary). The thousands of mitochondria found in each oocyte are probably derived from very few (<10) mitochondria found in primordial germ cells [11]. This phenomenon has been termed as ‘bottleneck’ or ‘sampling and amplification’ [12].

With the exception of certain bivalve families, maternal inheritance of mtDNA is the rule in all animal species [12], but ways of preventing paternal transmission vary [13]. For example, in certain crayfish species, the sperm lacks mitochondria. In some tunicates, the mitochondria do not enter the egg, whereas in mammals up to 100 paternal mitochondria enter the egg but are destroyed during the

Glossary

Control region: contains the major regulatory elements for the replication and transcription of vertebrate mitochondrial genomes. The control region is usually the most rapidly evolving part of a mitochondrial genome.

Doubly uniparental inheritance: female offspring inherit the mitochondrial DNA of their mother, whereas males inherit the mitochondrial DNA of both parents, leading to male offspring being heteroplasmic; found in the mussel families Mytilidae, Veneridae and Unionidae.

Heteroplasmic: having more than one type of mitochondrial DNA molecule in one individual.

Homologous recombination: genetic exchange occurs between any pair of DNA sequences that are identical by descent (homologous).

Homoplasmic: having only one type of mitochondrial DNA molecule in one individual.

Hybridogenic: where an ancestral genome from the maternal line is transmitted to the egg without recombination, whereas paternally derived chromosomes are discarded premeiotically, only to be replaced each generation through fertilization by sperm from a related species.

Intermolecular recombination: recombination occurring between mitochondrial DNA molecules.

Intramolecular recombination: recombination occurring within a mitochondrial DNA molecule.

Linkage disequilibrium: the degree of association between alleles at different loci.

Non-homologous recombination: genetic exchange takes place between DNA sequences that are not identical by descent (non-homologous).

Nucleoids: *in situ* observations suggest that mitochondrial DNA molecules within the organelle are organized in mtDNA-protein complexes called nucleoids. Each nucleoid comprises of two to eight mitochondrial DNA molecules. There are a few nucleoids in each organelle. Nucleoids are commonly found in many organelles and bacterial cells.

first few hours following fertilization. Selective destruction of mammalian sperm mitochondria occurs upon entrance to the egg cytoplasm because they are tagged with ubiquitin, a universal proteolytic marker [14]. Although these mechanisms exist to ensure that the zygote contains only maternal mitochondria, maternal inheritance of mtDNA in animals is often a quantitative phenomenon [12]. More specifically, situations exist in which the mechanism for recognition of paternal mitochondria in the fertilized egg can fail, thus enabling PATERNAL LEAKAGE to occur. Paternal leakage has indeed been observed in a taxonomically wide variety of invertebrate and vertebrate species [15–19], and, very recently, a case of paternal inheritance of mtDNA has also been demonstrated in humans [20]. Unfortunately, the rate of paternal leakage in animals has not been studied in any systematic way; the only available crude estimates (10^{-3} – 10^{-4} instances per fertilization) come from mice [16] and *Drosophila* [15,21]. However, failure in the recognition mechanism for paternal mitochondria might be more common in hybrid populations, in which specificity in the recognition process might be relaxed [14,19,22,23].

A more extreme exception to the ‘standard’ maternal transmission of mtDNA is DOUBLY UNIPARENTAL INHERITANCE [24,25], which is observed in several bivalve families. Under doubly uniparental inheritance, female offspring inherit the mtDNA of their mother, whereas males inherit the mtDNA of both parents, leading to male offspring that are HETEROPLASMIC.

mtDNA recombination: the ‘consensus’ view

The absence of recombination has been supported by several independent lines of research. Many studies of mitochondrial diversity in natural populations of a variety of animal species have failed to find recombinant haplotypes [1]. In addition, mitochondria in somatic cell hybrids do not harbor recombinant haplotypes ([26], but see [27]), and there is also evidence that mtDNA molecules become sequestered into clusters, thereby preventing physical contact of unrelated molecules [9]. The elevated mtDNA mutation rate in animals has also been interpreted as an indication of the absence of recombination in animal mtDNA [28].

The molecular toolkit for mtDNA recombination

One of the most important questions regarding the study of recombination in animal mtDNA is whether the molecular and biochemical toolkit for recombination is present in the organelles. Recent work suggests that human mitochondria have both HOMOLOGOUS RECOMBINATION [29] and NON-HOMOLOGOUS RECOMBINATION [30] activities because mitochondrial protein extracts can catalyze both types of recombination *in vitro*. In addition, DNA ligase III, a key enzyme in replication, recombination and DNA repair, has been shown to localize also in mitochondria [31]. In agreement with the *in vitro* results, Tang *et al.* [32] have demonstrated that HOMOPLASMIC populations of duplicated mtDNAs in humans can produce wild-type, as well as deleted, mtDNA through the action of INTRAMOLECULAR RECOMBINATION (see also [33]). However, although INTERMOLECULAR RECOMBINATION must

have been involved in the generation of the duplicated mtDNA in the first place, Tang *et al.* [32] failed to detect any intermolecular recombination among wild, duplicated and deleted mtDNA types.

A potential explanation for the failure of Tang *et al.* [32] to detect intermolecular recombination could be the lack of physical proximity of the three mtDNA types. For recombination to occur, physical proximity of mitochondrial genomes is, of course, an absolute requirement. Fusion of mitochondria has been demonstrated in *Drosophila*, and one of the genes (*fuzzy onions*) mediating the process has been identified [34]. *fuzzy onions* is highly conserved with homologs found in taxa as diverse as humans (mitofusins *Mfn1* and *Mfn2*) [35] and yeast (*Fzo1p*), suggesting that at least some of the components for mitochondrial fusion are conserved not only within animals, but even between animals and fungi. Despite the evolutionary conservation of at least one component of the molecular pathway via which mitochondrial fusion is achieved, early results on whether or not mitochondrial fusion in animal cells is a general intrinsic property of these organelles are contradictory. For example, on the basis of experiments in which human mitochondria carrying different mtDNA mutations failed to complement one other, Enriquez *et al.* [36] have suggested that human mitochondria fuse very infrequently. By contrast, using fluorescence microscopy, Legros *et al.* [37] have suggested that mitochondrial fusion in human cells is an efficient process and is mediated by the mitofusins (overexpression of *Mfn1* stimulates mitochondrial fusion); this is in agreement with studies on mitochondrial fusion in rat liver cells [38].

Constraints in the detection of mtDNA recombination in natural populations

The conclusion that animal mtDNA does not recombine based on absence of recombinant haplotypes in natural populations does not consider the probability of a mtDNA recombination event producing a detectable recombinant haplotype. This probability could be very small for the variation surveyed in typical population studies. The strict maternal inheritance of mtDNA means that, in most cases, recombination would occur in homoplasmic cells, making the detection of recombinants impossible (although in certain cases, intramolecular recombination has been shown to generate size heteroplasmy [32]); it is only in heteroplasmic cells that recombination can be detected. An individual could become heteroplasmic either by inheritance of mtDNA from both parents or by mutations in the mitochondrial genome of germ-line cells. However, heteroplasmy in animals is rare and recombination leading to new haplotypes is expected to be even more so. The situation is complicated further by the fact that the sperm:egg mitochondria ratio in the one-cell zygote is very small (perhaps $1:10^4$), which makes the occurrence of recombination and the detection of its products highly susceptible to random drift.

On the practical side, it is difficult to demonstrate strict maternal inheritance in natural populations because of the large sample sizes required [39]; in most cases to date, too few offspring have been examined to enable the

Table 1. Freely distributed software packages testing for recombination under a variety of assumptions and types of data

Name ^a	Optimality criterion	Description	Website	Refs
PLATO	Maximum likelihood	Tests for spatial variation in the evolutionary process, such as variation caused by the action of recombination	http://evolve.zoo.ox.ac.uk/	[59]
LAMARC	Maximum likelihood	Package of programs for estimation of population parameters, including recombination rate, from molecular data	http://evolution.genetics.washington.edu/lamarc.html	[60]
TOPAL	Least squares	Employs a sliding window on a multiple sequence alignment; changes between different sliding window topologies are tested statistically	http://www.bioss.sari.ac.uk/~frank/Genetics/topal.html	[61]
LDhat	Maximum likelihood	Package of programs that uses the coalescent for analyzing patterns of linkage disequilibrium and estimating the population recombination rate	http://www.stats.ox.ac.uk/~mcvean/LDhat/LDhat.html	[62]
PIST	–	A test designed to detect recombination on the principle that rate heterogeneity among sites is inflated in the presence of recombination	http://evolve.zoo.ox.ac.uk/	[63]
RETICULATE	–	Calculates compatibility matrices for detecting recombination	http://jcmr.anu.edu.au/dmm/humgen/ingrid/reticulate.htm	[64]
PHYLPRO	Distance	Uses a sliding window to determine the pairwise distance of all sequences in the windows and to evaluate, for each sequence, the degree to which the patterns of distances in these regions agree	http://life.anu.edu.au/molecular/software/phylpro/	[65]
RECPARS	Parsimony	Tests whether different subsets of a multiple alignment have different histories	http://www.daimi.au.dk/~compbio/recpars/recpars.html	[66]
HOMOPLASMY TEST	Parsimony	Tests recombination in a set of closely related sequences	http://www.biols.susx.ac.uk/home/John_Maynard_Smith/	[67]

^aThe list provided is an introduction to the field and is far from exhaustive. A more complete list of programs can be found at http://evolve.zoo.ox.ac.uk/Grinch/RAP_links.html; a comparative evaluation of some of the software listed here for recombination detection can be found in [40].

detection of low levels of paternal inheritance [12]. In addition, existing methods for detecting recombination are not very powerful [40,41]. Success depends heavily on the level of sequence divergence (modest detection success is achieved for sequences that have diverged by >5%) and number of recombination events (>3); the larger these two parameters are, the more successful the detection method will be [41]. In spite of (or perhaps because of) the shortcomings in detecting of mtDNA recombination, several computer packages have been developed for recombination detection in molecular sequence data (Table 1).

Recombination in animal mitochondrial genomes: the experimental evidence

The first direct demonstration of recombination in animal mtDNA was provided by Lunt and Hyman [5] in the nematode *Meloidogyne javanica*. The authors showed that the mtDNA CONTROL REGION can self-recombine, creating a figure-of-eight structure that results in two circular molecules: a large one that contains all the genes and parts of the control region, and a small one that contains only parts of the control region. This type of non-homologous recombination results in molecules that differ in size from the parental molecule, creating size heteroplasmy within individuals. Size heteroplasmy in the control region of mtDNA of several other animal species has also been attributed to non-homologous recombination [42–45]. Although similar mtDNA insertions or deletions could also be generated by slipped mispairing [46], this mechanism fails to explain the presence in the same individual of mitochondrial genomes with different deletions at the same region of the molecule [32].

Recombination in the control region might be easier than in the remainder of the mtDNA genome because of the presence, in some species, of small motifs of repetitive DNA on both sides of its core. The distribution of two types of such motifs (both types having the same length but differing at two nucleotide positions) was investigated by Hoarau *et al.* [8] in populations of the flatfish *Platichthys flesus*. Although the control region of most individuals contained arrays of just one motif or the other, one individual contained an array consisting of tandem repeats of one motif followed by tandem repeats of the other motif, providing evidence for recombination. Unfortunately, the two variable sites differentiating the two motifs were not found in recombinant fashion in any of the motifs examined, making it difficult to determine whether the recombinant array has resulted from homologous or non-homologous intermolecular recombination.

In certain bivalve families in which doubly uniparental inheritance is the rule, male offspring are heteroplasmic. Male heteroplasmy in these species offers an ideal system for the detection of recombination because two divergent molecules coexist in the same individual, and both in large numbers. Looking in male gonads of the marine mussel *Mytilus galloprovincialis*, Ladoukakis and Zouros [6] detected recombinant sequences coexisting with the parental molecules, which suggests that recombination occurred in the same individual in which the recombinant molecules were detected. Furthermore, Burzynski *et al.* [47] found two recombinant molecules in appreciable frequencies in a population study of the related mussel species *Mytilus trossulus*, suggesting that the recombinant molecules can be transmitted to the next generation.

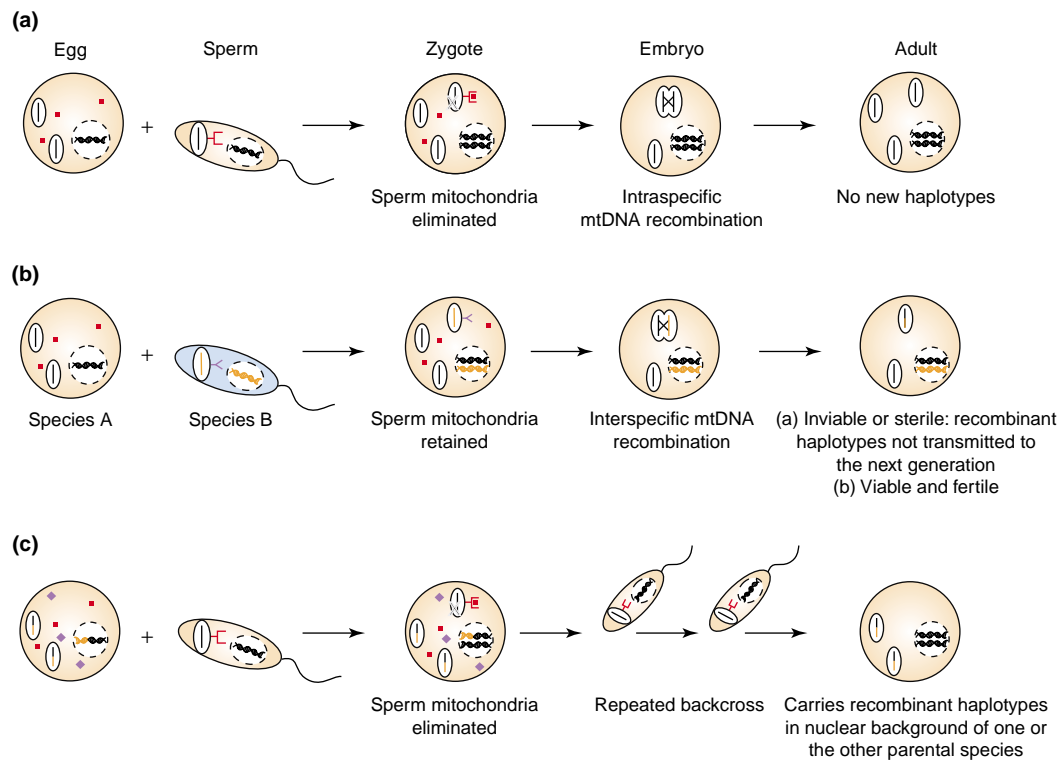
Box 1. Hybridization: a narrow path to recombinant mitochondrial DNA haplotypes

The rate with which recombination will produce new haplotypes in the population depends on the frequency of co-occurrence of two different mitochondrial genomes in the same cell and on the degree of their genetic divergence.

In intraspecific crosses, the mechanism that prevents transmission of paternal mitochondrial DNA (mtDNA) is very effective, securing the homoplasmy of the embryo (Fig. 1a). Even in the rare cases where leakage of paternal mtDNA occurs, the low degree of divergence between the two parental mtDNA genomes (e.g. the degree of divergence in humans is estimated to be 0.0028 nucleotide differences per site [52]) makes distinguishing between recombination and homoplasmy difficult [40].

By contrast, in interspecific crosses, the mechanism enforcing paternal mtDNA elimination can break down, resulting in heteroplasmy (Fig. 1b). In this case, the degree of genetic divergence between the mtDNA molecules of the two hybridizing species could play a crucial role in the production of recombinant molecules. mtDNA molecules will not recombine if the degree of genetic divergence is too high (data from

bacteria suggest that the rate of recombination decreases as the divergence between sequences increases [68]), although this possibility will arise only rarely given that highly divergent species will not hybridize in the first place. By contrast, for two species with a very low degree of divergence, the mechanism of sperm mtDNA recognition and elimination is probably as effective as in intraspecific crosses, resulting in the prevention of heteroplasmy. As the degree of genetic divergence between the species increases, the probability that sperm mitochondria are recognized and eliminated decreases, but so is the probability that the F1 hybrid will be viable or fertile. Therefore, the path to producing recombinant haplotypes in the population through hybridization might be narrow. However, once a fertile female hybrid carrying a recombinant haplotype is produced, this haplotype can be transmitted to the next generation by backcrossing with a male from either species. Similar events of repeated backcrossing can potentially fix the recombinant mtDNA haplotype against the nuclear background of one or the other parental species (Fig. 1c).



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Fig. 1. Hybridization as a path for generation of recombinant mtDNA haplotypes. In intraspecific crosses (a), egg factors (shown as red squares) recognize the identification factors carried by sperm mitochondria (shown as red brackets), leading to the elimination of paternal mitochondria. As a result, the embryo is homoplasmic and recombination events will not generate new haplotypes. In interspecific crosses (b), the egg factors from one species (shown as red squares) will not recognize the identification factors carried by sperm mitochondria from the other species (shown as blue brackets), leading to heteroplasmy and the possibility of production of recombinant mtDNA genomes. If the female hybrid is inviable or sterile, it will not leave any descendants and the recombinant genomes will be lost. If it is viable and fertile (c), repeated backcrossings to one or the other parental species could lead to the fixation of a recombinant mtDNA genome. Mitochondria are shown as solid line ellipses and nuclei as dotted circles; straight bars denote mtDNA genomes and helices nuclear genomes.

Hybridization events, especially recurring ones, such as those seen in hybrid zones or in HYBRIDOGENIC species (e.g. *Bacillus* stick insects, *Poeciliopsis* fish, *Rana* frogs or *Heteronotia* lizards [48]), could provide the opportunity for heteroplasmy and recombination to take place (Box 1). In support of this argument, molecular evidence suggests that destruction of sperm mitochondria

through ubiquitination did not occur in hybrid embryos created using domestic cow *Bos taurus* ova and wild cattle *Bos gaurus* sperm, arguing that sperm mitochondria destruction might be species-specific [14]. Similar results have been obtained from murine (mouse) hybrids [22,23]. However, early studies of the hybridogenic fish species *Poeciliopsis monacha lucida*

Box 2. Consequences of mitochondrial DNA recombination for human disease

Although the first descriptions of pathogenic mitochondrial (mtDNA) mutations were reported as late as 1988, some fifteen years later over one hundred mtDNA base-substitution mutations and hundreds of mtDNA insertions or deletions have been associated with common myopathies, neurodegenerative diseases and aging [69]. Examples include a sudden onset blindness disease known as Leber's hereditary optic neuropathy, which is associated with at least three point mutations in the ND genes [70], and the Kearns-Sayre syndrome, which is associated with numerous multiple deletions or duplications ([71], see also The Human Mitochondrial Genome Database website at <http://www.mitomap.org>).

In non-recombining genomes, the number of deleterious mutations is expected to increase over time (a process known as 'Muller's ratchet' [72]); by contrast, recombining genomes have the ability to purge deleterious mutations by recombination. Therefore, the occurrence of

recombination in human mtDNA could be reducing the mutational load associated with human mtDNA diseases significantly.

However, irrespective of whether or not human mtDNA recombines, there are several other potentially important characteristics distinguishing mtDNA diseases from those associated with nuclear loci. Human mtDNA diseases show a marked genetic, biochemical and clinical heterogeneity not found in diseases associated with most nuclear loci [73]. Explanations of this heterogeneity might be provided by the peculiarities of mtDNA inheritance (such as predominantly maternal inheritance, mitochondrial bottleneck, heteroplasmy, tissue variation and haplotype selection) [71]. The plethora of factors governing the pathogenicity of certain mtDNA mutations in humans makes theoretical modelling of mtDNA-associated diseases a difficult enterprise. Certainly, it will be interesting to test whether presence or absence of recombination can shift the balance against pathogenicity.

and *Poeciliopsis monacha occidentalis* failed to detect paternal leakage based on restriction fragment length polymorphism (RFLP) data [49].

Recombination in animal mitochondrial genomes: the statistical evidence

Perhaps the most celebrated example of the discussion about whether animal mitochondrial genomes recombine is the considerable (and constructive) debate regarding recombination in human mtDNA [7,50–52]. Under clonal reproduction, allelic combinations among variable sites are preserved, leading to strong LINKAGE DISEQUILIBRIUM. Recombination will cause the decay of the disequilibrium. An initial analysis [7] showed that the amount of linkage disequilibrium among pairs of mitochondrial sites decreased as the distance between the sites increased, suggesting the presence of recombination. However, this study was criticized on several grounds [50,51]. Further analysis of more datasets [52,53] suggested lack of statistical support for mtDNA recombination in human mitochondria. Irrespective of whether recombination can be detected statistically in human mtDNA genomes, intramolecular recombination of duplicated mtDNA has been demonstrated [32,33].

Extant mtDNA genomes can contain the signatures of past recombination events; however, these will be difficult to detect because mutation will have changed both the recombinant and parental genomes, mixing the signature(s) of recombination with the 'noise' of subsequent substitutions. The signature of recombination might be more conserved at the (less variable) amino acid sequence level. On this rational, a recent study [54] surveyed several datasets and argued for the presence of mtDNA recombination in three animal taxa: amphipods (*Gammarus fossarum*), field mice (*Apodemus*) and frogs (*Rana*). However, a different analysis of the same dataset [55] reached somewhat different conclusions; it rejected mtDNA recombination in *G. fossarum*, and provided weak evidence for recombination in *Apodemus* and very strong evidence in *Rana*.

Consequences of mtDNA recombination for molecular evolution research

Currently, all major methods for phylogenetic reconstruction assume a single evolutionary history for all mtDNA

genes (but see [56]). However, under recombination, the different parts of a given sequence will have different evolutionary histories. Given that many studies in molecular population and evolutionary biology are based on mtDNA (~70% of phylogeographic studies have involved analyses of mtDNA either primarily or exclusively [1]), it is essential to understand the effects of recombination on phylogenetic reconstruction.

A phylogeny obtained from a dataset in which recombination has occurred will be, by definition, different from the true histories underlying the data. This issue has been addressed by two recent studies, one focusing on how recombination will affect the shape of the phylogeny [57], and the other on how it will affect the branching pattern (topology) of the phylogeny [58]. These simulation studies include several implicit assumptions; recombination in natural populations is bound to show a more complex, and perhaps less predictable, behavior.

Schierup and Hein [57] focused on the effects of recombination on the shape of the phylogenetic tree and highlighted several artifacts that can be generated. For example, phylogenetic analysis of recombinant sequence data can generate phylogenetic trees resembling trees obtained from analysis of sequence data from a population undergoing exponential growth. The latter trees are characterized by longer terminal branches, a longer total tree length and less time to coalescence to the common ancestor. Therefore, failure to detect recombination can lead to overestimation of the length of terminal branches and the total branch length, underestimation of the time to the most common ancestor of the sequences, and incorrect rejection of the molecular clock.

Posada and Crandall [58] generated datasets by simulation under two different topologies, combined them (controlling for the proportion of each of the datasets in the combined pool) and analyzed the combined matrix under the assumption of no recombination. In simulations where the proportion of the datasets was 3:1 or larger, the tree contributing the majority of the alignment was recovered (or with some slight differences) [58]. Topologies lacking resemblance to either simulated topology were generated only in the cases where the proportion was 1:1.

The confounding effects of recombination on phylogenetic reconstruction revealed by these two complementary

studies show that failure to account for recombination can seriously mislead population genetic and phylogeographic inferences of a study organism. It is clear that datasets should be tested for the presence of recombination before every phylogenetic analysis (Table 1).

Conclusions and outstanding questions

mtDNA owes its popularity and usefulness as the marker of choice in animal evolutionary studies to several characteristics, prominent among which is the absence of recombination. However, recent work in several fields has questioned the validity of this characteristic. Studies of natural populations of several animal species have provided convincing experimental evidence for the presence of mtDNA recombination in at least some taxonomic groups [5,6,8,47], whereas detailed biochemical and molecular studies have shown that animal mitochondria do possess the necessary enzymes for recombination [29–31]. Furthermore, important insight has been gained in key areas for animal mtDNA recombination research: (1) on the mechanisms underlying mtDNA inheritance and persistence in animal cells [14–16,20,23–25], (2) on the exact location and structure of mtDNA genomes within mitochondria [9,12], and (3) on the behavior of the organelles themselves [34,37]. Finally, in the past few years a plethora of efficient algorithms for detection of recombination have been developed (Table 1) and the behavior of recombinant data has been explored [57,58].

Although there is no doubt that certain animal mitochondrial genomes do recombine, we remain basically ignorant as to how common mtDNA recombination might be in the animal kingdom. Shifting the research focus on populations in which maternal inheritance is broken often or has been compromised in the past might prove to be a most fruitful approach. Such an effort will be aided greatly by further understanding of the mysteries surrounding the molecular machinery regulating mitochondrial inheritance and behavior, and by the genomic revolution. Currently available data for most organisms (model systems excluded [2,52]) comprise small parts of the mtDNA genome from relatively few individuals, yielding analyses lacking in vigor and which are vulnerable to sampling error. Finally, an open question of great significance is the relevance of mtDNA recombination to our understanding of mtDNA-associated human diseases (Box 2). Although existing knowledge about the etiology of these diseases is strongly suggestive of complex genetic and molecular mechanisms, knowledge about whether human mtDNA recombination occurs, and at what rate, is bound to improve our knowledge about the inheritance and evolutionary dynamics of these important diseases.

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