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Intraclonal genetic variation: ecological and evolutionary aspects.
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Rapid changes in clonal lines: the death of a 'sacred cow'

HUGH D. LOXDALE[1,*] and GUGS LUSHAI[2]

[1]*Plant & Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JQ, UK*
[2]*Agriculture and Agri-Food Canada (AAFC), Eastern Cereal and Oilseed Research Centre (ECORC), Ottawa, Ontario K1A OC6, Canada*

It is well established that asexually reproducing viruses and prokaryotes mutate rapidly. In contrast, the eukaryotic clone is often still treated as if it is genetically homogeneous within and between populations, i.e. that it is assumed to show genetic fidelity. However, such fidelity has rarely been tested empirically using the range of high-resolution molecular markers now available, culminating with direct sequencing of the DNA. If such a biological entity as a 'clone' really did exist, it would be a fantastic entity, differing from everything else known in biology, i.e. it would possess a population mean but no variance for any particular trait. It would not be amenable to selection and adaptive variation and would thus be unchanging in time and space. In this paper, we argue that the general acceptance of clonal fidelity is a scientific convenience, since the rate of asexual reproduction of eukaryotes is not as fast as that of bacteria and hence it is easier to accept fidelity as a 'fact' rather than test for it. We propose that part of the acceptance of fidelity may have a cultural basis and thereby is a kind of 'pre-Darwinian relic'. Instead, a clonal genotype is perhaps largely a function of marker resolution, i.e. dependent on the number and type of markers employed. If this is so and were enough of the genome explored, perhaps each individual within a clone would be found to differ genetically at particular regions of the chromosomes. The question of what constitutes a clone is not just a semantic one and impacts directly on recent attempts to understand and produce 'artificial' clones, especially of mammals. New research is already confirming that mutations and epigenetic influences play a crucial role in the success of cloning attempts. *Biological Journal of the Linnean Society,* 2003, **79**, 3–16.

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"The process of evolution depends on the occurrence of hereditary variation. If DNA replication were always perfect, life could not have evolved and diversified; the same kinds of organisms, and no others, would be living today that existed 3 billion years ago, unless these had become extinct in the meantime." (Francisco J. Ayala, 1978)
"In the past, researchers assumed that genomes evolve to minimize mutation rates and prevent random genetic change. But the new findings are persuading them that the most successful genomes may be those that have evolved to be able to change quickly and substantially if necessary." (Elizabeth Pennisi, 1998)
"Chance favours the prepared genome." Lynne Caporale

*Corresponding author. E-mail: hugh.loxdale@bbsrc.ac.uk

INTRODUCTION

All individuals age, even individual parthenogens such as aphids. After giving birth to her offspring (perhaps 30–60 in total; Blackman, 1971), a female asexually reproducing aphid will senesce and die. In contrast, the clonal lineage (a vertically produced lineage, i.e. between generations) seemingly has some degree of 'immortality', although somatic and germ line mutations are assumed to occur and perhaps accumulate, as hypothesized in accordance with Muller's ratchet (Muller, 1964). Reference to clones as 'genetically identical' is still commonplace in the literature and media (e.g. Leutwyler, 1998; Cross, 2002), often despite a lack of supporting empirical evidence. Thus non-fraternal mammalian twins (an example of horizontal clonality, i.e. within a generation) are

known as 'identical' (Airhart, 1998), but this is not proven. Some mammals have even larger numbers of horizontally produced 'identical' offspring, up to 12 in the case of armadillos, whilst some parasitic wasps can produce thousands of offspring from a single fertilized egg. In this article, we briefly review current opinion concerning intraclonal genetic variation in a range of taxa, especially in relation to aphids. We also set the scene for the more specialist papers which follow in this volume of the journal, all contributions to the joint Royal Entomological Society–Linnean Society international symposium 'Intraclonal Genetic Variation: Ecological and Evolutionary Aspects', held at the Linnean Society, 11–12 April, 2002. Before so doing, we also provide a brief philosophical perspective concerning the possible reasons why clones are seen by many to have the special property of genetic fidelity and homogeneity.

PHILOSOPHICAL PERSPECTIVES

It is surprising that well over a century after the theories of evolution of Charles Robert Darwin (1809–82) and Alfred Russel Wallace (1823–1913) and the acknowledgement among biologists that living things mutate and adaptively radiate into novel ecological niches (Wright, 1988), eukaryotic clones are still perceived to maintain genetic fidelity over time. Certainly this misconception appears to be held by the public at large, although mutation in viruses and prokaryotes, particularly in relation to the rapid evolution of antibiotic resistance in pathogenic bacteria, is an established fact (Neu, 1992; Walsh *et al.*, 1996; Maiden, 1998). Even some biologists, although doubtless aware of mutational processes in general, accept the notion of eukaryotic clonal genetic fidelity in time and space, without rigorously questioning the fundamental premise of this unlikely state. Indeed, ideas of clonal fidelity of eukaryotes may be seen as a kind of last bastion of pre-Darwinism. How did this come about? It may, in part, be explained by the fact that viruses and prokaryotes have very fast rates of reproduction even compared with fast asexually propagating eukaryotic species such as aphids and nematodes. Genetic changes or entire genetic revolutions are thus comparatively more readily detected in these relatively simple organisms within a much shorter time frame. Another reason may perhaps relate to the fact that it is easier during a scientific study to *assume* clones have genetic homogeneity rather than test for this empirically using molecular markers; in many instances, useful specific markers (e.g. microsatellites) do not exist for the organism/s under study. A third reason, certainly as far as the wider public is concerned, may be a cultural one, as briefly detailed below, i.e. partially religious/historical in nature, which may ultimately have its roots in a continuing failure, wittingly or unwittingly, to fully appreciate 'What evolution is' (Mayr, 2002).

Much of modern science (but of course by no means all), has its origins in Europe, more especially since the Scientific Revolution of the 16th and 17th centuries. Scientists of this period (the best-known example being Galileo) were often in direct and serious conflict with the Christian church for promoting ideas and theories at variance with the accepted beliefs and dogmas of the day. Even as late as the mid-19th century, Darwin delayed publication of his thoughts on evolution because it conflicted with his high Anglican upbringing. He knew, quite rightly as it turned out, that the established church would not approve of his ideas, which were deemed heretical – and still are to a great many people today (Desmond & Moore, 1991; White & Gribbin, 1996). In the Judeo-Christian world view, there is one unchanging God, and the world was created as described in Genesis: the species are fixed. It is a hierarchical, anthropocentric system with mankind, God's divine creation, created as a facsimile of the creator, at the apex of the tree of life and with an immortal soul. This was the philosophy especially espoused by the Spanish Jesuit Francisco Suarez (1548–1617), founder of the idea of 'Special Creation' (White & Gribbin, 1996).

Contrastingly, in other religions, such as Hinduism and Buddhism, which had their origins in Asia, a different vision exists. Here, individual organisms live and die and age, and while mankind *may* be superior to other creatures in terms of ability, especially mentally, it is not held to be necessarily better than the rest of creation. As an organism dies, its life essence passes to other creatures; slowly, over many such life-and-death episodes, it eventually attains the dignity of humankind (Hindu concept of *Punarjanma*, or rebirth). There is thus the cultural idea and acceptance of transformation and continual change.

Sometimes the soul or spiritual essence is accompanied by a physical manifestation of change; in Béla Bartók's 1930 musical masterpiece, *Cantata Profana*, based on Romanian folklore, humans change into stags – a concept essentially pagan in origin (Ujfalussy, 1971). Similar ideas are also found in other cultures at other times, including the present. In Greek mythology, animal transformation or certainly hybridization can be seen in creatures like the sphinx, centaur, chimaera, minotaur and harpy; there is even a hybrid god, Pan. Hybrid organisms are found in other Mediterranean and Eastern cultures, including Persian (the gryphon) and Egyptian (the god Horus). Merfolk (mermaids and mermen) are ubiquitous in European, American, Asian and Polynesian folklore.

In ancient pre-Socratic Greek culture the notion of political and intellectual flexibility was accepted.

However, with Plato (427–347 BC) and his school such ideas declined and instead fixity in thought and political action became dominant (Popper, 2001). Prior to Plato, Empedocles of Agrigentum (495–435 BC) was the first person to propound an evolutionary origin of life. Here *parts* of animals budded off from plants and then assembled themselves. Only those creatures whose constituent parts were in harmony survived and the more 'monstrous forms' died out because they could not find mates. Aristotle (384–322 BC) in his treatise *Physics*, actually suggested that the fittest forms of life (in a modern Darwinian use of the term) could have arisen through chance, rather than design. He proposed a chain or ladder of evolution leading to the ideal form of perfection, Man.

In the European medieval world-view, animals and plants aged, but the seasons and indeed life itself (here equated with 'Nature'), were locked into a perpetual system dependent upon God's divine grace: it was a closed circle, essentially one of stasis. The Darwinian view of nature as one of flux, of change occurring over time, had its origin in the Enlightenment of the 18th century (Bury, 1982). Evolution can perhaps be likened to an open helix, with all the biological simile that this engenders.

Living entities have a special attribute that is widely acknowledged – that of variation, the stuff of natural selection, adaptation (including the process of genetic drift) and hence, ultimately, evolution. A population has, for any given character, be it genetic or phenotypic, a mean and its variance, either continuous or discontinuous (Sturtevant & Beadle, 1940; Shorrocks, 1978). The variation seen within a population of living organisms is an imperfect manifestation of an 'ideal' form, i.e. it is contrary to ideal notions of a divine unchanging entity itself equated with 'perfection' (Davies, 2001).

CLONES – IMAGINARY AND REAL

If all living creatures in the natural world are subject to mutation and selection, adaptive radiation and evolution, clones are surely also subject to such changes.

The term 'clon' (derived from the Greek *klon*, meaning twig or cutting) was originally coined by Herbert J. Webber in 1903, in reference to the vegetatively produced offshoot or descendant of a single plant being 'a colony of organisms derived asexually from a single progenitor' (Webber, 1903). It later evolved into 'clone', and came to be used more loosely and broadly over time (Mittwoch, 1998). Now two definitions exist. The first describes a clone as being an asexual lineage from a stem mother, the second additionally involves genetic identity (i.e. strict clonal fidelity) between members of the clone (Abercrombie *et al.*, 1990). In molecular biology, a clone is a piece of DNA inserted into a plasmid in order to produce multiple identical copies by a process of amplification. In immunology it denotes a protein molecule derived from a single hybridoma cell lineage with common immunological properties. If the second definition is accepted by biologists as having any particular biological meaning in relation to whole clonal organisms, then it needs to be tested. Prior to the advent of molecular markers (proteins, especially allozymes, and more recently, DNA), this was not possible, except for commonalties of colour and morphology. All the rest was assumption and, rather incredibly, a kind of triumph of hope over expectation. Clonal genetic homogeneity was rarely if ever tested empirically, so that when workers wished to describe a lineage as having genetic fidelity derived from a single founder, they used the term 'clone' when perhaps 'asexual lineage' was what they meant.

Now with the plethora of molecular (DNA) markers available, we can test the homogeneity of clones (Normark & Moran, 2000), both within and between lineages to discover: (1) whether there is genetic variability present in the genome; (2) if this is in (a) coding regions and hence potentially selectable, (b) in non-coding regions or (c) spread between; (3) if any of this variation has adaptive significance. This paper provides evidence, including empirical molecular data, indicating that clones have variance. We suggest that this variance may have biological significance (a topic more fully explored elsewhere in this volume; Lushai, Loxdale & Allen, 2003). But first we briefly explore a hypothetical world in which clonality in terms of exact genetic fidelity exists.

THE HYPOTHETICAL CLONAL ORGANISM

Selection may be the mechanism of evolution, but adaptive radiation is the main consequence of the evolutionary process, with all that this means in terms of ecological, morphological, physiological (e.g. pheromones), behavioural and molecular (DNA) changes. However, until recently there was rather little direct evidence for adaptive changes at the level of the gene and genome (see Carvalho *et al.*, 2002 for a review). The new area of 'genomics' ultimately concerns how the genome functions and changes in the face of selection and adaptation to novel selective forces and how changes in the DNA relate to phenotypic expression.

In a world without mutation and selection – with, in effect, genomic fixity – true clones would probably be unsuited to changing environmental conditions. This might not be so severe for organisms in a relatively stable environment, e.g. bacterial endosymbionts of insects. However, for organisms with short life spans living in a dynamic habitat with rapidly changing eco-

logical factors and novel selective pressures, such an environment would be seemingly detrimental to survival, both in the short and long term. Organisms would need to adapt quickly to altered circumstances (Vrijenhoek, 1998). An example here might be clonal aphids feeding on an ephemeral summer food plant; these, as described below, are liable to senesce.

In theory, all natural populations (and, one assumes, all laboratory populations) have a mean and variance for various characters (Fig. 1A). Presumably, laboratory populations, usually a subsample of the genetic variance in the wild population, may have less variance, often due to selection or drift (Unruh *et al.*, 1983). In the case of clones, if genetic identity really existed, then it could be graphically represented as a vertical 'pole' (Fig. 1B). Rather, one assumes that clones have variance, but that this is very restricted for various parameters, molecular and phenotypic. In the wild, as the clone evolves and responds to more and more environmental selective pressures and adapts accordingly, then the width of the variance may be assumed to increase as a result of the diverging lineage.

Asexual (= apomictic) aphid lineages e.g. the peach potato aphid, *Myzus persicae* (Sulzer), may be reared for long periods using a technique which involves artifical diets and the use of a membrane through which the aphids probe to obtain nutrients (Van Emden, 1988). Such aphids show variation for certain carboxylesterases and this may be selected to produce individuals *within a clone* with an esterase level greater than the typical clonal mean (Bunting & van Emden, 1980). Changes in esterase electrophoretic banding pattern have also been noted in aphids kept on similarly artificial diets, perhaps due to the effect of enzyme induction (Bunting & van Emden, 1981), although such changes are disputed (White, 1983). It may be posited that all individuals within the clone should have an identical lifespan and productivity (i.e. no variance), such that all members produce exactly the same number of offspring and all die exactly at the same time! Anecdotally, some plant clones like raspberries do have a similar lifespan world-wide (McLaren, 2000), but presumably with variance around a mean. A truly clonal entity may also have the distinct biological disadvantage of a lack of ecological flexibility (barring differential gene expression depending on environmental cues, e.g. various heterotrophic bacteria living on different carbon sources).

It is well known that over-specialization tends to lead towards extinction if the niche changes. True clones would be the ultimate in specialization, but they could not exist in the 'real' world of constant environmental change. This is a consequence of their life-cycle; unchanging clones have an adaptive peak and cannot cross between peaks within an adaptive landscape (Fisher, 1958; Wright, 1988). However, recent data provide evidence of variation, sometimes rapid, within eukaryotic clonal lineages or their analogues, i.e. highly inbred lines. In this respect it is worth commenting further on aphids, which clearly display the degree of biological flexibility that may be observed, starting at the level of the species and then progressing towards the so-called clone *sensu stricto*.

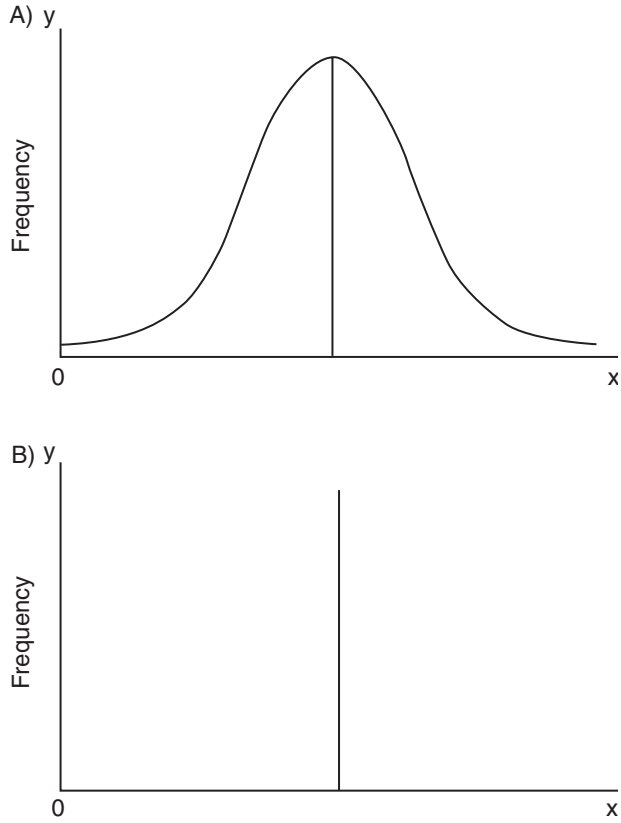

Figure 1. A. Normal distribution with mean and variance. B. Hypothesized 'clone' – the general perception.

Rapid genesis of genetic variation, with special reference to aphids

Various aphid species show a variety of polymorphisms in terms of karyotype, colour (discussed in further detail below) and other morphological features (e.g. anatomical ones such as siphunculi length and number of setae on abdominal tergites, rostral segments, legs, etc.; Foottit & Mackauer, 1990; Blackman & Eastop, 2000). Sometimes, as in the case of the corn aphid, *Rhopalosiphum maidis* (Fitch) which has two karyotypic forms ($2n$ = ~ 8 and 10), the polymorphism appears related to host adaptation (Brown & Blackman, 1988). In other species, such as *M. persicae s.l.*, translocation of autosomes may be related to actual

speciation events to form new 'asexual species', e.g. *M. antirrhinii* (Macchiati) which has a different chromosome number ($2n = 13$ or 14) compared with *M. persicae s.s.* ($2n = 12$; Blackman & Paterson, 1986). Besides the fact that such speciation events question notions of what a species is exactly (Claridge, Dawah & Wilson, 1997; Foottit, 1997), the production of chromosomally distinct forms may well be a one-way evolutionary ticket. Thus the newly evolved form may be unable to sexually reproduce with the founder population due to chromosomal non-disjunctions. Chromosomal translocation can also lead to the conferment of pesticide resistance (see below). Particular aphid species may also be more or less polyphagous, although undoubtedly some of this apparent variance is related to the existence of morphologically similar strains or even 'cryptic' species, as is also known in other Homoptera like whitefly, *Bemisia tabaci* (Gennadius) (Legg, 1996).

Within what one may term a 'good' aphid species, that is to say, one that appears to be homogeneous in respect of host adaptation, variance is still often apparent. This is clearly demonstrated in the case of cereal aphids of the genus *Sitobion*. Some species, e.g. *S. fragariae* (Walker), appear to show cryptic strain differences or sympatric speciation (Loxdale & Brookes, 1990; Sunnucks *et al.*, 1997), whilst others show chromosomal re-arrangements of potentially adaptive significance (Sunnucks *et al.*, 1998). Sometimes molecular DNA differences in electrophoretic banding profile (RAPDs [random amplified polymorphic DNA] or microsatellites) related to the host-plant have been noted, as with *S. avenae* (F.) (De Barro *et al.*, 1995; Sunnucks *et al.*, 1997), even on a microgeographical scale. In this aphid, host selection is associated with founder events of a colony produced when winged female migrants search out and land on various summer hosts such as Poaceae (grasses and cereals) (Lushai, Markovitch & Loxdale, 2002).

In addition to these 'adaptive' changes in geno- and phenotype, other changes are seen at the level of the clone itself. Blackman (2000) discusses clonal variability and adaptation in a paper entitled 'The cloning experts'. However, contrary to expectations, aphids do not seem to be too good at maintaining exact clonal phenotypic fidelity, let alone genetic fidelity. Besides the host adaptation alluded to above, and variance in anatomical features, an aphid clone can vary in the number of ovarioles (embryos) produced as well as reproductive rate (Dixon, 1989). Thus for example, alate exules (winged migrants) of the bean aphid, *Aphis fabae* Scopoli can have either 6, 8, 10 or 12 ovarioles. This variability is not related to weight, as it is in some Diptera (true flies) (Webber, 1955; Bennettova & Fraenkel, 1981). The range and frequency distribution of ovariole classes is characteristic for a species and even for particular morphs within a species (Dixon, 1989). Differences in reproductive rate (productivity) as well as longevity are also seen in highly inbred lines of the nematode, *Caenorhabditis elegans* (Maupas) raised under constant environmental conditions; these show a mean as well as variance for somatic traits (Vassilieva & Lynch, 1999; Fig. 2). Hence, the clone is by no means a fixed entity in terms of potentially adaptive changes, although the majority of such changes are not only 'silent', i.e. unobserved phenotypically, but probably neutral with only a very slight effect on fitness (~ 0.07%) (Davies, Peters & Keightley, 1999).

Within an aphid clone, and subject to crowding effects or plant nutritional status, winged morphs can be induced as a prelude to migration (Lees, 1967; cf. Dixon, 1998 for other details). Of these winged morphs, some will be short-range and others long-range 'flyers' (Kidd & Cleaver, 1984, 1986). Also, within a species, some clones are obligatorily asexual and can only produce winged and wingless asexual individuals (anholocyclic forms). In contrast, other clones or lineages can produce sexual morphs (gynoparae, presexual females), males and oviparae (egg-laying females which mate with males) dependent upon diurnal length and temperature conditions (holocyclic forms) (Lushai, Hardie & Harrington, 1996; Dixon, 1998). In turn, this polyphenism is usually associated with host switching between a primary woody host on which overwintering eggs are laid and a herbaceous secondary host on which asexual propagation occurs during the summer months. Between times, winged individuals migrate in spring and summer to colonize these different hosts during the different phases of the life-cycle (Moran, 1988). Other lineages still can produce morphs which are sexually intermediate or produce a few rare males amongst asexual females (androcyclic forms) (Dixon, 1998). In such multiple life-cycle species, genotypes from these partially asexual lines can mate with sexual lines increasing the genetic diversity of the lineages (Normark, 1999; Simon, Rispe & Sunnucks, 2002). These trends demonstrate that whatever a clonal lineage is, it is potentially a very versatile entity, and adapted to various environmental stimuli. Such a variety of life-cycle forms contribute to a complexity almost unparalleled in the natural world, accentuated by the alternation of sexual and asexual generations as well as a linkage between them (Delmotte *et al.*, 2001; Simon *et al.*, 2003, this issue; Wilson, Sunnucks & Hales, 2003, this issue).

Colonies of some gall aphids, often highly inbred, hence effectively clonal and showing little intergall migration, can produce morphologically very different 'soldiers' possessing mouthparts used to defend the other morphs against attack from predators and par-

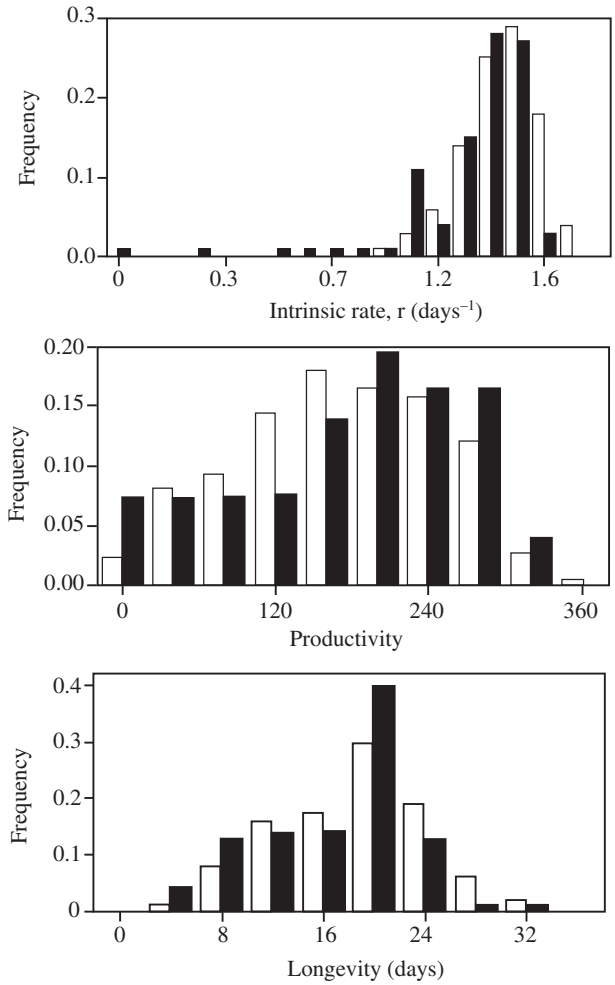

Figure 2. Graphs showing that highly inbred populations of the nematode *C. elegans* are not a 'perfect form'. Rather, such populations show variance for intrinsic population growth rate 'r', productivity and longevity (Figure 2 from Vassilieva & Lynch, 1999: The rate of spontaneous mutation for life-history traits in *Caenorhabditis elegans*. *Genetics* **151:** 119–129, reproduced with kind permission of the authors). The distribution of line means at generations 7 (white) and 49 (black). The phenotypic values for longevity were corrected for the changes in the controls.

asitoid wasps (Aoki, 1977; Dixon, 1998). Intraclonal morph changes (winged and wingless) are known to be under the influence of, and indeed regulated by, juvenile hormone titre (Lees & Hardie, 1981). This type of morphological change between apparently 'genetically identical' members of the same clone is also noted in polyembryonic wasps, *Copidosoma floridanum* (Ashmead) which parasitize eggs and larvae of moths in the subfamily Plusiinae (Lepidoptera: Noctuidae), sometimes producing more than 3000 embryos (Ode & Strand, 1995). There are two morphologically distinct castes, reproductive and defensive 'soldiers', the latter thin and worm-like (Fig. 3, which shows a closely related species, *C. sosares* Walker). There is found to be a distinct trade-off between these castes in response to intra- and interspecific competition within the parasitized host (Grbic, Nagy & Strand, 1998; Harvey, Corley & Strand, 2000). Geographically separate asexual populations of the spionid polychaete worm, *Pygospio elegans* (Claparède) also show different developmental modes as a function of environmentally induced reproductive flexibility (poecilogony) (Morgan *et al.*, 1999). Stress has been shown to influence the operation of genetic elements within the genome in bringing about change in function (Capy *et al.*, 2000). The influences of such mechanisms and those based on cytoplasmic inheritance (True & Lindquist, 2000) need to be studied further to better understand the molecular control of phenotypic variation.

Recent studies using molecular markers have revealed a plethora of mutational changes in aphids, including both direct alteration of the chromosomes, usually as determined electrophoretically with microsatellites, rDNA markers, etc., as well as epigenetic effects. Some species, for example *S. avenae* and the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), also show morph-dependent molecular genetic variance within clones. Thus, reproducible RAPD banding pattern differences were observed between winged, wingless and sexual (male or female) morphs of the same clone kept under conditions of strict clonal hygiene. One such band was found to discriminate between winged and wingless morphs (*S. avenae*) and sequence analysis showed it to have about 60% homology with a myosin gene promoter region (Lushai *et al.*, 1997). These *intra*clonal, *inter*morphic differences may relate to differential expression of repeated sequences between primer binding sites related to the genomic expression of different phenotypes (Lushai *et al.*, 1997) or, less likely, to methylation of the DNA. Empirical studies using a range of molecular markers – RAPDs, synthetic oligonucleotide probes, i.e. $(GATA)_4$ and AFLPs (amplified fragment length polymorphisms; cf. Loxdale & Lushai, 1998) – have also shown that clonal lineages of *S. avenae* and other aphids (e.g. grape rootstock phylloxera, *Daktulosphaira vitifoliae* Fitch) display banding pattern differences within a range of 5–15 generations (De Barro *et al.*, 1994; Lushai *et al.*, 1998; Forneck, Walker & Blaich, 2001a,b), mostly somatic, but in one case, in the germ line (Lushai *et al.*, 1998). In *S. avenae*, generation time is about 10 days. Whilst it is possible that the change noted using AFLPs in *D. vitifoliae* could be due to symbiotic bacteria (Moran, Baumann & von Dohlen, 1994; N. A. Moran, pers. comm.; Forneck *et al.*, 2001b), this seems unlikely in the other studies on *S. avenae*. Here positive controls (i.e. aphid heads only, lacking

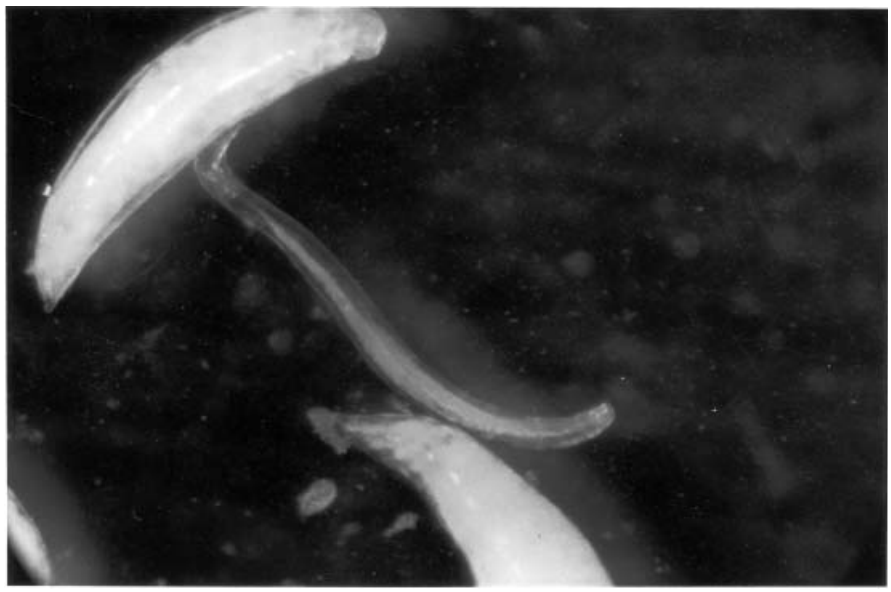

Figure 3. Two different clonal larval castes of the hymenopterous parasitic wasp, *Copidosoma sosares* (Walker). The thin thread-like larva is the 'soldier' or 'defender' morph, which protects the normal larval siblings from intra- and interspecific competition and dies when these pupate. The caste-ratio of soldiers to normal larvae is dependent upon the competitive pressure, and hence is an adaptive trait. (From: Hardy ICW, 1996: Precocious larvae in the polyembryonic parasitoid *Copidosoma sosares* (Hymenoptera: Encyrtidae). *Entomologische Berichten* (*Amsterdam)* **56:** 88–92. Reproduced with kind permission of the author, photographer Kees Hofker and journal).

symbionts) and cross-referencing using an aphid-specific probe supported the aphid origin of the band/s detected (cf. Lushai *et al.*, 1998 for details). These molecular studies demonstrate the rapid nature of molecular evolution in aphids in relation to potential adaptive significance, e.g. as seen with RAPD profiles in relation to specific hosts in *S. avenae* (Lushai *et al.*, 2002) and indeed, other markers.

Besides these changes, other longer term evolutionary changes have been noted in aphids in relation to host adaptation, more especially those affecting sequences of the mitochondrial DNA, e.g. cereal aphids like the greenbug, *Schizaphis graminum* (Rondani). These aphids have 'biotypes' that have been described in relation to their resistance to crop cultivars, although phylogenetic analysis appears to relate clustering to plant-host adaptation which predates agricultural practice (Shufran *et al.*, 2000; Anstead, Burd & Shufran, 2002). At some point, these and other molecular changes that occur within clonal lines lead to potentially great diversity (Blackman, 2000). This is possibly due to the telescoping of generations of aphids (Dixon, 1998) and the fact that in the absence of mortality factors like inclement weather, predators/parasites and pathogens, aphids can produce prodigious numbers of 'clonal' offspring in a relatively short while, e.g. around 14–20 asexual generations per annum from a single female founder (Loxdale & Lushai, 2003). All these factors contribute to adaptive changes that are well exemplified in the case of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) in California, which supposedly arose from one or a few founders. It has quickly multiplied and spread and produced numerous variants, including some resistant to pesticides (Dickson, 1962; Blackman, 1981, 2000).

With aphid resistance to pesticides, the problem has arisen in many countries in a relatively few species (Devonshire, 1989). Perhaps the mutations responsible for such resistance have only actually occurred a few times, but the clones have subsequently spread around the world in a very short time, either by their own means or, equally likely, by human agency (Loxdale *et al.*, 1993; Field & Blackman, 2003, this issue). In Britain, four species of aphid are resistant to pesticides, including *M. persicae* (Needham & Sawicki, 1971). All these cases arose in greenhouses or in situations where they have been subject to intense and repeated pesticide selection pressure, e.g. hop gardens in the case of the damson hop aphid, *Phorodon humuli* (Schrank). In *M. persicae*, high levels of carboxylesterase (E4) resistance is associated with an autosomal translocation (A1,3) which perhaps moves structural E4 genes away from a repressor gene (Blackman *et al.*, 1995). E4 regulation is also associ-

ated with methylation of the DNA which switches the genes on (unlike in vertebrates). How the genes are switched off when highly resistant clones spontaneously revert to lower levels of resistance, which they can do quickly in the absence of chemical selective pressure, i.e. one or two generations, is not clear (Hick, Field & Devonshire, 1996). Reversion does not appear to involve a change in the copy number of E4 genes as far as is presently known (see Field & Blackman, 2003 in this issue, for a detailed discussion of this phenomenon). Rather, epigenetic changes to the DNA influence the phenotype, which may direct feedback to its expression. In addition, the elevated esterases have pleiotropic effects on other resistance mechanisms, more especially knockdown resistance (*kdr*), which involves a mutation of the sodium channel gating system of the nervous system and which influences behaviour, including response to the alarm pheromone and movement (Foster, Denholm & Devonshire, 2000). These changes within clones clearly have adaptive significance (Lushai, Loxdale & Allen, 2003, this issue). Perhaps some of the 'phenotypic plasticity' observed in aphids, for example, changes in size in relation to host plant factors (Wool & Hales, 1997; Wilson *et al.*, 2003, this issue), are to a greater or lesser extent influenced by rapid genetic as well as epigenetic changes of the genome. Hence, an environmental-genomic feedback situation arises (as is also evident in the gene-switching mechanism concerned with the crowding of aphids earlier alluded to).

A final aspect of aphid clonality worth mentioning is colour. In some species like the rose grain aphid, *Metopolophium dirhodum* (Walker), a species with an autumn sexual phase, colour is genetically determined: females are apple green, males bright pink. The determining factor is the sex chromosomes, females being XX, males XO (Stroyan, 1949; Blackman, 1980; Dixon, 1998). Some species such as *S. avenae* come in a range of colours, from browns through to reds and pinks and greens of various shades, including a dark green form with a black dorsal patch on the abdomen (Jenkins, 1991). The holocyclic aphids (with sexual phase) are often brown. Under suitable conditions of light, temperature and diet, brown morphs can give rise to green offspring, showing that in this species, colour is not uniquely genetically determined but is also under environmental control (Jenkins, 1991; Jenkins *et al.*, 1999). The symbionts which are known to be involved in carotenoid synthesis (Jenkins *et al.*, 1999) may play a significant role in determining colour. It is highly probable that the different colours have adaptive significance, especially in relation to predation and parasitism by hymenopterous parasitoids (Ankersmit, Acreman & Dijkman, 1981; Losey *et al.*, 1997), or the colours are associated with protection from solar radiation (Jenkins *et al.*, 1999). Certainly, the inter- and intramorphic changes of colour, related to both quantitative and qualitative changes in carotenoid pigments (Jenkins *et al.*, 1999), demonstrate that an aphid clone may vary; it is not a fixed entity, whatever else it may be in a biological sense.

Rapid genetic changes in other taxa

Besides aphids, a wide range of other taxa have now been shown to undergo rapid genetic changes, from viruses, prokaryotes (bacteria) through to eukaryotes, including protozoans, plants, fungi and animals. Table 1 in Lushai & Loxdale (2002) illustrates clearly that clones are rapidly changing or have the potential to do so in the light of mutations of varying kinds. They thus fit in with the maxim of the ancient Greek philosopher Heraclitus (6th century BC): 'Everything is in flux, and nothing is at rest' (Popper, 2001).

However, even in the absence of direct selection (i.e. in a constant environment), organisms continue to spontaneously mutate, for example bacteria, aphids and nematodes (Moran, von Dohlen & Baumann, 1995; Moran, 1996; Lushai *et al.*, 1998; Vassilieva & Lynch, 1999). Such change is then seen simply as a property of the DNA, with its propensity to undergo point mutations directly, or the product of errors of replication. DNA repair mechanisms become operational in order to correct the majority of such changes; there are also parallel specific mechanisms of infidelity which need to be present for evolution to take place (Radman, 1999, 2001). There are sites where mutations fix, 'hotspots' often related to moieties (transposable elements, etc.) that have been implicated in the specificity of such sites, whilst other regions of the genome remain highly conserved. This promotes the hypothesis that after stochastic mutational events, some propagated by molecular mechanisms, a cascade of change may be in operation. In a creature such as *Drosophila* with ~ 13.5 thousand genes and ~137 megabases ($\sim 1.37 \times 10^8$ bases) of DNA (Adams *et al.*, 2000), it is inconceivable that chromosomes can be copied with strict fidelity each and every time a new individual is created. Certainly with bacteria (e.g. *Streptococcus pneumonae*) which divide within 20–30 minutes, the entire genome of some 2 million bases is copied, which means that around 1400 bases are copied a second! Here then, there is plenty of scope for error, as is well known, even with mutation and replication error rates of the order of 10^{-10}–10^{-9} per gene per generation.

Besides such fundamental changes to the base sequence of DNA, other larger scale mutational processes are well known (i.e. inversions and duplications), as well as duplications of repetitive sequences and slippage mediated events (e.g. microsatellites;

Goldstein & Schlötterer, 1999). Many of these polymorphisms are influenced by hotspots in the genome (Pennisi, 1998), more especially those mediated by transposons. Transposons are widely distributed amongst taxa, including *Drosophila* (P-elements, *mariners*, *copia*; Nuzhdin, Pasyukova & Mackay, 1996; Hartl, Lohe & Lozovskaya, 1997; Nuzhdin & Petrov, 2003, this issue) and aphids (*mariners,* L.M. Field, pers. comm.), although they appear to be absent in bdelloid rotifers, ancient asexuals which show the phenomenon of 'genome freeze' (Arkhipova & Meselson, 2000; Mark Welch & Meselson, 2000). In mosquitoes, MITEs (mini inverted repeat transposable elements) occur in some species at high frequencies throughout the genome ($>10^4$ copies) often at specific sites (Tu, 2001). Such elements are known to be involved in chromosomal re-arrangments in Diptera (*Drosophila*), some of which may have adaptive significance (Cáceres *et al.*, 1999a,b). Transposition rates are known to be high in *Drosophila,* up to 10^{-3}–10^{-2} per gene per generation (Nuzhdin *et al.*, 1996), whilst other non-coding regions such as microsatellites and minisatellite regions have mutation rates in the region of 10^{-5}–10^{-3} per gene per generation, sometimes up to 10^{-2}, whilst even the control region of insect mitochondrial DNA can mutate at around 10^{-4} per gene per generation (Lushai, Loxdale & Maclean, 2000 and Lushai & Loxdale, 2002 discuss mutation rates in a variety of living organisms; see also Klekowski, 2003, this issue). Lastly, many of the aforementioned duplicated regions are exceedingly abundant throughout the genome, for example in *Drosophila* (Schug *et al.*, 1998a, b), and occur as thousands of copies, sometimes tens of thousands (Goldstein & Schlötterer, 1999).

All of this makes it highly unlikely that clonal fidelity can be maintained for very long, i.e. over many generations, if at all (Lushai & Loxdale, 2002; Loxdale & Lushai, 2003). Thus, whilst the 50 offspring of a single asexual female aphid such as *S. avenae* may appear to be genetically homogeneous, this homogeneity is perhaps illusory. True, lineages may show a commonality of genotypes at, say, a range of microsatellite loci, which has been documented empirically (Haack *et al.*, 2000), but acceptance of clonality excludes the probability of mutational changes at other regions of the genome. It may be that each individual within the so-called clone varies at some region of its genome and such a possibility should not be ignored since this may have biological significance, particularly adaptive (Vrijenhoek, 1998).

Certainly, unicellular eukaryotic ciliates display large-scale chromosomal re-arrangements which may have adaptive significance. These changes appear rather unique in their operation, occurring at set sites, and may be under the influence of transposons (Yao, 1996; Meyer & Duharcourt, 1996). Similarly, the multiple nuclei of fungal spores, some of which show intraclonal changes in 18s and ITS regions of the rDNA (see Sanders *et al.*, 1995; Sanders, 1999; Sanders, Koch & Kuhn, 2003, this issue), may also have adaptive significance. In microorganisms, mutational changes are well known to be adaptive in controlled environmental experiments where evolutionary processes in the form of parallel and convergent evolution have been noted (e.g. Bull *et al.*, 1997) in response to both abiotic and biotic factors. Away from the experimental bench, bacteria have evolved rapidly in recent years under intense antibiotic selective pressure to develop resistance, rather as aphids have done in response to intense pesticide selective pressure, both in the laboratory and field (Neu, 1992; Foster *et al.*, 1998, 2000). In the case of bacteria, frequent recombination appears to be involved in some of the production of new mutations (Guttman, 1997). However, this seems much less likely in aphids such as *M. persicae* due to the relative scarcity in northern latitudes of its primary overwintering host peach (*Prunus persica* L.) on which mating occurs and eggs are laid (Blackman, 1974; Tatchell, Parker & Woiwod, 1983).

With all these fast reproducing clonal organisms, when new 'fit' genotypes evolve in novel habitats (e.g. on new hosts in the case of aphids), perhaps on a microgeographical scale (Guttman, 1997; Mopper & Strauss, 1998), selective sweeps rapidly purge populations of many of the genotypes present. When this happens, a rather structured population is likely to result, as with insecticide-resistant aphids (Brookes & Loxdale, 1987; Foster *et al.*, 2000), with relatively few main genotypes, and with an initially reduced capability to undergo further immediate adaptive switches. However, this is quickly offset by the capacity of such organisms to generate large populations that adapt quickly, and, of course, linkage to sexual lines promoting influxes of allelic variance (Delmotte *et al.*, 2001). In the case of animals like ancient asexual ostracods, perhaps the dearth of variance observed empirically is the result of the continuous purging of alleles via bottlenecks, selection or drift or a combination of these, in effect a severe series of selective sweeps (Schön *et al.*, 1998, 2003, this issue). We discuss the potential for adaptive significance of clonal mutations in another paper (Lushai, Loxdale & Allen, 2003, this issue).

CONCLUDING REMARKS

We now return to the concept of clonal fidelity and its graphical representation as a 'vertical pole in time and space without variance', i.e. the 'sacred cow' of clonal perfection. If such a biological entity really did exist, it would be fantastic and unlike any other living organ-

ism in the real world. We have argued that such an entity is extremely unlikely. Some may be tempted to counter-argue that this is still speculation. We believe, however, that the challenge in the years ahead is to prove or disprove the statement that all individuals in a clone vary – the reverse of the old, unproven and much cherished paradigm that they don't!

Already, the scientific and commercial drive to produce viable cloned mammals (Colman, 1999) is being adversely influenced by unexpected problems related to chromosomal/mutational changes (although even these are not true clones since their mitochondrial DNA differs from that of the nuclear genome donor female, Mittwoch 1998; see also Evans *et al.*, 1999). These include epigenetic effects in artificially produced offspring. Such changes cause a reduction in the survival of individual embryos or adults *per se*, the longevity of animals born to term, and their immunological competence (Wakayama *et al.*, 2000; De Sousa *et al.*, 2001; Humpherys *et al.*, 2001; Cibelli *et al.*, 2002). The epigenetic effects are associated with the proper switching of regulatory genes as a function of DNA methylation and are problems that have only very recently come to light (Kang *et al.*, 2001a, b). In addition, others appear to suggest problems relating to telomere length of implanted nuclei derived from fully differentiated somatic cells (Shiels *et al.*, 1999).

Clearly, a lot still has to be learnt about the nature of pro- and especially eukaryotic clonal organisms, including their levels of variance. This quest offers many new and exciting prospects for future discoveries, some no doubt of major fundamental and applied significance, whilst at the same time allowing old dogmas to be revised.

According to Huxley (1880), "it is the customary fate of new truths to begin as heresies and to end as superstitions". With clones, superstition seemingly predated the faltering steps on the path to heresy. The fact remains, however, that until the recent plethora of high-resolution molecular markers (particularly sequencing) became available in the last 20 years or so, clonal fidelity could not be rigorously empirically tested, only guessed at. We have argued elsewhere (Lushai & Loxdale, 2002) that clonality is merely a genotype resolution phenomenon dependent upon the resolving power of molecular markers (including type and number used), culminating with direct sequencing of the DNA. We concluded that it was a biological improbability. If so, what is a clone precisely?

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Note added in proof

A very recent paper by **Douhovnikoff V. & Dodd RS. 2003**. *Theoretical and Applied Genetics* (on-line) reveals intraclonal variation in clones of willow, *Salix exigua*, found using AFLP molecular markers; see http://link.springer.de/link/service/journals/00122/contents/03/01200

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