

Prokaryote and eukaryote evolvability

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Abstract

The concept of evolvability covers a broad spectrum of, often contradictory, ideas. At one end of the spectrum it is equivalent to the statement that evolution is possible, at the other end are untestable post hoc explanations, such as the suggestion that current evolutionary theory cannot explain the evolution of evolvability. We examine similarities and differences in eukaryote and prokaryote evolvability, and look for explanations that are compatible with a wide range of observations. Differences in genome organisation between eukaryotes and prokaryotes meets this criterion. The single origin of replication in prokaryote chromosomes (versus multiple origins in eukaryotes) accounts for many differences because the time to replicate a prokaryote genome limits its size (and the accumulation of junk DNA). Both prokaryotes and eukaryotes appear to switch from genetic stability to genetic change in response to stress. We examine a range of stress responses, and discuss how these impact on evolvability, particularly in unicellular organisms versus complex multicellular ones. Evolvability is also limited by environmental interactions (including competition) and we describe a model that places limits on potential evolvability. Examples are given of its application to predator competition and limits to lateral gene transfer. We suggest that unicellular organisms evolve largely through a process of metabolic change, resulting in biochemical diversity. Multicellular organisms evolve largely through morphological changes, not through extensive changes to cellular biochemistry.

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1. Introduction

Evolvability is a central concept in evolution but is easily misconstrued, hence its use must be defined carefully. At a basic level, evolvability is the fundamental concept of evolution. From the late-17th to mid-19th centuries it was generally assumed that species had an unchangeable ‘essence’. This Platonic concept was introduced in the late-17th century when it became increasingly clear that continuing spontaneous generation of larger life forms did not occur (see

Farley, 1977). If species had an unchangeable essence then, by definition, there could be no evolution, even if individual organisms deviated from the ‘ideal type’. ‘Evolvability’, by denying species have an unchangeable essence, is central to evolution. Since all evolutionists agree, this definition is not that interesting.

Burch and Chao (2000) offer a more limited definition, “the ability to generate adaptive mutations”. We consider the two aspects of this definition: ‘adaptive mutations’ and ‘ability to generate’. That adaptive mutations occur is the evolvability concept from the previous paragraph, but in modern terminology: some mutations are advantageous. In the early 19th century many accepted selection, but only in elimination of deleterious variants. Selection, by eliminating such

Abbreviations: ESND, evolutionarily-stable niche-discontinuity; PSF, periodically-selected function

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variants, tended to preserve the unchanging essence of the species. In contrast, the existence of adaptive variants and positive selection allows evolution through time and is an essential part of evolvability.

The ‘ability to generate’ adaptive mutations is more problematic, and is mirrored in Kirschner and Gerhart’s (1998) definition: ‘the *capacity to generate* (our emphasis) heritable, selectable phenotypic variation’. If it is simply the observation that advantageous mutations occur, then, again, the usage is uncontroversial, though uninteresting. If it implies that advantageous mutations can be generated ‘on demand’ (e.g. Cairns et al., 1988) then it is a specialised (and controversial) usage. Some discussions on evolvability appear to give the impression of ‘the more change the better’—yet most major change is highly deleterious. For instance, Radman et al. (1999) point out that selection for increased fidelity of DNA synthesis has been achieved with *Escherichia coli* in the laboratory (Fijalkowska et al., 1993), and that this demonstrates ‘there was no durable selective pressure in nature for maximal fidelity’.

However, the majority of discussions on evolvability (e.g. Wagner and Altenberg, 1996; Wagner, 1996; Kirschner and Gerhart, 1998; Partridge and Barton, 2000), acknowledge directed mutation is not required to understand evolvability. Nevertheless, confusion arises easily, as shown by reactions to work from Lindquist’s group (Rutherford and Lindquist, 1998; True and Lindquist, 2000). Other workers (Dickinson and Seger, 1999; Partridge and Barton, 2000) concluded that these authors favoured the idea that certain traits have been selected for their utility to contribute to organismal evolvability, *and nothing else*. While Lindquist points out that this was never her interpretation (Lindquist, 2000), the subsequent correspondence generated by this work (Dickinson and Seger, 1999; Partridge and Barton, 2000; Dover, 2000) illustrates how problematic this concept can be. There is no agreed definition for evolvability that explicitly avoids the problem of evolutionary forethought. Indeed, whenever the phrase ‘the evolution of evolvability’ is used, there is the possibility of it being misconstrued. This is not because evolvability cannot evolve through accepted processes of evolution. Rather, under known processes of Darwinian evolution, evolvability cannot evolve *in itself* because the origin and maintenance of a trait would have to precede selection for the trait.

Evolvability can be a by-product of selection however. For example, activation of a transposable element might lead to a mutation that is selected, thereby inadvertently leading to additional mutations (through additional element insertions) in the future. Such future mutations may be deleterious or advantageous; the increased mutation rate is a by-product of the transposable element hitchhiking with the selected mutation.

Still at issue is the evolutionary origin of traits that contribute to evolvability and adaptive mutations. Examination of the origins of such traits is an important step in alleviating controversy surrounding this area. This is particularly so with evolvability in multicellular organisms, where one gets the impression that we should be in awe of the exciting molecular and genetic mechanisms that contribute to eukaryote evolvability (Kirschner and Gerhart, 1998; Herbert and Rich, 1999). Other reviews on the evolution of evolvability (e.g. Partridge and Barton, 2000; Kirschner and Gerhart, 1998; Moxon and Thaler, 1997) identify mechanisms by which genome architecture can influence this (see also Table 2).

This paper is divided into sections that discuss the following topics:

Section 2: Selection versus neutral evolution.

Section 3: Parasites: evolvability or reductive evolution?

Section 4: Consequences of prokaryote and eukaryote genome architecture.

Section 5: The stress response and evolvability.

Section 6: An ecological perspective: evolutionarily-stable niche-discontinuity (ESND).

Section 7: Plasticity, learning and evolvability.

Briefly, Section 2 gives a theoretical frame of reference from which to consider the possibility that not all complex traits that impact on evolvability are necessarily a product of selection. In Section 3, we aim to emphasise that, despite parasitism having arisen multiple times in evolution, there was probably nothing about the ancestral groups from which various parasites emerged that made them inherently more ‘evolvable’. Rather, ecological limitations, and phenomena consequent to parasitism may better explain parasite evolution—parasites do not constitute a ‘special case’ with regards the ‘evolution of evolvability’. In Section 4 we consider the origins and impact of a range of phenomena that contribute to the genome

architectures and gene expression systems of eukaryotes and prokaryotes (bacteria and archaea). We conclude that eukaryote architecture largely arose through neutral mechanisms, while reductive evolution was central to the emergence of prokaryote architecture. The consequences of these mechanisms for evolvability in prokaryotes and eukaryotes are discussed. In [Section 5](#), the prokaryote–eukaryote division becomes blurred by considering the consequences of environmental stress for evolvability: constitutive multicellular eukaryotes with complex development are separated from ‘simple’ eukaryotes and prokaryotes. A contrast is drawn between periods where either genetic stability or change will be selectively advantageous. Experimental data, both with bacterial and metazoan models, point towards a general response to stress as being important in understanding how traits contributing to evolvability may have hitchhiked on survival of individuals. We focus on hypermutation (the elevation of mutation rate, either at a specific locus, or across the entire genome) and horizontal gene transfer (including meiotic recombination).

In [Section 6](#) we discuss how the physical and biotic environment limits evolvability, allowing a distinction to be drawn between potential and realised evolvability ([Fig. 2](#)). Our model, which we call evolutionarily-stable niche-discontinuity (ESND), describes how competition allows colonisation of a fitness peak, and subsequently, how intraspecific competition limits movement away from that peak ([Fig. 1](#)). Examples of interspecies competition and predator–prey coevolution are considered, and are aimed at understanding evolvability in eukaryotes. The likely effects of horizontal gene transfer on ESNDs in bacteria are also considered.

Finally, we briefly consider phenotypic plasticity in eukaryotes. We are not aware of clear examples of non-genetic components to phenotypic plasticity in prokaryotes. However, ‘social behaviour’ and the emergence of phenotype switching in bacteria may be interesting in this regard.

2. Selection versus neutral evolution

There are strong parallels in the evolution of complexity and the evolution of evolvability. Neither complexity nor evolvability can be directly selected for;

both impact future evolution, and hence are in violation of evolution as tinkering. [Szathmáry and Maynard Smith \(1995\)](#) point out that ‘There is no theoretical reason to expect evolutionary lineages to increase in complexity with time, and no empirical evidence that they do so’. Unlike with evolvability however, there is little apparent controversy here. It is accepted that complexity is sometimes a consequence of evolution, but not a predictable outcome of evolution. Reductive evolution in parasites and eukaryotic organelles are important counter-examples (see below).

How can we account for traits that contribute to complexity which are conserved in most eukaryotes when we know that, as with evolvability, complexity is not directly selectable? It is not sufficient to claim that a trait conserved across a broad range of species is evidence for selection. A recent example is the claim that junk DNA has a function because a survey of genome size shows that it correlates with cell size in cryptomonads ([Beaton and Cavalier-Smith, 1999](#)). The argument seems to be that selection for increased cell size has led to the expansion of junk regions because these take up space, and therefore the amount of DNA can ‘specify’ cell size. Correlation is ambiguous, and in this case it is unclear which is cause and which effect. Junk DNA may persist because it has not been selected against.

[Gibson \(2000\)](#) points out that the tendency for researchers to give post hoc adaptationist explanations is still alive and well in developmental biology. He writes that, ‘selection should only be invoked when the null hypothesis of neutrality cannot explain the data’.¹ In molecular evolution the importance of neutral evolution is often taken into account, and extremely complex traits such as the spliceosome, mRNA editing in trypanosomes, and the scrambled genes of ciliates have been argued to be neutral ([Stoltzfus, 1999](#)). While it is not certain if any of these traits originated through neutral evolution, the idea is an important one, since it shifts theorising away from post hoc explanations,

¹ This criticism of post hoc explanations is similar to [Gould and Lewontin’s \(1979\)](#) critique of the ‘adaptationist program’, that everything about an organism can be explained as aiding some aspect of its life cycle. Post hoc explanations may nevertheless be correct (equally, ‘good’ theories can be incorrect). The aim should be to reformulate them into testable hypotheses, and to look for explanations that account for a range of phenomena (not just the original observation that led to the hypothesis).

and frames the problems in the manner advocated by Gibson (2000).

3. Parasites: evolvability or reductive evolution?

Parasites are interesting in regard to evolvability because they represent a strategy common to eukaryotes, prokaryotes, viruses, and selfish elements. Parasites are often fast-evolving, and the move from a non-parasitic to a parasitic lifestyle has occurred frequently. We consider the following questions:

- Were ancestral groups from which parasites arose inherently more ‘evolvable’?
- If there is fast evolution in parasites, are they inherently more evolvable?
- Is the concept of evolvability useful here?

Parasitism is widespread—in plants, fungi, insects, worms, protists, bacteria, etc. Conspicuously absent are parasitic tetrapods (mammals, birds, amphibians and reptiles). Is this due to limited evolvability or an ecological limitation? We think the latter. The dependence of the eutherian embryo on the mother for nutrients is much like the dependence of endoparasitic larvae on the host for nutrition (Grbic, 2000). Suckling in mammals, and nutritional dependence of juvenile birds and mammals on parents might to a lesser extent be seen in this light. Indeed, juvenile parasitic stages in early development serve the same role as parentally-supplied nutrition, and it is worth noting that egg-yolk mass has become reduced in endoparasitic wasps (Grbic, 2000). Clearly, this modus operandi of early development has been made use of in mammals, and absence of true parasitism in tetrapods may simply reflect an absence of niches, though a few examples such as brood parasitism exist.

What distinguishes lineages that have become obligate parasites from those that are free-living? The discussion above suggests it is the presence of an available niche, not limits on evolvability. However, there must be adaptation in order to secure nutrients from the host, fine-tune development to coincide with host life cycle, and not kill the host before the parasite has matured or moved to the next host. Studies of unicellular parasite genomes suggest that the loss of traits no longer required in the parasitic lifestyle accounts for most change. For example, in the

Rickettsiae, adenosylmethionine synthetase is in the process of being lost from the genomes of this genus (Andersson and Andersson, 1999). Likewise, cases of loss from parasitic genomes of primary biosynthetic pathways, such as amino acid synthesis and de novo pathways for deoxyribonucleotide synthesis (Fraser et al., 1998; Andersson et al., 1998b) are consequent to the evolution of mechanisms for extracting these nutrients from the host.

Genome reduction and higher rates of evolution appear to be general features of parasitic genomes, being reported in leprosy bacillus (Cole et al., 2001), the obligate intracellular parasites *Chlamydia* (Kalman et al., 1999) and *Buchnera*, and other endosymbiotic bacteria (Moran and Baumann, 2000). Genome reduction is likely a consequence of redundancy, while the higher rates of evolution seen in parasites are attributable to Muller’s Ratchet, the fixation of slightly deleterious mutants within small asexual populations (Moran, 1996).

Genome reduction is extreme in chloroplasts (McFadden, 1999), mitochondria (Gray et al., 1999), and nucleomorphs (the remains of nuclei in secondary endosymbionts; Douglas et al., 2001). There is a difficulty in separating selection for evolvability per se from other potential selective pressures. Reductive evolution, and increased rates of evolution are consequences of parasitism or endosymbiosis, so while the process of adaptation can be extensively studied, the initial conditions cannot. What can be said is that to the parasite or endosymbiont, the host is a resource, so general models of evolvability are likely to be useful in understanding parasitism. In the following section, we consider this problem in greater depth.

4. Consequences of prokaryote and eukaryote genome architecture

In this section, we argue that many complexities of the eukaryote genome can be explained by the null hypothesis of neutralism, while the prokaryote genome cannot. It is almost universally assumed that eukaryotes evolved from ancestral prokaryote forms, an assumption that seems intuitively correct. However, it is just that—an intuitive bias that simple evolves to complex—and is taken as given by a large majority of researchers (see Forterre and Philippe, 1999 for

critique). An extensive body of literature and ongoing research challenges this notion (Reaney, 1974; Darnell and Doolittle, 1986; Forterre, 1995; Poole et al., 1998, 1999; Forterre and Philippe, 1999; Penny and Poole, 1999; Glansdorff, 2000). What is important for evolvability studies is that the assumption of a prokaryote to eukaryote transition effectively removes selection from discussions on the evolution of prokaryotes—they are by definition the ancestral state. Since the direction of change is assumed to be from simple prokaryote cells to complex eukaryote cells, the question becomes, by default, what drove eukaryote genomes to become so complex? We will argue that factors affecting the origin of prokaryotic genome organisation are equally important. This is an important point, since it changes our view of the evolution of genomic features contributing to evolvability.

Key aspects of eukaryotic genome architecture appear to be conserved from a very early period in evolution, pre-dating the last universal common ancestor (LUCA). In contrast, prokaryote genome architecture results from one or more periods of reductive evolution (Forterre, 1995; Forterre and Philippe, 1999; Poole et al., 1999; Penny and Poole, 1999). Our argument is based on extant genome architectures and the observation that the greatest diversity of RNA world relics (RNAs that appear to predate the origins of proteins and DNA) are found in eukaryotes. For prokaryotes, both the loss of ancient RNA genes and their compact genome architecture can be explained in terms of reductive evolution.

Some of our reasoning is given below, but it is not necessary to accept all our conclusions to accept our general argument on eukaryote and prokaryote evolvability. Our conclusions are consistent with Kirschner and Gerhart's (1998) description of prokaryote and eukaryote modes of evolvability. Prokaryotes, 'have undergone limited morphological change but instead have achieved extensive biochemical diversification'. Similarly, multicellularity in eukaryotes, specifically metazoa, 'achieved extensive control over the milieu of internal cells and generated many physiologically sensitive micro-environments in that milieu'. In this latter multicellular group, biochemical evolution is limited, and cells receive a more constant level of nutrition with little or no variation in the *type* of nutrients available. If evolution is biochemically conservative in metazoa and biochemically innovative in prokaryotes,

it is perhaps no surprise to find ancient biochemical traits conserved in eukaryotic cells, while these have been lost or replaced in prokaryotes.

Broad differences between eukaryote and prokaryote lifestyle have been described in terms of *r*- and *K*-selection (Carlile, 1982), terms derived from the equation for the rate of population growth (Box 1). Relative to prokaryotes, eukaryotes are *K*-selected, where *K*-selected organisms are broadly defined as having a relatively slow rate of reproduction and longer generation time, a stable (though limiting) nutrient supply, relatively stable populations, and are larger in size. In contrast, prokaryotes are relatively more *r*-selected, with faster reproduction and short generation times, small size, fast response times to a fluctuating nutrient supply, and with large fluctuations in population size. There is a spectrum of values with perhaps *E. coli* and yeast near the *r*-selection end, and elephants and oak trees near the *K*-selection end of the spectrum.

4.1. Prokaryote genomes

Prokaryote genomes possess only one origin of replication per chromosome. Consequently, genome size limits the rate of chromosome replication. Since fidelity is also affected by replication rate, replication rate will be constrained by the need to faithfully copy and maintain the genome.

Transient global hypermutation (Section 5) occurs in stationary phase (Table 1), whereas selection for fast replication operates during periods of exponential growth. There is no precedent for assuming that higher mutation rates will be selected for during exponential growth where proliferation of a successful strategy is required. Rather, a quick response to nutrient availability, followed by clonal proliferation, is advantageous. *r*-selection revolves around competition (during exponential growth) for resources that fluctuate in availability, and this places the reproductive rate under selection (Table 2).

That replication is rate-limiting during exponential growth has been documented for *E. coli*, where genome doubling takes 1 h, and cell number doubling occurs every 20 min (Alberts et al., 1994). The effect on the genome is straightforward—anything that can be lost will eventually be lost. Selection does not distinguish between junk, and what may be advantageous

Box 1. *r*- and *K*-selection

Rate of population growth, *R*, is given by the equation:

$$R = \frac{dN}{dt} = rN \left(1 - \frac{N}{K} \right)$$

where *r* is the maximum intrinsic rate of increase for a population; *N* the number of organisms and *K* the carrying capacity (of the environment).

r-selected organisms

Small
High reproductive rates
Short life cycles
Live in unpredictable environments
Fluctuation in resource availability
and type requires fast response times
Population size varies hugely

K-selected organisms

Large
Lower, more constant, reproductive rate
Longer life cycles
Live in more stable environments
Resources in more constant supply
(though limited in amount)
Population size relatively stable

r- and *K*-selection are best considered as relative measures. While specific application of this concept is problematic (organism A may be *r*-selected relative to organism B, but *K*-selected relative to organism C), it is no more problematic than fitness, which is also a relative measure. The concept is useful in general discussions such as this since it aims to explain many aspects of prokaryotes and eukaryotes, rather than invoke special explanations for each feature.

later (e.g. on a new nutrient source), so even essential functions required only periodically may be lost from the genome. It is therefore of little surprise to find that, in both *E. coli* and *Salmonella enterica*, genome size varies within species by around 20%. Similar variability is found in *Helicobacter pylori* and *Neisseria meningitidis*, and is interpreted as different genes being maintained in different isolates, which often inhabit different niches (Lan and Reeves, 2000).

Periodically-selected functions (PSFs) are regularly lost from individuals, but are maintained in bacterial populations through lateral gene transfer. PSFs are essential in the long term, given that environmental fluctuation is normal and that organisms must continually cope with such fluctuations. In a completely clonal population where replication time is rate limiting, PSFs would be irreversibly lost. Constant selection of PSFs within a population, coupled with lateral transfer is likely central to prokaryote genome architecture and evolvability, permitting maintenance of PSFs crucial to long term survival under conditions where these are frequently lost.

Plasmids are a complete transferable unit that can be immediately expressed, but do not increase the replication time of the genome. While a genomic copy of a PSF must be lost through gene decay (mutations and deletions) and reestablishment requires reinsertion, a plasmid can be lost without gene decay (this would be advantageous during exponential growth), is readily reacquired, and can be replicated in parallel with the genome. Supernumerary chromosomes in fungi have been likened to plasmids, as they are not permanent and in several cases have been found to carry genes for pathogenicity, detoxification of host antimicrobials, and antibiotic resistance (Covert, 1998).

An obvious solution to the prokaryote dilemma is to distribute genes across several chromosomes (i.e. multiple *chromosomes*—not multiple copies of the same chromosome—constitute the haploid genome) and with multiple origins of replication, thereby permitting a larger genome without slowing replication. A number of prokaryote genomes are spread across multiple chromosomes, and some may possess more genes than yeast (Bendich and Drlica, 2000). Circular

Table 1
Examples of stress response which may affect evolvability

Mechanism	Activating stress	Organism(s)	Notes	References
Prokaryotes				
Global hypermutation	Occurs in stationary phase, so likely to be a starvation response	<i>E. coli</i> ; <i>Pseudomonas putida</i>	Hypermutation is transient, recombination-dependent and in stationary phase	Torkelson et al., 1997; McKenzie et al., 2000; Kasak et al., 1997
Local hypermutation (contingency loci)	Recurrent selection, such as in host–parasite coevolution	<i>Haemophilus influenzae</i> ; <i>E. coli</i> ; <i>S. typhimurium</i>	Phenotypic switching of surface antigens, hypermutable virulence factors, V(D)J hypervariability	Moxon et al., 1994; Hood et al., 1996; Muramatsu et al., 2000; Revy et al., 2000
Gene amplification	Occurs in stationary phase, thus likely to be a starvation response	<i>S. typhimurium</i> ; <i>E. coli</i>	Requires residual activity at amplified locus; in late arising colonies	Andersson et al., 1998a; Powell and Wartell, 2001; Hastings et al., 2000
Genetic competence (DNA uptake)	Occurs in stationary phase	<i>B. subtilis</i> ; <i>Streptococcus pneumoniae</i> ; <i>H. influenzae</i>	Extracellular signalling molecules indicate a cell density ‘quorum’ which establishes competence	Solomon and Grossman, 1996; Tortosa and Dubnau, 1999
Sporulation		<i>B. subtilis</i>	Sporulation controlled by the same pathway as competence.	Levin and Grossman, 1998
Cell–cell interaction	Starvation	<i>Stigmatella auantiaca</i> ; <i>Myxococcus xanthus</i>	Sporulation in response to starvation in myxobacteria	Ward and Zusman, 1999; Plaga and Schairer, 1999
Eukaryotes				
Sexual sporulation	Starvation	<i>Saccharomyces cerevisiae</i> ; <i>A. nidulans</i>	<i>Saccharomyces</i> enters meiosis upon nitrogen starvation; <i>Aspergillus</i> sporulates sexually at low glucose; at high glucose it switches to asexual sporulation (dispersal)	Banuett, 1998; Adams et al., 1998
Supernumerary chromosomes		Fungi	Not usually stably maintained in the genome, cf. plasmids	Covert, 1998
Cell–cell interaction	Starvation	<i>S. cerevisiae</i> ; <i>D. discoideum</i>	In yeast, connecting filaments form between cells; starvation promotes fruiting body and spore formation	Varon and Choder, 2000; Crespi, 2001
PSI-dependent translation readthrough.	Heat shock protein-mediated	<i>S. cerevisiae</i>	PSI normally translation terminator; prion form results in readthrough	True and Lindquist, 2000
HSP 90-mediated phenotype exploration.	Heat stress, other stresses involving HSP 90	<i>Drosophila melanogaster</i>	Titration during heat stress may lift buffering, hidden phenotypes tested	Rutherford and Lindquist, 1998
Local (somatic) hypermutation	Host–parasite interactions	Mammals	Hypermutation of V(D)J genes in antibody formation	Jacobs and Bross, 2001

Table 2
Aspects of evolvability in prokaryotes, ‘simple’ and ‘complex’ eukaryotes

Prokaryotes	‘Simple’ eukaryotes	‘Complex’ eukaryotes
Reductive evolution Ancestral biochemistry replaced		Complexity, cell specialisation Ancestral biochemistry conserved
Fast doubling times during exponential growth	Relatively fast doubling times during exponential growth	Relatively constant reproductive rate
Rapid biochemical responses to environment	Rapid biochemical responses to environment Some nutrient storage	Fast response to environment usually behavioural Stable internal environment Nutrient storage (adipose tissue, glycogen, starch)
Limited RNA processing Coupled transcription and translation	Extensive transcript processing Physically separated transcription and translation	Extensive transcript processing Physically separated transcription and translation
Small, compact genomes Single origin of replication strongly limits chromosome size ‘In-series’ copies of genes selected against ‘Parallel’ copies (polyploidy) permit ‘genome stockpiling’ Redundancy may buffer deleterious mutation during stationary phase hypermutation (Finkel and Kolter, 1999)	Intermediate genome size, compact Multiple origins of replication per chromosome	Size limit determined by replication fidelity Multiple origins of replication per chromosome ‘In-series’ copies of genes not inherently disadvantageous Ploidy number relatively stable*
PSFs lost and regained through horizontal transfer	PSFs lost and regained through horizontal transfer	Use once per generation is sufficient for retention (less for recessive and imprinted genes)
New functions arise through gene duplication and recruitment in polyploids	New functions arise through gene or genome duplication, junk recruitment	Many new functions arise through gene or genome duplication, junk recruitment
Cell specialisation rare	Division of labour among ‘obligate cooperators’ leads to cell specialisation Limited investment in specific structures for nutrient acquisition and storage	Extensive cell specialisation—irreversible developmental fates of cells, tissues and organs, larval and adult stages in metazoa, polyphenic insects Heavy investment in specific structures (organs, mechanical tools) for nutrient acquisition and storage, defence or competition)
Developmental pathway regulation linked to environmental cues (fruiting body formation, genetic competence, biofilm, regulation of virulence—Table 1 in Crespi, 2001)	Developmental pathway regulation largely linked to environmental cues	Regulation of developmental pathways less dependent on environmental cues, with greater internal control

The above are generalisations to which there must be exceptions. *r*- and *K*-selection are relative measures, best described in terms of a spectrum (schematically shown by bar at top). For instance, unicellular eukaryotes are *r*-selected relative to their multicellular relatives, and many of the points listed under prokaryotes apply to this group. Transcript processing and junk accumulation is less extensive in unicellular than multicellular eukaryotes, operons and PSFs are a feature of their genomes, and developmental regulation is tightly linked to environmental cues. Constitutive multicellularity makes horizontal transfer unlikely, while unicellular eukaryotes may acquire new functions through transfer.

chromosomes with single origins of replication nevertheless place limits on individual chromosome size.

That circular chromosomes are only found in prokaryotes (eukaryote organelles of prokaryote origin—mitochondria, hydrogenosomes and chloroplasts—excepted) may be historical accident. Forterre (1995) has argued that the prokaryote lineages arose through adaptation to high temperatures (the thermoreduction hypothesis). Currently this is the best explanation for the presence of circular chromosomes in prokaryotes; circular DNA is more thermostable than linear DNA (Marguet and Forterre, 1994). Other data are also consistent with thermoreduction (Poole et al., 1999; Penny and Poole, 1999), and while some prokaryotes possess linear genomes (Bendich and Drlica, 2000), this state appears derived (Poole et al., 1998, 1999).

The main effect of a single origin of replication and selection for fast cell division on evolvability is that single genes or even whole operons can be transferred. The consequence of horizontal gene transfer for organismal competition will be considered further in Section 6, and the effect and consequences of multicopy genomes are discussed in Section 5.

4.2. Eukaryotes

K-selected organisms have a steadier rate of reproduction, with relatively smaller population fluctuation, particularly in multicellular eukaryotes. Eukaryote chromosomes possess multiple origins of replication, and accumulation of repetitive elements largely accounts for the 80,000-fold genome size variation in this domain (Hartl, 2000). In many cases, increases in size are probably not a result of selection (Hartl, 2000), and consequently, some eukaryote genome sizes are probably only limited by the fidelity of replication (see Table 4 in Drake, 1999).

With few apparent constraints on genome size, gene duplication followed by divergence is an effective means for the evolution of new functions. Neither duplication, nor the presence of pseudogenes, is inherently deleterious in eukaryotes (in contrast to prokaryotes). Gene duplication and divergence has resulted in major expansions of developmental gene families, e.g. the homeobox family (Ruddle et al., 1999). Genome duplication is also considered a feature of eukaryote genome evolution (Wolfe and Shields, 1997; Ruddle

et al., 1999), a good example being polyploidy in plants.

Lack of constraint on genome size has enabled large numbers of ‘selfish’ elements to co-exist in eukaryotic genomes (Smit, 1999; Brosius, 1999). Such elements can occasionally be recruited into the cellular repertoire. Examples include dendrite-specific RNAs, rodent BC1 and primate BC200. BC1 has been recruited from tRNA^{Ala} and BC200 from an Alu element (Brosius, 1999). V(D)J recombination in the vertebrate immune system is another example. Proteins RAG1 and RAG2 mediate V(D)J recombination, forming a site-specific recombinase which recognises and cleaves DNA at conserved recombination signal sequences (Agrawal et al., 1998; Hiom et al., 1998). Similarities in gene organisation, signal sequences, mechanism of action, and the presence of a transposase DDE motif in RAG1 (Landree et al., 1999) suggests this system originated through a germline transposition event into a receptor gene in the ancestor of jawed vertebrates (Agrawal et al., 1998; Plasterk, 1998). An unforeseen consequence of the recruitment that gave rise to V(D)J joining is that it also appears to participate in at least some chromosomal translocation events, though probably at low frequency (Melek and Gellert, 2000).

Aspects of placental development in eutherian mammals appear similar to viral infection (Larsson and Andersson, 1998; Harris, 1998). Cell fusion, forming the placental syncytium, is also a feature of endogenous retroviruses (providing an efficient means of infecting new cells). In human placental development, an envelope protein from the endogenous retrovirus ERV-3 is responsible for cell fusion and other differentiation events during formation of the syncytium (Lin et al., 1999). Production of endogenous retroviral particles early in placental development increases the chance of germline insertion, but also provides immunosuppression, thereby preventing the maternal immune system from rejecting the foetus. Indeed, retroviral envelope protein expression suppresses the immune response (Mangeny and Heidmann, 1998).

These examples highlight the centrality of the tinkering concept in evolution (Jacob, 1977). As the above cases illustrate, occasional recruitment of selfish elements into new function appears a consequence of the lack of selection against genome size, making the genomes of higher eukaryotes more vulnerable to

intragenomic parasites. The neutrality of non-coding sequences in chromosomes with multiple origins of replication appears to contribute considerably to eukaryote evolvability at the genetic level.

4.3. Transcript processing

Extensive transcript processing is a feature of eukaryotes, and includes mRNA splicing (Sharp, 1994), editing (Smith et al., 1997), and snoRNA-mediated cleavage, methylation and pseudouridylation of RNA (Weinstein and Steitz, 1999). Splicing and editing are absent from prokaryotes, and snoRNA-mediated modifications are absent in bacteria (though methylation is present in archaea). Though disputed (Lafontaine and Tollervey, 1998; Sontheimer et al., 1999), splicing and snoRNA-mediated modifications probably predate the LUCA (Poole et al., 1998, 1999).

Under *r*-selection and a single origin of replication, spliceosomal introns and snoRNA-mediated modifications are expected to be reduced or lost. mRNA processing delays the expression of proteins, the transcript being processed largely by RNA-mediated reactions. Methylation and pseudouridylation of RNA is ubiquitous, though heavily reduced in bacteria. In archaea, methylation is extensive, and requires snoRNA-like sRNAs (Omer et al., 2000), smaller than in eukaryotes. Each sRNA guides two methylations (the majority of eukaryotic snoRNAs guide just one). Pseudouridylation is minimal in archaea, with numbers comparable to those for bacteria (Charette and Gray, 2000). Modifications in bacteria are limited to highly conserved regions of the rRNA, which may explain their maintenance, while methylation may be important in archaeal rRNA for stability at high temperature (Omer et al., 2000).

In scenarios of the evolution of snoRNAs post-LUCA, the argument has largely been post hoc, with the emphasis being on how these RNAs could have diversified in eukaryotes (Morrissey and Tollervey, 1995; Lafontaine and Tollervey, 1998). The finding of sRNAs in archaea requires a revision of that theory. The alternative, loss under *r*-selection in prokaryotes, is the best explanation for the current data.

Some snoRNAs are paternally imprinted in rodent and human brains, and do not direct methylation of rRNA or other functional RNAs (Cavaillé et al., 2000). One of these may regulate A-to-I editing and/or al-

ternative splicing of the serotonin 5-HT_{2C} receptor mRNA through methylation (Cavaillé et al., 2000; Filipowicz, 2000). Indeed, splicing and A-to-I editing, perhaps also modification by methylation and pseudouridylation, are central to the generation of multiple products from one mRNA (Herbert and Rich, 1999). It is unclear how A-to-I editing of nuclear mRNAs arose in evolution, but the targets have largely been found in signalling in the nervous system of both invertebrates and vertebrates (Reenan, 2001). The role of splicing in generating alternative protein products, and in regulating developmental fate (Graveley, 2001), is possibly a consequence of its maintenance in the absence of selection to remove this apparatus long after its hypothesised role in early genomes would have become redundant. RNA processing pathways have clearly not evolved for evolvability, but nevertheless contribute to evolvability in two main ways. Editing and splicing results in multiple RNA or protein products from a single gene, while modifications serve to fine-tune RNA function. Both contribute to a more complex relation between genotype and phenotype.

4.4. Cytosine methylation, a double-edged sword

Another form of modification which may impact on evolvability is cytosine methylation at the level of DNA. This is widespread in eukaryotes, and is considered to provide a mechanism for gene silencing, and parental imprinting. Cytosine is an unstable base, readily deaminating to uracil, which, if unrepaired will result in a C:G to T:A mutation in one of two daughter copies. Methylation of cytosine produces 5-methylcytosine (5-meC) which deaminates more rapidly than unmethylated cytosine, yielding thymine (Poole et al., 2001). Cytosine methylation, while apparently providing a means of epigenetic control, also produces mutational hotspots, and this can potentially be beneficial or deleterious, depending on context.

Gene silencing has been considered to represent the main function of cytosine methylation, but Yoder et al. (1997) point out that evidence is limited. The majority of 5-meC residues are found in transposable elements, not promoters. They suggest that methylation is primarily a mechanism for silencing transposons, with the corollary that 5-meC to T deamination is largely beneficial because it results in faster inactivation of these elements through mutation. That this cannot be

the only function of cytosine methylation is supported by the existence of at least two repair mechanisms (Schärer and Jiricny, 2001; Poole et al., 2001). If both gene regulation and transposon inactivation are mediated by cytosine methylation, there is a trade-off because, in the former, 5-meC to T deaminations are potentially deleterious, whereas in the latter they are potentially beneficial. The presence of deamination repair mechanisms would therefore be important for repairing damaged genes, but weaken the potential for transposon inactivation (Poole et al., 2001).

The picture is further complicated, because methylation of transposable elements may contribute to epigenetic effects on adjacent genes (Whitelaw and Martin, 2001). Patterns of methylation are known to be inherited, and to have a phenotypic effect. An example is the *agouti* locus in mice, where coat colour is inherited epigenetically through the female line in the absence of genetic variation (Morgan et al., 1999). Whitelaw and Martin (2001) coined the term epigenotype for the effect that epigenetic inheritance has on phenotype, and excitingly, this may provide a means of exploring phenotypic space. However, work on *agouti* demonstrated that, even with selection for a given epigenotype, the original proportions of epigenotypes may reappear (Morgan et al., 1999; Whitelaw and Martin, 2001), making it hard to see how parental imprinting mechanisms could lead to genetic fixation of a phenotypic trait. However, Monk (1995) has proposed that 5-meC deamination may contribute to fixation, since this would make permanent the silencing effect at a given site. In this way, the epigenotype could permit exploration of alternative phenotypes that could then become 'hard-wired' in the genome.

Again, this mechanism impacts on evolvability, but did not evolve for evolvability's sake. Prerequisites for such complex regulation may instead have been the invasion of eukaryote genomes by transposable elements, and selection to silence these, given the apparent inability to prevent their insertion. The conflicting need to eliminate these and the recruitment of methylation into gene regulation, perhaps through adjacent transposons may have set up the requirement to repair 5-meC to T deaminations. Imperfect repair of these (Holliday and Grigg, 1993) may be the cost associated with the conflicting roles of methylation in the genome. However, it may provide a mechanism where 5-meC to T deamination gives rise to a heritable phe-

notypic trait from an epigenetic trait with limited heritability. Again, it is difficult to establish which came first, transposon inactivation or gene regulation, but the example serves to make the point that it is necessary to examine the origins of a process when considering the evolution of evolvability.

Another example is somatic hypermutation at the V(D)J locus in formation of the antibody variable region by C to U editing (Muramatsu et al., 2000; Revy et al., 2000). This is effectively enzyme-catalysed cytosine deamination at hotspots (contingency loci). The function is opposite to the uracil-DNA glycosylases, which are involved in repair of cytosine deaminations (Schärer and Jiricny, 2001), and is also seen in apolipoprotein B transcript editing (Herbert and Rich, 1999).

In summary, the effect of cytosine methylation on evolvability is potentially complex. Through elevated deamination rates at methylated sites, epigenetically-controlled traits may become genetically-encoded, with those that are selectively advantageous becoming fixed in the population. Such epigenetic-mediated exploration of phenotype space is in one sense analogous to receipt of a foreign gene by a bacterium—if the resulting phenotype is selected for, the gene will be retained, if not, it will be lost. Elevated deamination rates at unmethylated loci are also central to evolvability at contingency loci. Given that one of the roles of cytosine methylation is apparently in transposon inactivation, it is worth noting that inactivation results in conversion of a selfish element into a neutral sequence, which may on occasion develop into a functional sequence, as is the case for some promoter sequences (Whitelaw and Martin, 2001).

5. The stress response and evolvability

In this section, we consider how stress responses promote organismal survival. Under some circumstances (see Table 1), hypermutation (adaptive evolution), horizontal transfer, sex in organisms with an asexual cycle, recombination, cell–cell interactions, and cell specialisation can all be understood as stress adaptations. That they contribute to evolvability in prokaryotes and unicellular organisms is consequential—these traits have not been selected for their propensity to promote evolvability, and the

evolutionary origins of these phenomena need not be in the adaptation to stress. Rather, what is important is that they currently contribute to adaptation to stress in a range of organisms, and that this has an impact on evolvability.

We suggest that these mechanisms are important for understanding periods of genetic stability versus genetic change within the lifecycle of a range of organisms. Respectively, these might be described as ‘if it ain’t broke, don’t fix it’ and ‘adapt or die’ strategies. Switching between strategies is expected to be more effective in prokaryotes and unicellular eukaryotes than in multicellular eukaryotes since, as described below, mechanisms for alleviating lethal stresses exist in the first two groups, but not the third.

A range of starvation responses, which can be described as ‘adapt or die’ strategies, are seen in prokaryotes (Table 1). In *Bacillus subtilis*, sporulation and genetic competence (to take up DNA from the external milieu) are both controlled by an extracellular peptide, CSF (competence and sporulation factor). At low concentrations, CSF stimulates competence, and this occurs two–three generations prior to entry into stationary phase. At high concentrations, which arise shortly after entry into stationary phase, CSF inhibits competence, and stimulates sporulation (Lazazzera et al., 1999). Importantly, the SOS response and competence are coinduced and DNA uptake may provide a template for repair of endogenous DNA (Tortosa and Dubnau, 1999). Alternatively, formation of double-strand breakages may permit integration of foreign DNA concurrent with uptake. Perhaps favouring the first possibility is the observation that these ‘quorum sensing’ mechanisms are often strain-specific, which may favour uptake from closely-related strains.

Concurrent with competence (and controlled by the same pathway), degradative enzymes are expressed and these may act to increase the availability of extracellular nutrients (Tortosa and Dubnau, 1999). The same situation is seen in sexual sporulation in the fungus *Aspergillus nidulans*, where the α -(1,3)-glucan, which makes up the vegetative hyphal wall, is degraded to glucose (Champe et al., 1994).

A parallel to meiosis and sexual sporulation in fungi is evident here. Meiosis and competence precede sporulation, and DNA uptake in some bacteria may be most favoured between closely related strains,

thereby approximating sex. The response to starvation is to change from a mode of development where genetic change is minimised, to one where there is active change, before dispersal to a new environment.

In *Aspergillus*, hyphae are sent out into the medium in a radial pattern away from the centre of the colony. Closer to the centre, asexual spores develop, which allow dispersal to new nutrient sources. This strategy is analogous to exponential growth in bacteria. Sexual sporulation occurs later in the lifecycle of the fungus; sexual spores are formed, at the centre of the original colony, where nutrients will have been most exhausted (Champe et al., 1994), and this is equivalent to the stationary phase events of genetic competence and sporulation in *Bacillus*.

DNA uptake by prokaryotes is apparently not always an approximation of eukaryote sex. Distant transfers between archaea and bacteria have been documented (Nelson et al., 1999; Forterre et al., 2000), and both *Neisseria* and *Haemophilus* are apparently competent all the time (Solomon and Grossman, 1996), each containing well over a thousand copies of a DNA uptake signal sequence (Smith et al., 1999).

It seems unlikely that horizontal transfer is unbridled and without patterns, despite the vigour with which many in the phylogenetics community have taken on this idea as a post hoc explanation for current difficulties in explaining conflicting datasets (Woese, 1998; Doolittle, 1998). DNA loss, due to constraints on replication rate during exponential growth, suggests that any sequences taken up will only be fixed if they confer a selective advantage to the organism. Greater promiscuity permits greater sampling of environmental DNA, potentially bestowing a greater propensity to adapt to environmental change (greater evolvability). Greater promiscuity may also equate to greater parasite susceptibility, which might explain the existence of strain-specific competence factors.

There is now overwhelming evidence for transient hypermutation, induced by the SOS response to starvation (Torkelson et al., 1997; Foster, 1999; McKenzie et al., 2000). Metzgar and Wills (2000) argue that it may simply be a spandrel, that is, a by-product, not a directly-selected adaptation. The DNA polymerases involved in the response have been selected to copy highly damaged DNA, which constitutive polymerases (with higher replication fidelity) are unable to copy. The lower-fidelity polymerases

repair damaged DNA, but the lower specificity of polymerisation required to bypass lesions also results in a transient increase in mutation rate.

In the laboratory, global mutators have been successfully selected for, and tend to outcompete non-mutators (Sniegowski et al., 1997). Mutators can arise by chance, and, it has been argued that they could be maintained in asexual populations through genetic hitch-hiking on an advantageous allele created as a result of mutation. While it is thought that complete fixation of mutators would be rare, there seems to be a correlation between elevated mutation rate and virulence in pathogens (see Metzgar and Wills, 2000 for discussion). Perhaps this is not surprising, given that their hosts make use of somatic hypermutation in antibody formation, setting up a Red Queen race. However, the long-term side effects for bacterial mutators are potentially worse; mutational meltdown due to the accumulation of deleterious mutations.

Tenaillon et al. (2000) point out that horizontal transfer provides a potential mechanism for the spread of selectively advantageous mutations (such as those rare beneficial mutations arising during hypermutation) within a population. This might result in the advantageous allele being selected for while the mutator is selected against (due to an increase in deleterious mutations) and thus lost. Segregation of the beneficial mutation from the mutator phenotype may result in the elimination of mutator alleles from a population in the long term, though surprisingly this may not always be selectively advantageous in the short term. Where the cost of hypermutation is low and benefits (i.e. generation of many new advantageous alleles) are high, this may result in loss of sex (or other mechanisms that segregate mutator and newly arisen ‘beneficial’ alleles) in the short term (Tenaillon et al., 2000).

Prokaryotes with multiple copies of the genome are widespread (Bendich and Drlica, 2000), perhaps even the rule. For instance, *E. coli* is polyploid throughout its cell cycle (Åkerlund et al., 1995). Multiple genomic copies will serve as a buffer to deleterious mutation, minimising the detrimental effects of hypermutation, and at the same time, permitting new alleles to arise and be selected for (Koch, 1984). *Azotobacter vinlandii* maintains over 100 genomic copies in stationary phase (Maldonado et al., 1994), making it a potentially very interesting model organism for mutation studies.

Another mechanism contributing to adaptive evolution is transient gene amplification of the *lac* operons of *Salmonella typhimurium* (Andersson et al., 1998a) and *E. coli* (Hastings et al., 2000). Multiple copies of a mutant locus with residual activity produces an unstable ‘wild type’ revertant. At the same time, presence of multiple copies increases the likelihood of a true reversion event. This last point is important, since, in effect, multiple copies provide mutation with a bigger ‘target’ without deleterious changes being lethal. This mechanism (Andersson et al., 1998a) may be important in rescuing periodically-selected functions (PSFs) from loss during selection to reduce genome size. While Hastings et al. (2000) did not find such revertants in their studies on *E. coli*, this does not necessarily imply that this cannot occur.

An additional link between stress response and evolvability is reported in *Drosophila*. Rutherford and Lindquist (1998) mutated the *hsp83* locus (encoding the heat shock protein, HSP 90, which is involved in stabilising and refolding proteins at elevated temperatures), finding mutations of unrelated morphological traits in heterozygotes. The morphological mutations are stable even after subsequent crosses restore progeny to wild type. They argue that such a situation might arise in nature due to titration of HSP 90 during heat shock, or other stresses where heat shock proteins are expressed.

In contrast to the previous examples where change is immediate, the stress, and the release from HSP 90 buffering, would presumably have to be sustained across generations for an alternate phenotype to be expressed and for selection to act upon this. Developmental processes (formation of adult structures, for instance) must run before phenotype is expressed. The comparison highlights the difference in the nature of adaptation between unicellular and multicellular organisms. A relaxation of buffering in response to stress could promote survival through expression of new variants, but the stress must be sustained and non-lethal. A lethal stress such as application of an antibiotic can however be dealt with in unicellular organisms, where beneficial mutations or genes received through horizontal transfer confer instant alleviation of the stress.

A parallel system exists in yeast, where, under conditions of heat shock, the PSI protein, which has a role in translation termination, undergoes a

conformational change, becoming a prion (True and Lindquist, 2000). This conformational switch impairs translation termination, and there is extensive readthrough, producing alternative protein products. Reversion to the non-prion form is possible, and the process can result in heritable changes. As Metzgar and Wills (2000) point out, it is not possible to establish whether these examples are best described as spandrels, or whether there was selection for the buffering of variability in the absence of stress, and release from buffering during stress. The latter scenario is not incompatible with current evolutionary theory, as demonstrated by the above discussion of stress response in unicellular prokaryotes and eukaryotes, but given Rutherford and Lindquist's (1998) titration model, we favour the first possibility.

In Table 1 asexual sporulation, and cell–cell interaction are also listed as environmentally regulated and promoting survival during stress. Sporulation or cell–cell aggregation to form fruiting bodies, biofilms and other transient multicellular structures in response to environmental stress is not controversial. The difference between these, and the more controversial mechanisms is that the controversial mechanisms require mutation. If such responses can be selected for under lethal conditions, such as starvation, then so can the latter. However, that transient hypermutation and horizontal transfer are selected is best explained as occurring through hitch-hiking, not direct selection. The twist is that the fixation and subsequent maintenance of adaptive evolutionary traits through hitch-hiking may be on different loci at each round of selection.

To conclude this section, while the evolutionary origins of many of the stress responses in Table 1 are still obscure, it is nevertheless possible to identify selection pressures which result in their maintenance and heritability. These are all 'adapt or die' strategies with a short term survival advantage, consistent with standard evolution. As pointed out by Metzgar and Wills (2000) and Hastings et al. (2000) there is no requirement for evolutionary forethought. If the ultimate consequence of starvation (or other environmental stresses) is death, then individuals in which elevated mutation rates, genetic competence or locus specific amplification are induced, may survive. There are therefore two aspects: the ability to induce the mechanism to generate variability, and advent of a new function which may alleviate the stress.

6. An ecological perspective: evolutionarily-stable niche-discontinuity (ESND)

Between groups of taxa (complex multicellular ones in particular), there often appear to be long-term stable niche boundaries. In a fitness landscape these boundaries limit access to a single peak, or subset of peaks, and thus limit evolutionary potential. For example, the vertebrate flying insectivore niche has been occupied by birds at day and bats at night for over 55 million years (Novacek, 1985) with little crossover between nocturnal and diurnal niches. Dinosaurs and mammals may have provided niche boundaries for each other for over 150 million years until many of the great Mesozoic reptiles became extinct around the Cretaceous–Tertiary boundary (Bromham et al., 1999; Sereno, 1999).

Typically, niche restrictions are explained as dominance resulting from specialisation of the incumbent species (Rosenzweig and McCord, 1991), which may be viewed as the occupant of a peak on a fitness landscape. The role of interspecific competition has been emphasised for preventing individuals accessing fitness peaks occupied by other taxa (Mayr, 1963). Character displacement (e.g. Pritchard and Schluter, 2001), where taxa evolve to be more distinct with respect to each other when in sympatry than when allopatric, is clear evidence for the importance of interspecific competition in mediating niche discontinuity. However, we believe that the role of competition between species in restricting evolutionary potential is best understood as a property of the interaction of interspecific competition together with the intraspecific competition among members within each potentially competing species. For this purpose, we introduce the concept of ESND to explain the maintenance of niche boundaries.

Discontinuity between niches will be maintained if a shift in an individual (in either species) towards an alternative niche involves a deleterious trade-off between interspecific and intraspecific competition. As shown in Fig. 1, a small heritable shift away from the fitness peak of the individual's own gene pool results in a greater fitness reduction (due to intraspecific competition) than the fitness increase from increased resource access (via interspecific competition).

Fig. 1 depicts an ESND for two taxa (1 and 2) that specialise on different food resources, with each taxon

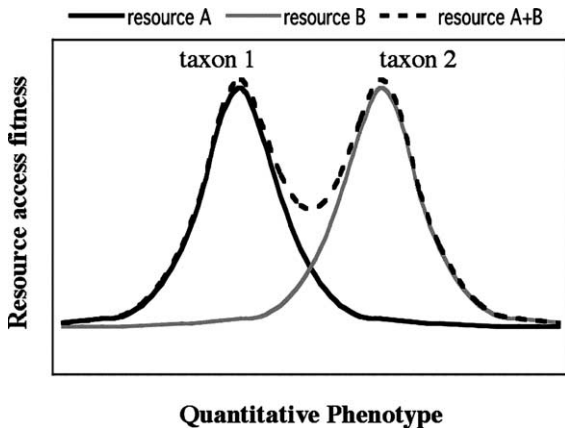


Fig. 1. Evolutionarily stable niche discontinuity between two taxa. The curves represent relative fitness contribution derived by an organism from access to resources A (black line) and B (grey line), as dependent on a quantitative phenotype. The sum of the curves for resources A + B (dashed line) represents the overall relative fitness of an organism with respect to the quantitative phenotype. The signature of an ESND is a selection pattern creating a valley of low fitness. This is expected to occur where a phenotype shift results in a deleterious trade-off between interspecific and intraspecific competition (i.e. towards the valley).

located near its own peak of fitness. The black and grey curves show the overall fitness derived from resources A and B, respectively, as a function of a given phenotypic character. The contributions sum (dashed line) to give the relative fitness for that character. If the strength of intraspecific competition among individuals of taxon 1 for resource A was weak, then the resource A peak would be reduced. Taxon 1 would still be a poor competitor for resource B, but would no longer be prevented from evolving the phenotype towards the resource peak B. This emphasises the importance of intraspecific competition for maintaining niche discontinuity. Models of resource partitioning among mammals (Phillips, in preparation) suggest that an ESND between two taxa can be maintained where potentially competing taxa specialise, respectively, on either side of an environmental discontinuity that may be physical (e.g. night versus day) or biological (e.g. different prey species).

Niche partitioning among large cursorial carnivores illustrates ESND maintained by specialisation in several characters. Throughout Eurasia, Africa and America, the cat and dog groups of carnivores fill niches for fast-burst and endurance predators, re-

spectively. As predators, cats and dogs have many differences (Jones and Stoddart, 1998). As fast-burst, first-strike predators, cats have a high proportion of fast twitch glycolytic muscle (like Olympic sprinters), powerful jaws and crushing canines, as well as having forelimbs as part of the killing mechanism. Conversely, as well as behavioural differences, large dogs, as endurance predators, have a low proportion of fast twitch glycolytic muscle (like marathon runners), slashing jaws and canines, and forelimbs (specialised for long-distance running) are not included in the killing mechanism.

Consider an individual dog (or cat) with a heritable shift in only one of the above characters towards the optimum phenotype of the other taxon, but without concurrent shifts in the other characters. This change will reduce fitness in its own niche, but will still be of little benefit in accessing the other niche. Additionally, we predict that coevolution between competitors and their prey (or predators) will strengthen ESNDs. A dog with a slightly higher ratio of glycolytic to oxidative muscle is unlikely to benefit as a fast-burst predator because potential prey has coevolved with the faster burst-predators (cats). Yet other dogs will leave this mutant dog behind before they reach their endurance limit—intraspecific competition is strong. A consequence of ESND development for coevolution with prey resources is that evolvability may be more affected by ESNDs among taxa that prey on live organisms, than taxa that are autotrophs or detritivores.

Given the prevalence in nature of physical and biological discontinuities, in the absence of extrinsic extinction and immigration of foreign (non-coevolved) competitors ESNDs should develop between coevolved taxa that compete for resources. Perhaps it is not surprising that catastrophic physical events have so often been suggested to catalyse evolvability (Jablonski, 1986; Roy, 1996). Although such events may not directly affect molecular and developmental mechanisms, they free lineages from ESND-restricted evolutionary trajectories.

The establishment of ESNDs may differ between eukaryotes and prokaryotes in that horizontal transfer may break such barriers down in prokaryotes. For example, pathogenic *Shigella* strains of *E. coli* appear to have multiple independent origins within *E. coli*, probably concurrent with receipt of a plasmid carrying pathogenesis genes, and subsequent convergent gene

losses (Pupo et al., 2000). Operons in both prokaryotes (Lawrence, 1999) and fungi (Walton, 2000) are also interesting in this regard, since, like plasmids, they represent a distinct, potentially transferable unit (e.g. an entire biosynthetic pathway, complete with regulatory sequences).

Horizontal transfer of genes that allow an organism to compete in a new niche may have a number of outcomes. (1) The incumbent is better adapted and the invader cannot colonise the niche. (2) The invader is better adapted (i.e. given the trait under selection in the niche is in both incumbent and invader, the genetic background of the invader results in it being better adapted than the incumbent). (3) Both have similar fitness, which may result in further competition, extinction of one or the other, or specialisation leading to two niches. In the context of evolvability it is not sufficient just to consider interspecific competition between a potential invader and the incumbent species. Evolvability also depends on intraspecific competition within the invader, and coevolution between different levels of the food chain.

Functional interactions between organisms and their environment necessarily invoke evolutionary constraints. Flowers which interact with pollinators are subject to greater evolutionary constraints than are parts such as leaves and bark, which are not required to interact specifically with other organisms (Raven et al., 1986). Evolutionary stability conferred on plant reproductive structures has made them more useful than (for example) bark or leaves in determining phylogenetic relationships.

Evolutionary constraint can also result when environmental interactions change during development. Many amphibian and reptile taxa experience dramatic shifts in their environment through development, essentially having to function in different niches. For instance, the komodo dragon (*Varanus komodoensis*) begins life as an arboreal predator of small insects, progressively moves onto larger insects, small vertebrates and eggs, then larger vertebrates and eventually fills a terrestrial large predator/scavenger niche (Auffenberg, 1981). Mutations providing a potential fitness advantage at any point along this continuum may be deleterious somewhere else during growth. This effect is less in mammals and birds because they typically feed their young until they can occupy the adult niche.

Compared with other vertebrates, mammals and birds are also notable for an increased emphasis on homeostasis, particularly endothermy (Ruben, 1995), so stabilising internal biochemical and physiological conditions. Both effects, reducing the range of niches during development and stabilising internal conditions, should enhance morphological evolvability. Indeed, while mammals and birds have diversified into widely different niches and morphologies from their ancestors that shared the planet with dinosaurs 65 million years ago, amphibians, turtles, lepidosaurs (snakes and lizards) and crocodylians typically have not (Benton, 1993).

7. Plasticity, learning and evolvability

Population genetics typically considers just the genetic contribution to the phenotype on the grounds that the genetic component is selectable. Phenotypic plasticity, such as the specific branching pattern of a tree that has grown into a gap of light in the forest, is not genetically determined—yet has an important bearing on evolvability. One suggestion, often called the Baldwin effect (Baldwin (1896), though also proposed by others), is that useful non-genetically acquired phenotypes will eventually tend to be determined genetically. Schmalhausen (1949) and Simpson (1953) explained the Baldwin effect genetically, without the inheritance of acquired characters. These explanations however assumed that the plasticity was eventually lost as the optimal phenotype became the only developmental possibility, and therefore heritable. However, this approach does not seem useful for all cases; a tree in the forest still needs to be able to grow into a new gap where there is light—plasticity needs to be retained.

Baldwin (1896) also proposed that learning tends to hasten the rate of evolution. Traditionally (e.g. Wright, 1931; Grant, 1991) learning, or any non-genetic component of phenotypic variability, has been thought to slow the rate of evolution by diluting the genetic component, thereby reducing the efficiency of natural selection in sorting genetic variance. However, quantitative genetic models (Anderson, 1995) suggest that after an environmental change, populations of individuals able to ‘search phenotype space’ and those that can learn, will tend to find fitness peaks faster. Using neural networks, Hinton and Nowlan (1987) showed

that non-genetically acquired phenotypes could allow an organism to find a fitness peak faster than networks that only had genetically determined variability. In terms of fitness landscapes, it is straightforward to produce models where a combination of phenotypic flexibility and genetic variants will find a new optimum faster than the same model with only the genetic component. Testing this hypothesis may be challenging, though we note the parallels with the earlier discussion on cytosine methylation and epigenotypes (Section 4).

Wyles et al. (1983) reported that land vertebrates had an increasing rate of morphological evolution with increasing brain size to body size (encephalisation). How could larger brain size lead, on average, to a faster rate of morphological evolution? Their suggestion was that the more flexible behaviour of larger-brained animals allows them to broaden, for example, their use of food sources. Because the behaviour of the species is more flexible, it is possible that a new morphological variant would be advantageous in using the new food source. In this suggestion there is no direct linkage between relative brain size and morphological evolution. Mutations leading to improved learning ability could be selected for if behaviour was more flexible, and quite independently this could allow a different mutation to be selected that modified some aspect of morphology. To follow the idea further, the plasticity of flowering plants in varying their growth form in response to their local environment is considered the plant equivalent of flexible behaviour (Trewavas, 2002). For example, the phytochrome pigment system by detecting the level of shade, produces etiolation in plants (Smith, 1974).

Little is known about phenotypic plasticity in the prokaryote lineages and ‘lower’ eukaryotes. The phenomenon of phenotypic switching (where an organism will switch on a suite of genes in response to an environmental cue, e.g. to evade detection by the host in the case of parasites) is interesting in this regard, though nevertheless appears genetically determined (Odds, 1997; Dybvig, 1993). What is perhaps interesting, with regard to the Baldwin effect, is how phenotype switching arose. In one sense it presupposes a degree of plasticity in searching phenotype space, presumably with the retention of the initial phenotype. The earlier example of non-genetically determined cytosine methylation patterns that may lead to a genetically inherited phenotype (Section 4), may

serve as an important model for further investigation, particularly in organisms such as the pathogenic yeast *Candida albicans*, where both regulation by cytosine methylation and phenotype switching are present (Russell et al., 1987; Soll et al., 1993).

Likewise, there is little known about ‘behavioural’ plasticity in prokaryotes. A major question is whether the ‘social behaviour’ of groups such as the myxobacteria is genetically determined, as generally expected, or whether there is a degree of plasticity (Crespi, 2001). Irrespective of whether non-genetically determined plasticity is a feature of some prokaryotes and ‘simple’ eukaryotes, compared to developmentally complex eukaryotes, plasticity (and searching of phenotype space), particularly in bacteria is most widely achieved through horizontal transfer. PSFs are only temporarily present in any given lineage, such that genome plasticity is an integral part of phenotypic plasticity in prokaryotes (Lan and Reeves, 2000).

8. Conclusions

In this paper, we have examined a wide range of biological phenomena relevant to the concept of evolvability. In agreement with most authors, we conclude that there is no need to explain evolvability as having evolved in itself; the evolution of phenomena contributing to evolvability can be explained by current evolutionary theory. It is important to base models for evolvability on a range of data, rather than establishing post hoc explanations for a single dataset. To this end, we have examined parasitism, genome architecture and gene expression, the stress response, ecological interactions and plasticity all in the context of evolvability.

We consider the fact that parasitism (Section 3) is absent from certain groups, such as tetrapods, to be best explained in terms of niche availability, not constraints on the evolvability of this group. Indeed, nutritional dependence of juveniles, and suckling mammals is as a resource strategy equivalent to parasitism. Our conclusion is that the ancestral lineages from which parasites have evolved do not constitute a special case—parasitism can in principle emerge in any lineage, subject to niche availability.

One outstanding question is whether parasitism results in lineages that are more or less ‘evolvable’.

Both prokaryotic and eukaryotic parasites and endosymbionts have repeatedly undergone reductive evolution, losing massive amounts of genetic material. This is a convergent feature resulting from redundancy subsequent to the evolution of mechanisms for nutrient import. There may be less pressure for loss of superfluous sequences in obligate intracellular parasites compared to free-living prokaryotes, as suggested by the 24% non-coding content of the *Rickettsia* genome, compared with around 10% for other bacterial genomes (Andersson et al., 1998b). In this respect there is no clear qualitative answer regarding parasite evolvability—on the one hand, more junk suggests a larger pool from which new functions can arise, but conversely, gene loss consequent to parasitism reduces the number of new functions that could arise via gene duplication. A potential difference is that horizontal transfer is more likely to enable prokaryotes and simple eukaryotes to switch niches (Section 6). This should in principle apply equally to parasitic groups, though may in some cases be limited by reduced encounters between these and other groups (e.g. the *Rickettsia conorii* genome (Ogata et al., 2001) possesses genes that may be involved in competence and DNA uptake, yet very few genes have entered the genome by horizontal transfer).

In prokaryotes, an *r*-selected lifestyle (Section 4) is characterised by exponential growth in response to an energy source, with competition driving shorter cell doubling times. That prokaryotes possess a single replication origin places pressure on chromosome size, since replication is the rate-limiting step in cell number doubling under exponential growth. Consequently, there is selection for elimination of superfluous DNA, including periodically-selected functions (PSFs). PSFs can be maintained by horizontal transfer, permitting more or less continual selection within a population or wider unit. Numerous prokaryotes maintain multiple genomic copies which may buffer against gene loss, provide a means of sidestepping the rate-limiting effect of replication by genome copy stockpiling, and may also permit the emergence of biochemical novelty through divergent evolution at identical copies of a given locus. This latter point, given the potential for additional catalytic activities in numerous enzymes (O'Brien and Herschlag, 1999), may explain how prokaryotes have become so biochemically diverse and colonised so many en-

vironments (Rothschild and Mancinelli, 2001), even with ongoing sequence elimination.

In general, eukaryotes are *K*-selected (Section 4) relative to prokaryotes (Carlile, 1982). They possess multiple origins of replication per chromosome, and, with relatively stable nutrient sources, doubling times are not the major component to competition. Genome size is therefore not limited by replication rate, but by replication fidelity. Consequently, the accumulation of junk DNA is not in itself selected against. In eukaryotes, neutral evolution appears to be central to understanding complexity and evolvability. Accumulation of junk DNA is neutral, and conducive to occasional co-option of junk or duplicated DNA into a new function.

No hard boundary delineates *r*- and *K*-lifestyles which are best considered as a spectrum, helpful in understanding general patterns, but problematic if used to compare specific taxa (where other sources of environmental adaptation are then neglected). The utility of describing an *r*-*K* spectrum can be seen when comparing unicellular and simple eukaryotes to prokaryotes and complex multicellular eukaryotes. Unicellular eukaryotes appear to make use of horizontal transfer and tend to lose and gain PSFs, as supernumerary chromosomes in fungi (Covert, 1998) demonstrate, but the eukaryote translation apparatus makes for response times on the order of an hour in yeast compared with minutes in *E. coli*.

Where prokaryotes and, to a lesser extent, unicellular eukaryotes have diversified through biochemical adaptation to a wide range of environmental extremes, multicellular eukaryotes have tended to colonise niches without major alterations to the basic biochemistry. In the latter, new niches can be reached by virtue of changes in structures, rather than the underlying biochemistry (e.g., the beaks of Darwin's finches (Lawrence, 1999)). The emergence of an internal biochemical environment that can be regulated in response to starvation (e.g. by release of large reserves of stored energy) may have been a prerequisite to the emergence of morphological evolution in multicellular organisms, permitting the colonisation of new niches, but precluding access to ancestral niches. It helps to view the question of differences in prokaryote and eukaryote evolvability in terms of the evolutionary history of these groups—the consequence of *r*- and *K*-selection is that at the extremes

of the spectrum, the mechanisms that contribute to evolvability, and therefore the nature of evolvability, are very different. In prokaryotes, genome size constraints, population-wide maintenance of PSFs, fast reproduction and response times are central, while in multicellular eukaryotes, junk accumulation, complex genotype-phenotype relations, cell specialisation and internal control are central (Table 2).

As discussed in Section 5, mechanisms for dealing with environmental stresses are also different between eukaryotes and prokaryotes. On the whole, changes in environment which are lethal to the organism will result in extinction in specialised multicellular eukaryotes whereas adaptation to non-lethal, sustained changes in environment may be possible. The process of heritable adaptation cannot happen within-generation because developmental programs cannot be re-run to produce new, slightly modified structures in an adult. In prokaryotes, unicellular eukaryotes, and to some extent plants (which produce multiple centres of reproduction from vegetative tissue), there is the possibility of within-generation adaptation through immediate expression of a beneficial mutation or acquired gene. Viewed in these terms, prokaryote ‘adapt or die’ strategies make them more evolvable in response to environmental stress, while mechanisms to stabilise the internal environment in complex multicellular eukaryotes serve as a buffer to the external environment. Unicellular and simple multicellular eukaryotes are perhaps somewhere in the middle.

An important consequence of this is that the extensive biochemical change seen in prokaryotes and unicellular eukaryotes, together with reductive evolution, may explain the observation that *r*-selected organisms appear to have lost more early biochemical relics than multicellular eukaryotes (Poole et al., 1998, 1999). Much more of multicellular biochemistry may in fact be a frozen accident, though many processes would have been lost because of the diminished requirement for interaction with fluctuating environments. The relevance of organisms in extreme environments as models for the earliest organisms (Nisbet and Sleep, 2001) must be reconsidered within this framework.

The effects of stress have been very important in experimental studies relevant to evolvability (particularly in prokaryotes), but we emphasise that we have still not covered all aspects of evolvability. Questions

such as redundancy and modularity need more consideration, and other aspects of the system will affect potential evolvability in more ways than those described in our treatment of genome architecture and environmental interactions. A formal treatment of time scale, from within generations, to millions or billions of years, is also required.

Our ESND model (Section 6) emphasises the difference between potential and realised evolvability (Fig. 2), the latter including limits placed on organisms from constraints in their environment. Lateral transfer in prokaryotes may break down some ESNDs in a way that is similar to the niche competition when organisms adapted to previously isolated niches are able to interact (e.g. geological changes allowing interaction of isolated biota, or the introduction of exotic

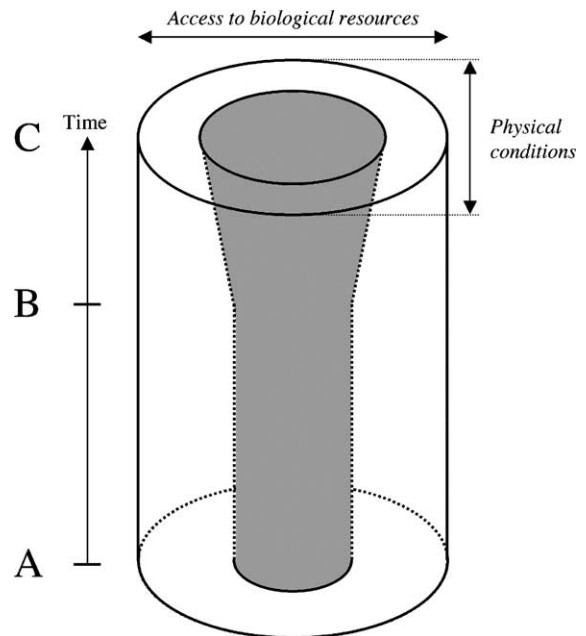


Fig. 2. Traces the relationship between the potential (transparent) and realised (shaded) niche through time for a hypothetical organism. The potential niche includes the full range of physical (axis 1) and biotic (axis 2) conditions for which the organism can survive and reproduce. The effect of competition and predation on fitness contracts this range, leaving the realised niche (shaded) that naturally occurs. Extinction of a predator at time B allows the expansion of the realised niche (within the bounds of the potential niche). Changes to the potential niche may follow due to alteration of the fitness landscape owing to the expansion of the realised niche.

species into an environment). Likewise, ESNDs can break down in some cases where complex behaviour is a trait in one organism, humans being the prime example. The emergence of plasticity, including complex behaviour, further separates organism from environmental changes because this allows a wider range of responses for a given genotype. The effect of organisms restricting the potential evolvability of others needs more consideration, as does plasticity (including learning). Indeed, there are qualitative similarities between genetic plasticity through horizontal transfer in prokaryotes and lower eukaryotes, behavioural plasticity through learning in animals and behavioural plasticity in plants.

In contrasting the extremes of the *r*- and *K*-selection spectrum, we have highlighted many different mechanisms that contribute to evolvability in terms of a general set of selective pressures. The consequence of this is that the nature of evolvability is quite different at the extremes of the spectrum. One interesting aspect of the ESND model for the *r*- and *K*-selection spectrum is that it seems likely that the spectrum is not uniform—there is arguably an ESND-like separation between prokaryotes and simple eukaryotes and between the latter and multicellular eukaryotes. While both horizontal transfer and behavioural plasticity may result in breakdown of ESND on a local scale, we suggest that these large scale discontinuities are evolutionarily stable. As a final comment, evolvability, in one sense, never needed to evolve because information transfer is always error prone—early biological systems were of much lower fidelity, and therefore inherently ‘evolvable’.

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