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Intraclonal genetic variation: ecological and evolutionary aspects.
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The dynamic clonal genome and its adaptive potential

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Populations of clonal organisms are often represented as being evolutionary inert with persistent genetic fidelity. The advent of molecular methods and the corresponding increased genetic resolution of clonal populations forces a reconsideration of this viewpoint. We review molecular data from viruses, prokaryotes and eukaryotes to support the argument that clones possess a highly dynamic and adaptive genome. *Biological Journal of the Linnean Society*, 2003, **79**, 193–208.

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INTRODUCTION

In the debate over the evolution of sex, important questions are raised by the longevity of asexual species (Judson & Normark, 1996; Normark, Judson & Moran, 2003). In particular, given that sex is beneficial because it generates genetically variable offspring, how do asexual genomes survive? By definition, evolution is dependent on genetic variance, even if operative for a relatively short time in the life cycle (Gorokhova *et al*., 2002).

The advent of molecular technologies has resulted in an increased ability to detect mutational change and thus quantify the levels of variation in asexual populations (Lushai & Loxdale, 2002). Changes within non-coding, repeat DNA and synonymous or silent mutations are probably effectively neutral (although their effect on secondary structuring and spacing of genes is not well understood) but most mutations with observable phenotypic effects are deleterious. Work on *Drosophila* has suggested that deleterious mutations arise at the level of about one per individual per generation, although this is considerably less for other organisms (Lynch, Blanchard & Postlethwait, 1999).

In an asexual population, unless very large, no individual will be free of harmful mutations. Hence the mean fitness of an asexual population is expected to decrease with time (Lynch *et al*., 1993) and the population should eventually become extinct. As a result of mutation and subsequent isolation, an asexual population may consist of several clones, each adapted to a particular niche, but in a stochastic environment mean fitness will often be severely reduced (Haldane, 1932, 1990). Yet, despite these points, asexual lineages persist in numerous independent taxa, and some of these lineages are very ancient indeed (Judson & Normark, 1996; Normark, Judson & Moran, 2003). However, we here take a step back from such interclonal genetic variation and its ecological consequences (Vrijenhoek, 1998) and concentrate instead on intraclonal variation.

EMPIRICAL EVIDENCE FOR RAPID CHANGE IN CLONAL ORGANISMS

Recent molecular studies have highlighted the levels of intraclonal genetic variation in both unicellular (e.g.

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Elena, Cooper & Lenski, 1996) and multicellular organisms (Lushai *et al*., 1998). Phages, bacteria, yeast and mitochondrial lineages are some of the groups that have provided insights on evolutionary processes in relation to molecularly identified changes in the genome (see Table 1a for an overview). Most of the data derive from studies on strains and populations under environmental stresses of nutrition and temperature. For an insight into the experimental utility of such study organisms, see Bull *et al*. (1993; bacteriophage-T7) and Lenski & Travisano (1994; *Escherichia coli*).

Studies on the complexity of evolution in asexual species is exemplified by work on bacteriophages (Bull *et al*., 1993, 1997; Cunningham *et al*., 1997; Crill, Wichman & Bull, 2000; Wichman *et al*., 2000). These reveal a high level of parallel molecular evolution within independently evolving lines derived from a common ancestor, and show similarities in deletion events and related nonsense mutations in loci adjacent to the points of breakage (T7, Cunningham *et al*., 1997). Similar work on the bacteriophage-øX 174 has also revealed such parallelisms, as well as reversals to the ancestral type; in fact the convergence made the lineages appear more closely related than they were in reality (Bull *et al*., 1997; Wichman *et al*., 2000). In these experiments, the purity of the lineages was ensured by allowing them to evolve in geographically distant laboratories; however, trends across these geographically dispersed lineages were consistent. The convergence strongly indicates selection (Bull *et al*., 1997). Fitness of a mutation was genome-dependent; when the same genome was subjected to the same selection, the same mutations were favoured.

Rapid evolutionary changes in bacteriophages have also been illustrated by host alternation studies, where in some lineages *Salmonella enterica* was substituted for the typical *E. coli* host. Typically, the highest levels of convergence were observed among lineages grown with the same species of host (Wichman *et al*., 2000) to the extent of a depressed ability to grow when phages from an *S. enterica* lineage were returned to the ancestral *E. coli* (Crill, Wichman & Bull, 2000). Other significant trends included rates of substitution that were far more rapid during the initial stages of the evolutionary experiment, culminating in an adaptive plateau, and specific sites of reversion at 'gene F' that appeared responsible for host-change. Other beneficial mutations have been found in this system, two involving gene F, and a third affecting the amino acid sequence of the internal scaffolding protein, gene B (Bull, Badgett & Wichman, 2000). Selection coefficients for these were between 0.03 and 0.14, where 0.01–0.1 are generally considered large.

Stimulated by the classic experiments of Luria & Delbrück (1943), there is now a wealth of information about mutational change and adaptation in bacterial lineages. We focus on recent work by Lenski and colleagues using their cryogenic 'fossil-bed' *E. coli* experimental lineages (Lenski & Travisano, 1994; but see Dykhuizen, 1990). Their work has revealed how rare beneficial mutations sweep through a standing population of purely asexual lineages causing bursts of change moving the population to new 'adaptive' plateaus, whereupon stasis ensues until the onset of the next selective-sweep (Elena *et al.*, 1996; see Elena, Codoñer & Sanjuán, 2003, with respect to viruses). Over approximately 10 000 generations, Lenski & co-workers have, for example, observed stepped increments (punctuated evolution?) of cell size in a fixed-resource environment.

Further studies have identified the evolution of 'mutator genotypes' that help maintain levels of variation up to two orders of magnitude greater than the norm (Elena & Lenski, 1997; Sniegowski, Gerrish & Lenski, 1997). The effect of mutator genotypes in a population was most significant when they originated in rare rather than common lines, suggesting strong frequency-dependent selection. The data showed an upper limit to evolutionary change in these asexual lineages, recognized as a plateau of adaptive change after the initial rapid rate of evolution from the ancestral state when placed in a new environment (De Visser *et al*., 1999; Bull, Badgett & Wichman, 2000). This eventual steady-state is perhaps evidence for clonal interference. In strictly asexual lineages, advantageous mutations that have arisen in separate lines that cannot be combined lead to interclonal competition checking further adaptation (Gerrish & Lenski, 1998).

Verification of such experiments by the use of insertion sequence (IS) probes have validated many of the trends (Papadopoulos *et al*., 1999). Microsatellite markers have been used to confirm the trends and to predict a rate for rare beneficial mutations, i.e. 4×10^{-9} (Imhof & Schlötterer, 2001). Another study on *E. coli* demonstrates the dynamic nature of these organisms in a static environment, a 'constant batch' culture system devoid of periodic renewal or thinning out of growth media (Finkel & Kolter, 1999). After a few days in culture, 'growth advantage in stationary phase' (GASP) phenotypes that were different from their original ancestor evolved and stabilized at constant population levels. Interestingly, serial collections from the stock culture indicated that the GASP cultures were continually adapting, with newer collections being progressively fitter than previous ones. DNA restriction fragment length polymorphisms (RFLPs) and phenotype variation all indicated that selection favoured a series of different genotypes over time

Table 1. (a) Literature is cited in alphabetical order describing three broad areas of mutational change, mechanisms of change and adaptive evolution in simple clonal lines with an onus on empirical molecular data*. Note temperature = 'T°'. (b) Literature in alphabetical order that describe similar traits in multicellular clonal lineages

Reference	A short description of the paper
(a)	
*Austerberry, Snyder & Yao (1989)	*Tetrahymena*, site-specific variation
Bennett & Lenski (1999)	*E. coli*, adaptation to T° and evolution
Bohannan & Lenski (2000)	*E. coli* & bacteriophage, relating genetic change to community evolution (review)
*Bull *et al*. (1993)	Bacteriophage (T7), C > T and G > A changes (0.5–1.5% genomic changes) with T°
*Bull *et al*. (1997)	Bacteriophage (øX174: genome = 5386 bp), T° -adaptation, convergent evolution, 119 substitutions at 68 sites
*Bull *et al*. (2000)	Bacteriophage (øX174), fitness effects (13.9–3.8%) of big-benefit mutations under T°-stress
*Coyne, Chalker & Yao (1996)	Ciliates, genome downsizing
*Coyne & Yao (1996)	*Tetrahymena*, sequence specific chromosomal breakage
*Crill *et al*. (2000)	Bacteriophage (øX174), host adaptation, 79 substitutions at 55 sites (1% of genome), 2 base insertion, 27 base deletion, evolutionary reversals
*Cunningham *et al*. (1997)	Bacteriophage (T7: genome = 39.937 bp), parallel molecular evolution, nine independent 1.5 kb deletions, also 14 independent *nonsense* in parallel positions at 7 amino acid sites
De Visser *et al*. (1999)	*E. coli*, mutator genotypes, 'speed limit' = upper limit to adaptive evolution in a fixed environment indicating 'clonal interference'
Denamur *et al*. (2001)	*E. coli*, control of mutation rate, horizontal gene transfer
Elena *et al*. (1998)	Bacteriophage, mutation accumulation and fitness decline through bottlenecks
Elena & Lenski (1997)	*E. coli*, genetic variability, mutator adaptive phenotypes
Elena *et al*. (1996)	*E. coli*, punctuated evolution due to rare beneficial mutations
*Finkel & Kolter (1999)	*E. coli*, fitness selection over time even in static environments
Freidberg *et al*. (2002)	Enzymes of evolutionary change (*trans*-lesion synthesis, TLS, SOS and Y-family polymerases) (mini-review)
Giraud *et al*. (2001)	Costs and benefits of high mutation rates
*Imhof & Schlötterer (2001)	*E. coli*, microsatellite analysis of beneficial mutations: rate = 4×10^{-9}. Selective sweeps were predictable, no apparent 'speed limit' to mutation noted, but experiment only ran 1000 generations
Jain *et al*. (1999)	Prokaryotes, horizontal gene transfer among genomes, 203 operational, 109 informational genes investigated supporting 'complexity hypothesis'.
Korona *et al*. (1994)	*Comamonas* sp. (soil borne bacteria), multiple adaptive peaks in a structured habitat; see Riley *et al*. (2001)
*Lake *et al*. (1986)	Evolution mapped with 3D structure
*Lerat *et al*. (1999)	Tracing the historic pattern of evolution in TE moieties
Lenski & Travisano (1994)	*E. coli*, adaptive peaks, macroevolutionary dynamics
Lenski (1989)	Are some mutations directed? (review)
*Lynch (1997)	Nuclear and organelle tRNA genes accessed, 26 taxonomic groups, 44 complete organelle sequences, 2 complete genomes, neutral mutation accumulation, gradual decline +100 MY
*Lynch & Blanchard (1998)	As in Lynch (1997), tRNAs, rRNAs and proteins accessed for plants, fungi and animals
*Lynch *et al*. (1999)	Spontaneous mutations (review)

Table 1. *Continued*

Reference	A short description of the paper
*Lynch & Conery (2000)	Duplicate genes, evolve at 0.01% Myr^{-1}; few evolve, most silenced over time and may be involved in forming species barriers
*Moran (1996)	Endosymbiont bacteria, +100 Myr lineage, 16S rDNA indicated high silent mutation load with A + T bias in comparison to free-living bacteria
*Papadapoulos *et al*. (1999)	*E. coli*, IS variation (not very sensitive), selective sweeps in a 10 000 generation experiment
Radman (1999)	Enzymes of evolutionary change (mini-review)
*Rand & Kann (1998)	mtDNA-*Drosophila*, accumulation of silent mutations
Riley *et al*. (2001)	*Ralstonia (*soil-borne bacteria) rapid phenotypic change and diversification in structured environment vs. unstructured one (solid and liquid culture mediums)
Schneider *et al*. (2000)	*E. coli*, insertion sequence (IS elements) mediated mutations and rearrangements
*Szafraniec *et al*. (2001)	*S. cerevisiae*, hidden mutational load revealed by stress
Sneigowski *et al*. (1997)	*E. coli*, high rates of mutations caused by mutator genes
*True & Lindquist (2000)	*S. cerevisiae*, chromosomal rearrangements linked to TE ([*Ty*1]
Volkov *et al*. (2002)	*S. cerevisiae*, novel prion [ISP^+] control of translation
*Vulic, Lenski & Radman (1999)	*E. coli*, mutations in mismatch repair promote mutations recombination
*Wichman *et al*. (2000)	Bacteriophage (øX174), 126 substitutions, 90% encoded amino-acid changes, 62% occurred in parallel
*Yao (1996)	Paramecium, DNA rearrangement
(b)	
*Arkhipova & Meselson (2000)	Retroviral variation in asexual and sexual lineages
*Blackman *et al*. (2000)	Aphids, karyotype diversity: *Trama troglodytes* (2n = 14–23); *T. caudata* (9–12); *T. maritima* (10–14) paralleled by rDNA distribution noted using FISH
Butlin *et al*. (1998)	Ostracods, clonal diversity, ancient asexual lineages (review)
*Cáceres *et al*. (1999)	*Drosophila*, implication of TEs in the origin of chromosomal rearrangements
Capy *et al*. (2000)	Impact of TEs on host genome under stressful conditions (review)
*Davies *et al*. (1999)	*C. elegans*, cryptic deleterious mutations (96% = 0.07% fitness effects)
*De Sousa *et al*. (2001)	Sheep, epigenetic changes in development, telomeres
*Devonshire *et al*. (1998)	Aphid, *Myzus persicae*, insecticide resistance, mechanisms and DNA diagnostics
*Denver *et al*. (2000)	*C. elegans*, high rates of mutation in the mtDNA genome of 74 lines; 26 mutations; 16 base substitutions; 13 transitions; 3 transversions; 10 insertions-deletions
*Fenton, Malloch & Germa (1998)	Aphids, rDNA ITS variation
*Forneck, Walker & Blaich (2001)	Aphids, AFLP variation
*Foster *et al*. (2000)	Aphids, insecticide resistance, fitness costs
*Foster *et al*. (2002)	Aphids, pleiotropic effects, fitness
*Fry *et al*. (1999)	*Drosophila*, lower deleterious mutation rates (0.02) than previously noted (see Crow, 1994)
*Gorokhova *et al*. (2002)	*Daphnia*, correlation of IGS size variants with major life history traits indicating rapid adaptation
*Hedges *et al*. (1992)	Salamanders (*Ambystoma*), mtDNA study of gynogen-clonal polyploidy, nuclear and mtDNA decoupling, 3.9 ± 0.6 Myr lineage
Hick *et al*. (1996)	Aphids, methylation of DNA and genome function
*Humpherys *et al*. (2001)	Mice, epigenetic changes in development

Kidwell & Lisch (2000)	TEs and genome evolution, review
*Kuhn *et al*. (2001)	Fungi (arbuscular), FISH and ITS probes suggest multiple genomes in individual lineages, with some nuclei containing ITS-T2 (40%) vs. a rarer type ITS-T4 (17%); also cases of co-occurrence (~8%)
*Lerat *et al*. (1999)	*Drosophila*, modular TE evolution, functional domains accessed and patterns of deletion events noted
*Lushai *et al*. (1997)	Aphids, intraclonal phenotype variation, RAPD pattern variation within some clones
*Lushai *et al*. (1998)	Aphids, intraclonal variation, RAPD band variation suggesting somatic (n = 14) and germ line mutations (n = 1) over 32 generations
Lynch *et al*. (1998)	*Daphnia*, mutational variation, mutational meltdown and life history variation
Lynch *et al*. (1999)	Spontaneous mutation rates, generation length, genome size effects on mutation rate (review)
*Mandrioli *et al*. (1999)	Aphids, intraclonal NOR heteromorphism
*Mark-Welch DB & Meselson (2000)	Bdelloid rotifers, genetic variation between alleles at a locus
*Meyer & Duharcourt (1996)	*Tetrahymena*, chromosomal rearrangements
Normark (1996)	Weevils (*Aramigus*) species complex, one group COI (4.5% indicate 2 Myr asexual)
*Normark (1999)	Aphids (*Trama*) rapid karyotype change but low EF1 divergence suggesting rare sex (see Blackman *et al*., 2000)
Normark & Moran (2000)	Using sequence data to track mutations (review)
*Phillips *et al*. (1999)	Aphids, facultative asexuals persist past ecological 'dead-ends' by behavioural modification in their lifecycle
*Sanders (1999)	Fungi (arbuscular), rDNA multiple genes, ancient asexuals, hyphal anastomosis
Santelices (2001)	*Rhodophyta*, phenotypic plasticity resulting from intraclonal variation and strain selection (review)
*Santelices *et al*. (1996)	*Rhodophyta*, sporelings growing together to produce unisexual chimeras, colour variants and RAPDs indicate mixed tissue. Chimeras showed increased intraclonal variation.
*Schön *et al*. (1998)	*Darwinula stevensoni* (ostracods: ancient asexuals 25–60 Myr), no divergence in ITS1, COI (3.8%), suggesting slow nuclear evolution, highly efficient DNA repair mechanism
Schultz *et al*. (1999)	*Arabidopsis*, spontaneous deleterious mutation rate in a plant (0.1), fitness decline (0.9%)
*Spolsky, Phillips & Uzzell (1992)	Salamanders (*Ambystoma*), gynogenetic asexuals, COI divergence (5.2–8.5%), suggests ~5 Myr divergence
Sommerfeldt & Bishop (1999)	Ascidians, adaptive chimerism
Stewart & Nilsen (1995)	Cranberry, phenotypic plasticity, genetic variation
*Sunnucks *et al*. (1998)	Aphids, microsatellites detail chromosomal re-arrangements
Tinti & Scali (1996)	Stick insects, hybridogenesis, sexual linkage to asexual lineages
*Turner *et al*. (1992)	*Rivulus*, selfing hermaphroditic fish, high clonal diversity generated by mutational changes
*Van Doninck *et al*. (2002)	Ostracods, the probability of a general purpose genotype in an ancient asexuals is tested against ostracods species with mixed lifecycle strategies
Vassilieva & Lynch (1999)	*C. elegans*, spontaneous mutation rates affect lifecycle traits (0.05) and low mutation rate (average 0.0034)
Vassilieva *et al*. (2000)	*C. elegans*, mildly deleterious alleles and fitness effects (0.03 per generation)

following trends noted in the experiments where cultures were sequentially renewed.

Another group of bacterial lineages that are buffered from environmental changes are the ancient endosymbiotic associations of bacteria living within specialized polyploid host cells (mycetocytes), for example the 100–250 million year old association of *Buchnera aphidicola* with its aphid host (Baumann *et al*., 1993; Moran, 1996). Infection is through cytoplasmic inheritance. A comparison of the 16S rDNA from a range of mutualistic symbionts (*Buchnera* from aphids, P-symbionts from whitefly and mealybugs and *Wolbachia pipientis* from tsetse flies) with *E. coli* and *Salmonella typhimurium* showed that the symbionts have evolved faster than their free-living relatives (Moran, 1996). Minimum rates of evolution were 1.7–2.7 (16S rDNA) and 1.4–3.2 times faster (eight additional genes compared between *Buchnera* and *E. coli*). Synonymous substitutions between these bacteria were similar, indicating that these were largely mildly deleterious (Ohta, 1992). The rate of non-synonymous substitutions was particularly high in the *Buchnera* lineages, and resulted from increased third position codon substitutions favouring A + T changes, and confirms the prediction of the fast accumulation of deleterious mutations in small populations of asexual endosymbionts, as predicted from Muller's ratchet (Moran, 1996). That these ancient asexual lineages have existed for over 100 Myr suggests the existence of strong compensatory processes overriding the deleterious effects, or of selection that is too weak or difficult to detect.

Similar trends for the accumulation of mildly deleterious mutations have been reported from comparison of tRNA genes in asexually propagating organelle genomes of animals, plants and fungi. Such mutational load should lead to slow declines in mean fitness (over 10–100 Myr) and could cause eventual extinction (Lynch, 1997; Lynch & Blanchard, 1998). The accumulation of mild deleterious mutations has been reported for genes from *Drosophila* mitochondrial DNA (mtDNA) (Rand & Kann, 1998), but whilst some show high genetic polymorphism (cytochrome-β and ATPase 6 genes), others show little or no variation (ND3 and ND5) (Rand & Kann, 1996). However, such genomes are perhaps not entirely non-recombinant as there is mounting evidence for rare recombination in mtDNA (Wallis, 1999). Many pieces of the puzzle remain unclear. Details of why differential loads occur in the mtDNA genome need to be clarified to understand how these systems remain intact even under high mutational stress. One possibility is that genomes can carry high genetic loads provided certain regions remain unaffected (Redfield, 1999).

Endosymbiotic asexual bacteria such as *Buchnera*, together with examples of ancient asexuals (Judson & Normark, 1996), directly contradict evolutionary theories that suggest that clonal lines should be short-lived (Table 1b). Included here should be the arbuscular mycorrhizal fungi (asexuals for >300 Myr) that have a unique multinuclear genomic complement (Kuhn, Hijiri & Sanders, 2001). Evidence from the fossil record suggests that darwinulid ostracods may have persisted without sex for about 100 Myr (Butlin, Schön & Martens, 1998; Butlin *et al.*, 1999). DNA sequence variability can also be used to test the duration of asexuality in such lineages since both nuclear and mitochondrial data should theoretically be in congruence in truly asexual lineages. As the mitochondrial DNA divergence within and between species of darwinulids range, respectively, from 3.8 to 27.7% (Schön *et al*., 1998, 2003), current invertebrate molecular clock models (~2.0% = 1 Myr) imply a minimum of 14 Myr of divergence from a common ancestor. Mitochondrial DNA divergence (~2.5%) among clonal samples of another ostracod species have been equated to 5 Myr of divergence based on rates of 0.5% variation per million years (Chaplin & Herbert, 1997); therefore, a similar estimate would put the divergence rates of some of the darwinulids at approximately 60 Myr. Irrespective of exact dates, both fossil and molecular divergence records within the ostracods suggest that old asexual lineages are prevalent in this group (Butlin *et al.*, 1998). In contrast, nuclear sequence data from the ribosomal DNA (rDNA) internal transcribed spacer-1 (ITS1) region show no variation in one of these species, *Darwinula stevensoni* (Brady & Robertson), in comparison to 3.8% divergence for mtDNA. This may suggest the rare occurrence of sex (Normark, 1999), but this seems highly unlikely when we take into account the lack of variation among populations from Finland to South Africa. Instead, slow molecular evolution with a propensity to highly efficient DNA repair may explain these observations (Schön *et al*., 1998).

Further examples of ancient asexuals include the brine shrimp, *Artemia salina* L. with an estimate of 30 Myr (mtDNA, Perez *et al*., 1994), and the bdelloid rotifers (Mark-Welch & Meselson, 2000, 2003). All members of the class Bdelloidea are believed to reproduce wholly asexually (Mark-Welch & Meselson, 2000). Part of the observed invariance stems from the absence of retroviruses in this group (Arkhipova & Meselson, 2000). In the absence of any records of sexual forms, the occurrence of bdelloids in 35–40 Myr amber suggests that they are 'ancient asexuals'. One important indication for ancient asexuality within diploid genomes would be the progressive divergence between homologous alleles at the same locus in paired chromosomes (Mark-Welch & Meselson, 2000). This unique signature of genetic divergence from the time of ancient ancestral asexuality or 'genome freeze'

with regards to recombinant events has to date only been noted in bdelloids. Other references of old asexual lineages include the weevil, *Aramigus tessellatus* (Say) +2 Myr (mtDNA, Normark, 1996) and salamanders of the genus *Ambystoma*, ~4–5 Myr (mtDNA, Hedges, Bogart & Maxson, 1992). The study of such phenomena is fundamental to our understanding of genomic evolution and adaptation (Normark, Judson & Moran, 2003).

FACTORS AFFECTING RAPID CHANGE IN THE CLONAL GENOME

Estimates for rates of deleterious mutations in flies, nematodes and plants are given in Table 1b and are as high as one per individual per generation (Crow, 1997), but much lower levels are normal (Fry *et al*., 1999; Schultz, Lynch & Willis, 1999; Vassilieva, Hook & Lynch, 2000; but see Lynch *et al.*, 1999 and Kondrashov, 1999). Most of these mutations are recessive and decrease fitness by around 1–2% per generation (Houle, Morikawa & Lynch, 1996), with 0.07% being attributed to barely detectable 'cryptic' mutations as identified in the nematode *Caenorhabditis elegans* (Maupas) (Davies, Peters & Keightley, 1999). Such mutations will eventually be eliminated by natural selection, but many questions still remain about the detrimental effects of all mutations in ecological settings, especially with regards to the effects of compensating mutations and back mutations (Bull *et al*., 1997). The estimate for the rate of beneficial mutations, 4×10^{-9}, has allowed workers to predict selective sweeps in bacterial populations (Imhof & Schlötterer, 2001).

Two well-described mechanisms of genetic change are: (1) those involving the activation of mutagenic activity (e.g. *trans*-lesion synthesis, TLS, SOS and Y-family polymerases) or the inhibition of an antimutagenic (proofreading) system (MRS) (Radman, 1999; Friedberg, Wagner & Radman, 2002); and (2) those involving transposable elements (TEs) (T*n* in bacteria; LINE-like elements and *hobo* in *Drosophila*; T*y* in yeast; Capy *et al*., 2000). Because of the function of the first class of mechanisms, it has been suggested that point mutations that are maintained (as most damage is repaired) should not necessarily be looked upon as genetically erroneous because variation is accurately and actively assimilated by this group of DNA polymerases. Such phenomena are changing the interpretation of apparently chaotic genetic change in individual genomes to one of events that are adaptive (De Visser *et al*., 1999; Radman, Matic & Taddei, 1999). Analogues of both systems have been found in prokaryotes (TLS; see Friedberg, Wagner & Radman, 2002; IS changes, e.g. Schneider *et al*., 2000) and eukaryotes (TLS: see Friedberg *et al.*, 2002; TEs: Nuzhdin, Pasyukova & Mackay, 1997; Cáceres *et al*., 1999; see Nuzhdin & Petrov, 2003). Interestingly, different elements of the TLS and TE mechanisms correspond to hotspots, sites of inversions, deletions and gene rearrangement (for TEs, see Table 1b).

How do such mechanisms work? In the case of TEs, metabolic and environmental cues (stresses) on genomes trigger an intricate response by these latent mutator mechanisms to 'reveal' the genetic variability from amongst the soup of genetic variation that hitherto lies hidden by buffering proteins such as *Hsp90* (Rutherford & Lindquist, 1998). These agents then promote adaptive mutations with large effects on fitness (Capy *et al*., 2000; Friedberg, Wagner & Radman, 2002). When the stress is alleviated, populations revert back to non-mutator or 'hidden' states associated with favourable genotypes. Because of their mode of operation, these factors involve explosive evolutionary events that can be thought of as near-Lamarckian or 'directed mutations' rather than genetic mechanisms mediated by fast selective processes (see Lenski & Mittler, 1993).

There is a significant amount of relevant research on mutational changes (Drake, 1991) and adaptation (Szafraniec, Borts & Korona, 2001) in brewing yeast *Saccharomyces cerevisiae*. The latest results include mechanisms of change affecting genomes that are not genetically inherited. Instead, cytoplasmically inherited prions, *PSI*$^+$, *PIN*$^+$ and *ISP*$^+$, have been implicated as epigenetic modifiers. In a manner similar to the factors described above (polymerase mutases, TEs), prions provide the means to activate hidden genetic variation and produce new heritable phenotypes (see True & Lindquist, 2000; for [*ISP*$^+$], Volkov *et al.*, 2002). The difference from that described for mutase enzymes and TEs is that the epigenetic influences allow heritable changes to be operational in a single step or generation rather than through a process of selection over generations. Hence, such factors appear to allow the organisms to adapt to new niches without 'closing the door' on their ability to occupy the previous one. A lineage with such modifying elements acquires the ability to ascend or descend multiple adaptive peaks. A molecular mechanism showing such an immediate effect could be described as functioning in an adaptive homeostatic fashion with respect to metabolic/environmental change.

The discovery of TEs and prion-like elements enables us to move away from searching for 'linear affects' in strings of sequences effecting rapid change and start to search for associations of secondary and tertiary structure in functional genomes (Wolffe & Matzke, 1999). Future research should address where and when these aspects interact with specific sites of change, leading to chromosomal breakage (ciliates: Meyer & Duharcourt, 1996; Yao, 1996), chromosomal

rearrangements (e.g. Sunnucks *et al*., 1998), karyotype variation (Blackman, Spence & Normark, 2000), and the production of duplicate genes (e.g. in bacteria: Force, Lynch & Postlethwait, 1999; Lynch & Conery, 2000). There may be a cascade or temporal association in the way genetic change is fixed through the evolution of a lineage and studies on this are already beginning to emerge (e.g. Lerat *et al*., 1999). A proviso also has to be made to the natural assumption that complexity through epigenetic interactions gives rise to significant adaptive changes over time. There are many examples of point mutations effecting gross phenotypic changes (e.g. in mtDNA of *C. elegans*; Denver *et al*., 2000).

The short generation time and potential for rapid growth in many clonal populations are a key aspect of their ability to exploit heterogeneous environments. In the case of aphids, an asexual clonal population derived from a founder has been described as a 'thinly spread single individual with many pieces' (Janzen, 1977). In the phage studies described earlier, the longest experiments lasted 33 days and the number of evolutionary generations was estimated as 1.0×10^7–10^8 phage/host populations 100 times a day (Bull *et al*., 1997). The reproductive potential of asexual multicellular clonal organisms such as aphids and nematodes is also enormous. Aphids could theoretically generate up to 7.6×10^{28} individuals per year from a single founder in perfect conditions (Harrington, 1994). If such *r*-type strategy was to be coupled with, for example, an aphid's ability for 'telescoping of generations' (Dixon, 1998) (involving a process whereby an adult parthenogenetic female not only has daughter embryos developing inside her, but these in turn have their own progeny developing within them), then the potential for adaptation is huge. This is because such populations also have the capacity to become preconditioned to external environmental changes, both by maternal metabolic factors and in some situations, directly by environmental influence through the mother's abdominal wall on the developing embryos, allowing adaptive changes to take place very quickly. Not all asexuals ascribe to this enormous reproductive potential and, in their case, mutation-based models, e.g. mutational meltdown within a finite number of generations (less than 100 in water fleas, *Daphnia pulex* (Leydig): Lynch *et al*., 1998), Red Queen and other models supporting frequency-dependent factors (Van Valen, 1973; Charlesworth, 1987; Hamilton, Axelrod & Tanese, 1990) cannot be ignored in accounting for the extinction of many distinct clonal lines.

Clonal adaptation has been perceived, as a consequence of its mode of reproduction, as being 'locked' within lines (Fisher, 1930). Clonal organisms in a heterogeneous environment can be envisaged as a set of lineages each close to its 'adaptive peak'. Each lineage, locked into its specialized niche, is unable to shift horizontally between peaks (Wright, 1988; Cruzan, 2001), as described for clonal populations of the freshwater snail, *Potamopyrgus antipodarum* (Gray) (Fox *et al*., 1996; Jokela *et al*., 2003).

THE DYNAMIC CLONAL GENOME

However, this is not the whole story. 'Multiple-generation complex lifecycles' occur in several multicellular taxa (Moran, 1994). One example is the bird cherry-oat aphid *Rhopalosiphum padi* (L.). Its complex lifecycle includes sexual phases, where apomictic parthenogenetic lineages are intermittent with the production of sexual forms (males and oviparae); asexuals with continual parthenogenesis irrespective of environmental cues; androcyclic with continual parthenogenesis and the inclusion of rare males, and intermediate forms where parthenogenesis is occasionally interrupted by the production of a few sexual females in response to specific cues (Tatchell & Parker, 1990). Irrespective of lifecycle, a number of behaviourally and physiologically distinct phenotypes can be produced by the same clonal lineage. For example, aphids can exhibit up to seven distinct phenotypes in a single holocyclic lifecycle (see Dixon, 1998). These clonal phenotypes often have very different physiology and ecology in relation to one another, and can occupy quite different niches over time.

Variation or plasticity, even in a single phenotype, is a separate issue and has been described in highly inbred lines of *C. elegans* for the traits of productivity and longevity (Vassilieva & Lynch, 1999; Loxdale & Lushai, 2003). Such variance has adaptive significance when environments are changing. In the gall-forming lettuce root aphid *Pemphigus bursarius* (L.), behavioural modifications in facultative asexuals have meant that clonal populations that would have normally been driven extinct persisted past expected ecological dead-ends (Phillips, Bale & Tatchell, 1999). Similarly, there are adaptive differences within individuals of artificial clones of red-spotted cherry salmon *Oncorhynchus masou macrostomus* (Berg) (Iguchi, Matsubara & Hakoyama, 2001). Intraclonal variation is the basis of such phenomena and the potential for somatic mutations to drive such variation is well-described in plants (Gill *et al*., 1995; Klekowski, 2003). However, what is lacking is the molecular support. An example is the recent correlation of rDNA intergenic space (IGS) size variants with major life history traits in *D. pulex*, indicating rapid adaptation in clonal lineages (Gorokhova *et al*., 2002).

All the examples above involve vertical propagation with offspring produced in different generations. Horizontal propagation can also occur when a single fertilized egg cleaves into several embryos. Classic

examples include mammalian 'identical' siblings such as Armadillos, which have up to 12 offspring (Nowak, 1991), and some parasitic hymenoptera, e.g. *Copidosoma floridanum* (Ashmead) with several thousand offspring (Grbic, Nagy & Strand, 1998). During development, such embryos are under very similar metabolic and environmental conditions; however, in the case of the wasp, after birth, development can be channelled into either a normal larva or a defensive soldier morph (Harvey, Corley & Strand, 2000; see Loxdale & Lushai, 2003, for a photograph of these morphs).

Analysis of eukaryotic genome databases suggests that duplicate genes arise at a high rate (0.01 per gene per Myr) (Lynch & Conery, 2000). In time, most become functionally silenced; however, the accumulation of such duplications may act as barriers to interspecific gene flow and thus promote speciation. In largely asexual lineages, a high level of karyotype variation has been noted that surpasses intraspecific sequence differences (Blackman, Spence & Normark, 2000). In some species, karyotype variation has been correlated with host shifts. Thus the corn-leaf aphid *Rhopalosiphum maidis* (Fitch) has karyotypic forms specific to barley (2n = 10) and maize *Zea mays* L. (2n usually = 8) (Brown & Blackman, 1988). Hence such karyotypic variation could be an early stage in sympatric speciation.

Horizontal gene transfer between genomes would also allow non-recombinant genomes to change, as occurs in both prokaryotes and eukaryotes (Jain, Rivera & Lake, 1999; Jain *et al*., 2003). It appears that universal 'housekeeping' genes are more likely to cross species boundaries than informational genes governing processes such as transcription and translation because the latter relate to large complex systems. Sequence comparisons indicate that the transfers consist of singular large-scale events that could have led to the evolution of new taxa.

Some species have multiple genomes. For example, fluorescent *in situ* hybridization (FISH) probes have been used to trace the distribution of two divergent sequences of the rDNA ITS2 in arbuscular mycorrhizal fungi (Kuhn, Hijiri & Sanders, 2001; Sanders, 2003). The molecular probe shows that the ITS2 copies can occur alone in a proportion of nuclei whilst some nuclei support both types, suggesting that several independent genomes exist within individual asexual lineages (with the potential for recombination between the different nuclear genomic populations). Such genomic variation within coenocytic hyphae raises the possibility of intraclonal genome competition as the organism adapts to changing environments. Chimerism, the physical merging of two separate clonal organisms, has been verified by phenotypic characters and randomly amplified polygenic markers (RAPDs) in red algae, rhodophyta and ascidian zooids (Ascidiacea, Tunicata) (Sommerfeldt & Bishop, 1999; Santelices, 2001; Sommerfeldt, Bishop & Wood, 2003). The evolutionary analysis of such complex associations awaits further molecular analysis.

Part of the success of asexuals has been attributed to the possible existence of general purpose genotypes ('GPGs', Lynch, 1984). The ancient asexual ostracod *D. stevensoni* (with an extensive geographical distribution) was compared with asexual populations of *Heterocypris incongruens* (Ramdohr), a cypridinid species with mixed reproduction, and another ancient asexual darwinulid species with a limited geographical and ecological distribution, *Vestalenula molopoensis* (Martens & Rossetti) (Van Doninck *et al*., 2002). Salt and temperature tolerances were greater in *D. stevensoni* compared with both *H. incongruens* and *V. molopoensis* and one interpretation of this is that the *D. stevensoni* clonal population has a GPG giving the ostracods a selective advantage in a range of environments. Such entities are thought able to adopt several niches in a heterogeneous environment due to their wider tolerances to change.

CLONAL LINKAGE

In species with multiple lifecycles, there are opportunities for individuals within clonal lineages to benefit from alternative sexual lifecycle gambits, emphasizing that asexual lineages are not necessarily stuck in evolutionary 'dead ends'. As molecular markers continue to be applied to population genetic studies, possible linkage between asexual and sexual lineages has been suggested in a growing number of species – aphids of the genus *Trama* (Normark, 1999) and *Sitobion* (Delmotte *et al*., 2001), water fleas, *Daphnia* spp. (Crease, Stanton & Hebert, 1989) and non-marine ostracods (Schön *et al*., 2000) (see also Simon *et al*., 2003). Such linkage may involve interspecies hybridization, as suggested between the predominantly asexual grain aphid *Sitobion avenae* (F.) and the holocyclic (with sexual phase) sister species, the blackberry-grain aphid *S. fragariae* (Walker). This appears to result in habitat specialization in the largely clonal summer populations (Sunnucks *et al*., 1997; for ostracod examples, see Havel, Hebert & Delorme, 1990; Chaplin, Havel & Hebert, 1997).

To describe the adaptive clone in this synthesis, the focus has been on pure asexual reproduction and apomictic parthenogenesis. Yet the different types of clonal systems are in themselves evolutionary strategies that promote genetic variability (Hughes, 1989). 'Gynogens' is a process whereby clonal egg activation is dependent on sperm produced by ancestral or related species. This is a remarkably diverse system, for example, in salamanders of the genus *Ambystoma* (Hedges *et al.*, 1992). Here mtDNA studies have

revealed that in all-female salamanders incorporating the nuclear genome of several sympatric bisexual species, hybrids are produced with unusual ploidy. The result is that maternal inheritance has become uncoupled from the nuclear genome, to the extent that in certain species of this complex the prevalent gynogenetic maternal lineage derives from an ancient line for which there is no contemporary equivalent nuclear bisexual species. 'Hybridogenesis' is maintained by heterospecific fertilization, where an invariant haploid maternal genome is combined with a recombinant paternal genome. Some vertebrates, e.g. Mexican poeciliid fish (Quattro, Avise & Vrijenhoek, 1992), and invertebrates, e.g. stick insects of the genus *Bacillus* (Tinti & Scali, 1996; see Pertoldi, Scali & Loeschcke, 2001; Scali *et al*., 2003), demonstrate this phenomenon and certainly the fish appear to derive from old maternal lineages (~0.1 Myr). All these modes impart genetic variation involving a basic clonal component.

CLONAL ADAPTATION IN THE FIELD

In peach-potato aphids *Myzus persicae* (Sulzer) collected from natural populations, adaptation to environmental selective pressure is well-documented in the form of resistance to pesticides. In the highly insecticide-resistant strains of this insect (R_2 and R_3), tolerance to organophosphates (OPs) and carbamates is conferred by overproduction of a carboxylesterase enzyme following amplification of the E4 and FE4 genes, a factor associated with a translocation event between autosomes 1 and 3 in the case of E4 (Blackman *et al*., 1995). In the absence of the pesticide the phenotype may revert to a lower esterase expression due to loss of methylation, yet the number of E4 genes involved is apparently not affected (Hick, Field & Devonshire, 1996; Field *et al*., 1999; Field & Blackman, 2003). In these strains resistance to OPs and carbamates is also conferred by altering enzyme catalytic site conformation (MACE, modified acetyl cholinesterase) or other structurally important proteins such as the domain IIS6 transmembrane in the case of the insect *para*-type sodium channel gene responsible for knockdown (*kdr*) resistance to pyrethroids (Devonshire *et al*., 1998; Foster, Denholm & Devonshire, 2000). In such cross-resistant forms in asexual species, the multiple resistance is often in strong linkage disequilibrium (Hick *et al*., 1996; Devonshire *et al*., 1998), perhaps as a consequence of 'selective sweeps'. The clonal genotype has not altered (except for epigenetic influence in the case of E4/FE4; Field & Blackman, 2003), yet the change brought about by a selective environment benefits the organism in the short term in the face of continued pesticide selective pressure (Foster *et al*., 2000). These changes have been noted to have fitness costs, notably in sensitivity to alarm pheromones, overwintering survival and migration, which makes these genotypes less successful over the course of time (Foster *et al*., 2000, 2002) (Table 1b).

Several other examples of fitness effects are evident from molecular studies of aphids in the field or greenhouse. Use of molecular markers has revealed high intensity of selection driving adaptation in new habitats or hosts. Examples include *Sitobion* aphids moving between native grasses and cereals (Sunnucks *et al*., 1997; Wilson, Sunnucks & Hales, 1999), and the cotton-melon aphid *Aphis gossypii* Glover moving between cucurbit and non-cucurbit hosts (Vanlerberghe-Masutti & Chavigny, 1998; Fuller *et al*., 1999; see Wilson, Sunnucks & Hales, 2003, for an overview). Such exploitation of new resources may initially be successful because exploitation of a novel resource reduces intraspecies competition or clonal interference.

Lastly, the combination of genetic changes, prodigious growth rate and rapid developmental responses to environmental cues along with geographical displacement to new habitats are probably the main reasons for the successful establishment of several well-reported clonal pests, such as the spotted alfalfa aphid *Therioaphis maculata* (Buckton) in North America. Here a large number of novel adapted clones, including insecticide-resistant strains, appear to have evolved within a few generations from a small introduced asexual founder population. It has been estimated that from 1.7×10^{11} aphids in one Californian valley, a rare variant would have evolved 170 000 times (Dickson, 1962). The transition from clonal variation (intraclonal variants) to the establishment of interclonal variation is a population phenomenon involving persistent selection. Once established, such systems are susceptible to environmental change (Charlesworth, Morgan & Charlesworth, 1993; Howard & Lively, 1994; Waxman & Peck, 1999). Rare genotypes may tend to survive when selective sweeps strike the majority (Vrijenhoek, 1998) and may help to maintain population dynamics (Sasaki, Hamilton & Ubeda, 2002). Molecular studies are already revealing rare genotypes that appear to be maintained within asexual populations (Lushai, Markovitch & Loxdale, 2002).

CONCLUSIONS

Theoretical biologists continue to stress the longevity, and thus high adaptedness of lineages, although extinction is inevitable for all lifecycle types (Raup & Jablonski, 1993). Intense discussion of the persistence of sex tends to override further debate on genetic variability and adaptation in asexual populations (Falush, 1999; but see Fagerström, Briscoe & Sun-

nucks, 1998). Furthermore, variation in asexual organisms in agricultural, horticulture, forestry and medicinal industries have very real applied significance. Artificial cloning is a new and expanding field that will also benefit from a better understanding of the dynamic nature of clonal lineages. These points are exemplified by recent developments in cloned mammals where such lineages are corrupted due to epigenetic-related genomic malfunctions (De Sousa *et al*., 2001; Humpherys *et al*., 2001; Kang *et al*., 2001; Taeyoung *et al*., 2002). Much still needs to be understood about the basic mechanisms of change in clonal genomes, along with a continual need to upgrade our preconceptions.

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