Population Size Does Not Influence Mitochondrial Genetic Diversity in Animals

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Within-species genetic diversity is thought to reflect population size, history, ecology, and ability to adapt. Using a comprehensive collection of polymorphism data sets covering ~3000 animal species, we show that the widely used mitochondrial DNA (mtDNA) marker does not reflect species abundance or ecology: mtDNA diversity is not higher in invertebrates than in vertebrates, in marine than in terrestrial species, or in small than in large organisms. Nuclear loci, in contrast, fit these intuitive expectations. The unexpected mitochondrial diversity distribution is explained by recurrent adaptive evolution, challenging the neutral theory of molecular evolution and questioning the relevance of mtDNA in biodiversity and conservation studies.

Genetic diversity is a central concept of evolutionary biology that has been linked to organismal complexity (*1*), ecosystem recovery (*2*), and species ability to respond to environmental changes (*3*). A lack of diversity is typically considered as evidence for a small or declining, potentially endangered population (*4*, *5*). Population genetics theory tells us that, for a neutral locus, the expected polymorphism at mutation-drift equilibrium is proportional to the effective population size, the equivalent number of breeders in an ideal, panmictic population. Other factors can of course affect the genetic polymorphism, including population structure (*6*), population bottlenecks (*3*), and natural selection [either directly or through genetic linkage (*7*, *8*)], life cycle (*9*), and mating systems (*10*). These multiple influences complicate any attempt to interpret the genetic diversity of one particular species in terms of population size (*11*). Population size, however, presumably varies by several orders of magnitude between species and taxa, so that one would typically predict that abundant species should be, on average, more polymorphic than scarce ones despite the noise introduced by other evolutionary forces.

Meta-analyses of allozyme polymorphism studies were mostly consistent with this theoretical prediction (*12*, *13*). In particular, invertebrate animals were found to be more polymorphic, on average, than vertebrates (*13*). It was noted, however, that the expected proportional relationship between diversity and effective population size was rarely met (*14*). DNA-based markers have now replaced allozymes in population genetics studies. Among these, the supposedly nonrecombining and evolutionary nearly neutral mitochondrial DNA (mtDNA) has been the most widely used marker of population history and diversity (*15*, *16*), the general belief being that mtDNA diversity should reflect effective population size more accurately than allozymes (*17*). In this study, we approach the taxonomic and ecological determinants of effective population size by analyzing the distribution of the genetic polymorphism across animal taxa, focusing on mtDNA and comparing it to allozymes and nuclear DNA data.

Three exhaustive within-species polymorphism data sets were used: an allozyme data set (912 species) taken from the compilation by Nevo *et al*. (*12*), a nuclear sequence data set (417 species), and a mitochondrial sequence data set (1683 species), the latter two both built from the Polymorphix database (*18*, *19*). We first calculated the average genetic diversity in eight largely represented animal taxa (hereafter called "groups"). The allozyme and nuclear data sets yielded highly similar results (Fig. 1): The average within-species diversity in all four invertebrate groups was higher than that of vertebrates, mollusks being the most diverse and mammals the least diverse, on average. This is essentially in agreement with our intuition about species abundance in these taxa. The mtDNA data diversity, however, was highly variable between species within a group, but remarkably homogeneous between groups (Fig. 1). Insect or mollusk species did not appear more polymorphic, on average, than mammals or birds, contradicting our prior beliefs about relative population sizes in these taxa. The average invertebrate mtDNA diversity (7.67%) was not appreciably different from the vertebrate one (7.99%), whereas the nuclear invertebrate average (2.46%) was four times as high as the vertebrate one (0.60%).

A series of within-group analyses were conducted to examine the influence of specific ecological variables (Table 1). Allozyme data again agreed with our intuition about population sizes: Among mollusks, the terrestrial pulmonates were substantially less polymorphic than marine bivalves or gastropods, consistent with the enormous dispersal potential of the latter; among crustaceans, the microscopic, planktonic branchiopods (e.g., *Artemia* and *Daphnia*) appeared much more diverse than the larger decapods (shrimps, lobsters, and crabs); among fish, marine species showed a significantly higher heterozygosity than the geographically restricted freshwater species. The mtDNA diversity, in contrast, failed to reflect these differences in average population size. Again, a homogeneous average nucleotide diversity was found, irrespective of body size and ecology (Table 1). Freshwater fish species were even significantly more polymorphic than marine ones.

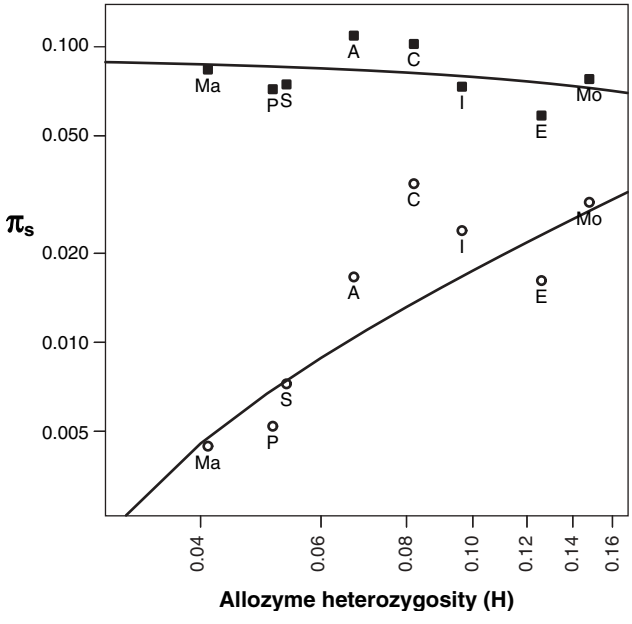

Fig. 1. Average allozymic, nuclear DNA, and mtDNA diversity in eight animal taxa. *x* axis: allozyme average heterozygosity. *y* axis: circles, nuclear DNA average synonymous diversity (kendall test: $\tau = 0.87$, $P < 0.05$); squares, mtDNA average synonymous diversity (kendall test: $\tau = -0.14$, not significant). Ma: Mammalia (allozymes: 184 species; nuclear: 30 species; mtDNA: 350 species); S: Sauropsida (reptiles and birds: 116, 20, 378); A: Amphibia (61, 4, 96); P: Pisces (bony fish and cartilaginous fish: 183, 22, 270); I: Insecta (156, 73, 511); C: Crustacea (122, 2, 78); E: Echinodermata (sea stars and urchins: 15, 14, 47); and Mo: Mollusca (46, 9, 125). The nuclear averages of the little-represented Amphibia (four species) and Crustacea (two species) are shown but were not used for the statistical test.

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Variations in mitochondrial mutation rate among phyla could be invoked to explain the discrepancy between animal mtDNA diversity and effective population size. The mutation rate, however, would have to be inversely related to population size throughout animal taxa to explain the data—a pattern very unlikely to appear by chance. Demographic stochasticity, e.g., recurrent population bottlenecks, could remove the effect of equilibrium population size on genetic diversity (*20*). Demographic effects, however, should affect the nuclear genome as well, which is not what we observe. Natural selection, either purifying or adaptive, must therefore be invoked to explain the locus-specific behavior of mtDNA.

Purifying selection against deleterious mutations (so-called background selection) decreases the diversity at linked loci through hitch-hiking. The strength of this effect depends on the distribution of fitness effects among mutations, and one generally still expects an increase of diversity with population size under background selection (*21*), which is not consistent with the homogeneous mtDNA diversity distribution. Our analytical results confirmed this statement: The conditions under which background selection can lead to a more or less independent relationship between diversity and effective population size appear implausible (fig. S2).

The mtDNA pattern, however, appears to be in good agreement with the hypothesis of recurrent fixation of advantageous mutations leading to frequent loss of variability at linked loci (*7*, *22*), a process recently named "genetic draft" by Gillespie (*23*). The population number of advantageous mutations per generation obviously increases with population size and compensates the decrease of genetic drift in Gillespie's (*24*) simulations, which predict an essentially flat, even negative, relationship between genetic diversity and population size. The gene-dense, nonrecombining context of the animal mitochondrial genome maximizes the potential impact of the genetic draft, as compared with that of the nuclear genome (*25*).

To firmly distinguish between the two selective models, we examined the pattern of nucleotide substitution between species. The neutrality index (NI) (*26*) was first calculated when outgroup sequences were available. This index aims at comparing the ratio of nonsynonymous (amino acid–changing) to synonymous (silent) changes within species (π_N/π_S) and between species (d_N/d_S): NI is 1 when evolution is neutral, greater than 1 under purifying selection, and less than 1 in the case of adaptation. A significant shift toward values less than 1 was detected in invertebrate mtDNA loci, consistent with the adaptive hypothesis (Fig. 2 and fig. S1).

This result, limited to the genes for which polymorphism data are available, was confirmed by a whole-genome mitochondrial analysis. The d_N/d_S ratio was calculated for the 13 mitochondrial protein-coding genes in various animal taxa (Table 2). The average genomic d_N/d_S was significantly higher in invertebrates than in vertebrates. This is not consistent with a model invoking solely purifying selection, because the rate of fixation of deleterious mutations is expected to decrease with population size. Observing a higher rate of nonsynonymous substitution, but not a higher level of diversity, in large populations strongly corroborates the hypothesis that positive selection drives mitochondrial evolution in animals: Neither negative selection (which should decrease d_N/d_S and increase NI) nor a relaxation of constraints (which should increase the diversity) can explain this pattern. The additional amino acid substitutions detected in invertebrates would correspond to adaptive changes, plus the deleterious ones hitch-hiking to fixation—the rate of deleterious substitution is expected to increase with population size in the genetic draft model (*24*).

This study reveals that the mitochondrial diversity of a given animal species does not reflect its population size: No correlation between mtDNA polymorphism and species abundance could be detected, despite the large body of data analyzed. Nuclear data, in contrast, are fairly consistent with intuitive expectations. We conclude that natural selection acting on mtDNA contributes to homogenization of the average diversity among groups, in agreement with the genetic draft theory. mtDNA appears to be anything but a neutral marker (*16*) and probably undergoes frequent adaptive evolution, e.g., direct

Table 1. Ecological determinism of allozyme and mtDNA genetic diversity. The numbers of species used are shown in parentheses.

Taxon		Allozymes (H, %)	mtDNA (π_s, %)
Fish	Freshwater	4.7 (71)	8.7** (123)
	Marine	6.1* (65)	3.7 (51)
Crustaceans	Large benthic	4.6 (81)	10.1 (26)
	Small planktonic	21.0* (8)	5.8 (6)
Mollusks	Terrestrial	7.4 (23)	7.8 (8)
	Marine	30.0** (17)	5.6 (34)

*$P < 0.05$ (Student's t test). **$P < 0.01$ (Student's t test).

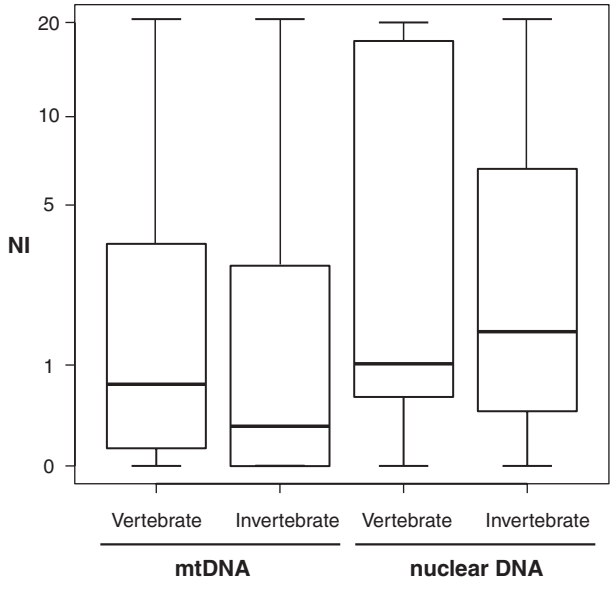

Fig. 2. Neutrality index (NI) distributions (logarithmic scale). Medians are indicated by thick horizontal bars. Boxes include 50% of the distributions. The invertebrate mtDNA median NI (0.42) is significantly lower than the vertebrate one (0.88; $P < 10^{-3}$, Mann-Whitney test). NI values greater than 20 were forced to 20 for clarity. Low-frequency (<0.125) polymorphic sites were excluded from the analysis.

Table 2. Mitochondrial genomic d_N/d_S ratio in animals.

Taxon	Data sets	d_N/d_S
	Vertebrates	
Mammalia	21	0.080
Sauropsida	9	0.121
Amphibia	12	0.086
Teleostei	44	0.065
Chondrichthyes	2	0.077
Average		0.086
	Invertebrates	
Insecta	4	0.198
Crustacea	5	0.084
Mollusca	2	0.122
Echinodermata	1	0.106
Nematoda	2	0.219
Chelicerata	6	0.138
Platyhelminthes	1	0.140
Urochordata	1	0.188
Cnidaria	2	0.167
Average		0.151 **

**$P < 0.01$ (Student's t test).

selection on the respiratory machinery (*27*), nucleo-cytoplasmic coadaptation (*28*), two-level selection (*29*), or adaptive introgression, perhaps hitchhiking with a maternally transmitted parasite (*30*). mtDNA diversity is essentially unpredictable and will, in many instances, reflect the time since the last event of selective sweep, rather than population history and demography. Low-diversity mitochondrial lineages, typically disregarded as important from a conservation standpoint, might sometimes correspond to recently selected, well-adapted haplotypes to be preserved.

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31. This work was supported by Action Concertée Incitative IMPBio MODEL_PHYLO from the French Ministère de la Recherche. Many thanks to K. Dawson, N. Bierne, and four anonymous reviewers for their comments on an earlier version of this manuscript. The authors declare that they have no competing financial interests.

Supporting Online Material

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Materials and Methods
Figs. S1 to S5
References and Notes

31 October 2005; accepted 28 February 2006
10.1126/science.1122033

Proapoptotic BAX and BAK Modulate the Unfolded Protein Response by a Direct Interaction with IRE1α

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Accumulation of misfolded protein in the endoplasmic reticulum (ER) triggers an adaptive stress response—termed the unfolded protein response (UPR)—mediated by the ER transmembrane protein kinase and endoribonuclease inositol-requiring enzyme–1α (IRE1α). We investigated UPR signaling events in mice in the absence of the proapoptotic BCL-2 family members BAX and BAK [double knockout (DKO)]. DKO mice responded abnormally to tunicamycin-induced ER stress in the liver, with extensive tissue damage and decreased expression of the IRE1 substrate X-box–binding protein 1 and its target genes. ER-stressed DKO cells showed deficient IRE1α signaling. BAX and BAK formed a protein complex with the cytosolic domain of IRE1α that was essential for IRE1α activation. Thus, BAX and BAK function at the ER membrane to activate IRE1α signaling and to provide a physical link between members of the core apoptotic pathway and the UPR.

Cell viability depends on the functional and structural integrity of intracellular organelles. Multidomain proapoptotic BAX and BAK proteins function in concert as essential gateways to intrinsic cell death pathways operating at mitochondria (*1*). Several anti- and proapoptotic BCL-2 family members also localize to the ER and modulate steady-state calcium homeostasis (*2–4*). In higher eukaryotes, ER stress stimulates three distinct UPR signaling pathways through sensors that include IRE1α (also described as inositol-requiring transmembrane kinase and endonuclease 1α), PERK (protein kinase–like ER kinase), and ATF6 (activation of transcription factor 6) (*5*, *6*). IRE1α is a serine-threonine protein kinase and endoribonuclease that, on activation, initiates the unconventional splicing of the mRNA encoding X-box–binding protein 1 (XBP-1) (*7–9*). Spliced XBP-1 is a potent transcriptional activator that increases expression of a subset of UPR-related genes (*10*). The cytosolic domain of activated IRE1α binds the adaptor protein TRAF2 [tumor necrosis factor (TNF)–associated factor 2], and triggers the activation of the c-Jun N-terminal kinase (JNK) signaling pathway (*11*, *12*). Activated PERK directly phosphorylates and inhibits the translation initiation factor eIF2α (thus decreasing protein loading into the ER) and induces expression of the transcription factor ATF4, which increases expression of certain UPR genes such as *Chop* or *GADD153* and *Grp78* or *BiP* (*5*, *7*). BiP is a chaperone that maintains PERK and IRE1α in an inactive state. However, in cells undergoing ER stress, BiP preferentially binds to misfolded proteins, thereby releasing the stress sensors to undergo activation by homodimerization and autophosphorylation (*13*, *14*).

Double knockout (DKO) cells from BAX-BAK–deficient mice are resistant to proapoptotic agents that induce the UPR (*1*) and also show a defect in steady-state ER calcium homeostasis under nonapoptotic conditions (*3*). A validated in vivo model for ER stress uses intraperitoneal injection of tunicamycin (Tm, an inhibitor of N-linked glycosylation) (*15–17*). This treatment triggers a stress response in the liver and kidney that causes extensive cell death in these organs after several days of treatment. Most DKO mice (more than 90%) die during embryogenesis (*18*), as a result of developmental defects. We generated a conditional BAX-BAK DKO model in which a *bax* allele flanked with LoxP sites was targeted in *bak*-null embryonic stem cells (*19*). To achieve inducible deletion of *Bax* in adulthood, MxCre^{+}*bax*$^{fl/-}$*bak*$^{-/-}$ and control MxCre^{+}*bax*$^{+/-}$*bak*$^{-/-}$ mice were treated with poly(IC). Mice were subsequently injected with Tm (1 μg/g body weight) and

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