Was Dan Janzen (1977) right about aphid clones being a 'super-organism', i.e. a single 'evolutionary individual'? New insights from the use of molecular marker systems

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"…The countless Aphides, prolific tribe,
With greedy trunks the honey'd sap imbibe...
All these, increasing by successive birth,
Would each o'erpeople ocean, air, and earth."

Erasmus Darwin (1803)

"The truth is rarely pure and never simple."

Oscar Wilde (1895)

Abstract: Dan Janzen proposed in a paper in 1977 (*loc. cit*.), that a clone of aphids and for that matter dandelions consists, respectively, of one large 'super-organism'. In effect a single evolutionary individual able to exploit resources over an expanded geographical range, and sometimes with aphids also, a wider range of resources (different kinds of host plants), much more than if the organism concerned were a single individual. Such a view is of course based on the notion that an asexual lineage (clone) has strict genetic fidelity, that is to say, is genetically identical over its entire genome between clone mates. This seems a highly unlikely scenario and indeed, modern molecular markers have revealed a plethora of mutational events within such so-called clones. Here in this talk I provide evidence from aphids that they are not 'perfect forms' but rather show a range of variations, including evidence of hybridization events, and that they can and do adapt to environmental circumstances, sometimes swiftly. Hence that even as asexual lineages, aphids are able to exploit new ecological circumstances and flourish, e.g. host adapted forms, whilst some species, notably the highly polyphagous peach-potato aphid (*Myzus persicae*), have also evolved resistance to a range of pesticides, and by so doing, have managed to survive in the face of these poisons. However, there are fitness costs associated with such adaptation, more especially in the highly resistant aphids. Because of the variation and adaptation shown by particular aphid species and asexual lineages, they cannot be described as a single evolutionary unit in a 'Janzenian' sense. What they show is ecological plasticity and an ability to adapt quickly, in large part enhanced by their incredible rate of reproduction and population expansion. Some migrating winged aphids are constrained in their exploitation of new habitats by environmental factors – geographical, climatic and ecological, especially lack of suitable hosts. In contrast, some other aphid species have seemingly colonized large areas of the world (probably aided by human agency) so that deciding what a population is exactly is a difficult task. It may even be that certain 'super clones' detected using molecular markers have indeed spread far and wide, clones which appear to fit the description of being 'general purpose genotypes' in that they can feed on a range of plant hosts under a range of different geographical-climatic conditions. As such, they are nearest to Dan Janzen's views, although here again, strict genetic fidelity is not necessarily proven, only accepted from the application of a limited number of markers, e.g. multilocus genotypes in the case of microsatellite markers.

Key words: aphid, clone, genetic variability, molecular markers, DNA, adaptation

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In 1977, Dan Janzen in a famous paper entitled "What are dandelions and aphids?" argued that clonal lineages of both organisms could be considered as "Evolutionary Individuals" or "EI's", effectively as "super-organisms" with the ability to exploit resources over a wide geographical area. These multiple individuals thereby have a competitive edge over single organisms that lack the capacity to propagate parthenogenetically (apomictically) and to rapidly produce, it was assumed, large numbers of genetically identical copies, i.e. clones *sensu stricto* (*s.s*). For sure, aphids can and do reproduce quickly by such means: it has been calculated (Harrington 1994) that under ideal conditions (dearth of predators, parasites, pathogens and benign climatic conditions, especially including optimal temperatures of 20-25°C), a single asexual female could in theory produce in a single growing season 7.6×10^{28} offspring (with a generation given as 7 days, 50 offspring per female and 18 generations a year), enough to cover the Earth's surface to a depth of around ~ 150 kilometres! However, whether they can maintain genetic fidelity for long, if at all, and how long so-called clones persist either in the laboratory or field unchanged is a contentious issue, one for which there is little or no empirical evidence, certainly for the persistence of organisms which are, in effect, 'ideal' or 'perfect' forms, a view which clearly has Creationist overtones (Loxdale & Lushai 2003). On the contrary, there is a growing body of recent molecular biological evidence that clones (or more correctly, asexual lineages) rapidly mutate and that at least some of this variation has adaptive significance. Thus the view that aphid clones are genetically stable in time and space is outdated as well as erroneous and to a large extent is wishful thinking, it being experimentally convenient to assume that clones maintained in culture are genetically constant in terms of fidelity, even when there is little or no experimental proof that this is actually the case. Indeed, if a clone truly did exist it would be a strange entity indeed at a population level: thus for any given trait, it would have a population mean and no variance (see Fig. 1 of Loxdale & Lushai 2003). Clearly this is contrary to everything we know about natural populations in the real world, individuals of which mutate and undergo adaptive changes in the face of novel selective pressures, both negative and positive, in an ever-changing world. Since mutational change is a property of the DNA itself, whether by point mutations, errors of replication and repair, transposons (re-arrangements perhaps aided by transponation events, i.e. 'hotspots'; Pennisi 1998), etc., it is hardly surprising that all organisms, including clones, are subject to evolutionary pressures (Lushai & Loxdale 2002).

Another aspect of the clone issue is whether asexual lineages represent evolutionary 'dead ends', a view first put forward by Charles Darwin (Browne 1996) and emphasised by Simon & al. (2003a) who suggest that this is so because they often appear at the terminal nodes of phylogenetic lineages. However, recent evidence from bdelloid rotifers, asexual for aeons, shows that they have adaptively radiated and speciated to produce some 350 species, despite their renowned celibacy (Birky & al. 2005). Lushai & al. (2003) review the evidence for adaptation in asexual lineages.

Clonal definitions

The ongoing debate about the definition of clones and clonality, that is to say, what exactly a clone is, is often perceived as largely one of semantics. Thus to some, a clone is just the asexual progeny from a single female foundress whilst to others, the aspect of genome-wide genetic fidelity is crucial (Abercrombie & al. 1990). But then again, clonality is a complex phenomenon. For example, to begin with, clones may be generated within a generation (horizontal clones, e.g. monozygotic mammalian twins) or between generations (vertical clones, e.g. as in aphids and nematodes). Then there is the genetic nature of asexual reproduction itself, ranging from simple budding and apomictic forms of reproduction to automixis, gynogenesis, and hybridogenesis (see Hughes 1989, Simon & al. 2003a for details).

It may be desirable to use simplistic approaches in deciding upon clones and clonality, but whilst this may be perfectly useful in enclosed populations of asexual organisms in a laboratory controlled environment cabinet or glasshouse culture, e.g. in a cage or Blackman boxes (Blackman 1971) or Austin tubes (Austin & al. 1991), it is decidedly less useful and often useless in the field. In a nutshell, how is it possible to know that one is dealing with a particular asexual lineage unless discriminating morphological characters or molecular markers are available? Prior to the advent of molecular markers in the late 1970s, there were few intraspecific markers in aphids except colour variation, often polymorphic variants, typically greens, pinks, reds and browns, in – for example – the grain aphid, *Sitobion avenae* (F.). As discussed by Jenkins & al. (1999),

colour in aphids is also a complex phenomenon, governed by genetic (intermorphic difference) as well as environmental factors (intramorphic differences), the latter probably mediated via symbiotic bacteria. To confuse matters, it is known that the frequency of green and brown morphs of *S. avenae* changes over the course of the growing season as a result of transgenerational intramorphic differences within holocyclic (= with annual sexual phase) lineages (Chroston 1983, in Jenkins 1991, Jenkins & al., 1999). Various lineages with different lifecycle strategies exist, some of which display colour polymorphisms (i.e. anholocyclic (= obligate asexual); holocyclic (facultative asexual females which produce sexual males and females under suitable environmental conditions of reduced day length and low ambient temperature); androcyclic (asexual females which produce asexual females and sexual males only); and 'intermediate' (asexual females which produce asexual females and a few sexual males and females only; see Simon & al. 2002). Such lifecycle-related colour differences may have a demographic component (Simon & al. 1999).

The coloured asexual lineages of certain species, such as the pink and green forms of the pea aphid, *Acyrthosiphon pisum* (Harris), differ in their host preference (Via 1999). Perhaps too, biotypic differences (Eastop 1973) may also be distinguishable as a function of colour or higher levels still of evolutionary divergence, due to chromosomal re-arrangements like translocations, e.g. the snapdragon aphid, *Myzus antirrhinii* (Macchiati) (Hales & al. 2000), tends to be a darker green than the peach-potato aphid, *Myzus persicae s.s.* (Sulzer) (pers. obs.). The coloured clones or strains (here I mean a population of individuals of broadly similar genotype and probable origin too derived from a single female founder) may differ in their ability to transfer one or more pathogenic plant viruses (Terradot & al. 1999), and in this way, colour cues could be valuable at a field scale. In addition, different coloured clones or strains may differ in their size and intrinsic rate of increase and their susceptibility or resistance to predators and parasites (e.g. Ankersmit & al. 1986; Losey & al. 1997) and pathogens, e.g. entomopathogenic fungi (Judith Pell, pers. comm.). Even so, such traits do not prove aphid genetic fidelity-similarity, only infer it. Ultimately, only molecular markers, ranging from protein markers, especially allozymes, to high-resolution DNA markers, can provide evidence on the genotypic status of clones, and even then the information given is only a sample, often a small sample, of the variability potentially present throughout the entire genome (Loxdale & Lushai 2003).

Mutational events in clonal populations

One of the classic examples of mutation within an aphid population concerns the spotted alfalfa aphid *Therioaphis trifolii* forma *maculata* (Buckton), introduced into the USA from Europe in the early 1950s (Blackman & Eastop 2000). Considered to probably have arisen from one or a very few asexual founders, the species rapidly expanded, both in numbers and range. Dickson (1962) calculated that in one large valley in California over the course of two growing seasons, some 1.7×10^{11} individuals had arisen! Even at a conservative mutation rate of 10^{-7} per gene per generation, this could lead to something like 17,000 mutations. Certainly, resistance to organophosphorous insecticides developed very quickly in this species, aided no doubt both by chemical selective pressure, i.e. usage, and the insect's huge reproductive potential. The latter is of course aided by the telescoping of generations of aphids, in which asexual females have within them not only their daughters but also their granddaughters (Dixon 1998), the short generation time (~ 10 days), and number of generations per growing season (perhaps 14 in temperate regions, but more in warmer climes).

Application of molecular markers over the last ten years, especially including RAPDs, oligonucleotide probes, microsatellites and AFLPs, i.e. multilocus 'fingerprints' or genotypes, i.e. 'MLGs' (see Loxdale & Lushai 1998 and Behura 2006 for an overview of the type and use of molecular markers in entomology), have provided unequivocal evidence of mutational changes within asexual aphid lineages, mostly somatic, but sometimes in the germ line (e.g. De Barro & al. 1994, Lushai & al. 1998; see also Loxdale 2007 and Loxdale & Lushai 2007). In addition, as well as intraclonal differences *per se*, intraclonal, intermorphic differences have also been seen in clones of two cereal aphid species, mutations shown to be of aphid genomic origin using both Southern blotting and sequencing (Lushai & al. 1997). Such differences could be the result of mutational changes in priming sites, to repetitive insertions between priming sites or perhaps to transponation events. In some studies, mutated bands were observed within 5-12 generations, although in a recent laboratory study of grape root phylloxera (*Daktulosphaira vitifoliae* Fitch) using AFLPs, and

involving eight asexual lineages over 15 generations, mutated bands were seen in every generation from the first onwards, whilst a majority of individuals had one or more mutation (of 156 individuals tested, 123 showed mutated bands) (Vorwerk & Forneck 2007). Interestingly, the bands were not seen to accumulate in particular lineages as expected from the predictions of Muller's ratchet (Muller 1964); rather, they were found to be scattered at random within and between lineages, as may probably more realistically be expected. Microsatellite mutations within apparent clonal lineages have also been reported, usually by addition of repeats (e.g. Wilson & al. 1999 in *Sitobion* aphids, Kasprowicz 2006 in *Myzus persicae*, Moncada & al. 2006 in *D. vitifoliae*), whilst the evidence from these markers is usually consistent with descent from a common foundress (Miller 2000). Fenton & al. (2005) have also revealed that ribosomal DNA IGS (intergenic spacer) variation within Scottish field populations of *M. persicae* correlates with microsatellite profile, suggesting a common descent of such genotypes. As well as interclonal variability, some intraclonal variation was also detected, clearly showing mutation within lineages (Fenton & al. 2005). Since studies of Cladocerans (*Daphnia*) have shown such IGS variation to be adaptive (Gorokhova & al. 2002), the IGS variants found in aphids may similarly have adaptive significance. Shufran & al. (2003) have found intraclonal IGS variation in the greenbug, *Schizaphis graminum* (Rondani) related to pesticide selective pressure over generations.

As well as the aforementioned variations, other variations associated with intraspecific hybridization events have been documented. Thus in the case of ribosomal genes, Fenton & al. (1998a) found evidence for introgression between *M. persicae s.s*. and a closely related species, some clones of the former species having two ITS (internal transcribed spacer) haplotypes in the same individual, one probably derived from *Myzus certus* (Walker). Evidence from microsatellites and mitochondrial markers has likewise shown asymmetric introgression between aphids of the genus *Sitobion* on wild grasses (Sunnucks & al. 1997): thus for example, female lineages on cocksfoot grass (*Dactylis glomerata* L.) bearing microsatellite alleles from both the blackberry-grain aphid, *Sitobion fragariae* (Walker) and *S. avenae s.s* also had some 80% *S. fragariae* mitochondrial DNA (mtDNA). This unequivocally shows that there continues to be a meeting and mating of the two species which are morphologically similar, with diploid chromosome number 2n=18; Hille Ris Lambers 1939, Blackman & Eastop 2000), male *S. avenae* predominantly crossing with the oviparae of *S. fragariae*, and presumably on the primary overwintering host, blackberry (= bramble), *Rubus fruticosus* L. agg. (see also later). Of interest in this context is the fact that the sex pheromones are known to be similar in the two species (Goldansaz 2003). Delmotte & al. (2003) have similarly also shown, using both microsatellite and mtDNA markers in cyclically parthenogenetic and asexual lineages of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), that asexual lineages have arisen from multiple hybridisation events between *R. padi s.s.* and an unknown closely related species. The production of asexual lines by such means confounds earlier conclusions drawn from molecular analyses using mtDNA markers (Simon & al. 1996) that the asexual lineages had been effectively reproductively isolated from the cyclically parthenogenetic lineages for a very long time (0.4-1.4 MY); rather, this new evidence supports the view that the asexual lineages are of much more historically recent origin (see also Delmotte & al. 2001 and 2002).

Displacements in time and space

In the period 1950-1980, the prevailing view amongst aphidologists was that these small herbivores, because of their small mass and the fact that winged morphs were readily borne on air currents above their flight speed, were generally carried long distances. Now it is appreciated, especially as a results of the wind tunnel experiments of Hardie and co-workers at Silwood Park, Ascot, U.K. (Hardie 1993, Hardie & Campbell 1998), that different aphid species respond differentially in terms of time duration to both white and green light (targets) during teneral flights and orientate and land on suitable host plants below the boundary layer in still air (Taylor 1974). There are undoubtedly fitness benefits in terms of feeding and reproducing in maximising the early discovery of, and settling and feeding upon, a suitable plant host (Cockbain 1961a,b). Perhaps the classical evidence obtained for long distance flight in aphids, especially over deserts and the sea, is because the sensory-deprived insects keep flying due to the fact that the normal landing cues are absent (See Loxdale & al. 1993 and references therein). Molecular ecological evidence, especially using allozymes and microsatellites, has shown that aphid aerial displacements must be considered in a largely species-specific manner and appear to be related to migratory urge and ability, *viz.* to flight behaviour: thus long distance

migrants show spatially homogeneous gene frequency patterns, short distance fliers heterogeneous patterns (Loxdale 2007, Loxdale & Lushai 2007). Because many aphids are carried long distances, including globally via human agency (on vehicles, ships and aircraft on infested plant material), deciding what a population is exactly is difficult, if not impossible (Loxdale 2007). Some species such as *M. persicae* and *S. avenae*, are distributed globally and it may be that certain clones are also nowadays distributed around the world (e.g. Wilson & al. 1999, 2002, Figueroa & al. 2005), although they are of course still liable to mutation and selection, and hence rapid evolution.

Life cycle and clonal selection

In terms of lifecycle morph (anholocyclic, holocyclic, etc.), there is now strong evidence from both microsatellite and mtDNA markers, of latitudinal-based clines in cereal aphids (Martinez-torres & al. 1997, Simon & al. 1999, Llewellyn 2000, Llewellyn & al. 2003), probably the direct result of negative selection against asexuals in regions with severe weather (e.g. north of Scotland; see also Kasprowicz 2006 in the case of *M. persicae*). Variability of life cycle traits in the pea aphid, *A. pisum* is under climatic-photoperiodic (latitudinal-geographic) control and thus aphids experience "strong stabilizing selection for maximal developmental rate in this aphid which is already strongly *r*-selected" (Mackay & al. 1993) along with male alary production (there is a genetic basis to the production of males and with evidence for a geographically-based production of apterous males; Smith & Mackay 1989, 1990). That this is so possibly mitigates against long distance migration and survival by winged forms, certainly in this species and probably many others too. However, the noted latitudinally-based variation in the photoperiodic 'interval timer' (Lees 1960) found in host alternating cyclically parthenogenetic aphids (*R. padi*) is apparently not that crucial for the lifecycle (Lushai & al. 1996), although perhaps more research is required to confirm or dispute this.

Host preference and clonal selection

It was shown in the mid-1990s using high-resolution molecular markers that strains of aphids of certain species showed a preference for particular host plants. Thus De Barro & al. (1995a) using RAPD markers, demonstrated host stratification, with *S. avenae* genotypes appearing to prefer wheat or grass (*D. glomerata*) early in the growing season, although this clear relationship tended to break down as the season progressed. Such host preferences were confirmed by breeding experiments of *Sitobion* aphids, and chromosomal polymorphisms were found to be associated with some such preferences (De Barro & al. 1995b; Sunnucks & al. 1998). Later, this work was confirmed and extended using microsatellites, whereupon three largely reproductively isolated pools of genotypes were found, one specific to wheat, one occurring on both wheat and *D. glomerata*, and one specific to *D. glomerata* which, as earlier mentioned, had both *S. avenae*-like and *S. fragariae*-like alleles and predominantly *S. fragariae* mtDNA, suggesting gender symmetrical introgression at the very least and perhaps hybridisation itself (Sunnucks & al. 1997). If so, this appears to be an example of sympatric evolution in action. Work by Haack & al. (2000) also using microsatellites, showed that clear host preferences occur in *S. avenae* populations from France, with some 'specialist' genotypes existing, whilst other genotypes were more 'generalist' (e.g. two clones from maize, *Zea mays* - 'super-clones'), occurring over a wide geographic range and persisting over several field seasons. Other studies on *S. avenae* have shown the existence of such generalist genotypes, both in the U.K. and elsewhere (e.g. Chile; Figueroa & al. 2005), whilst similar generalist genotypes of *M. persicae* have also been discovered both in the U.K. (Fenton & al. 1998b, Fenton & al. 2005) and in Australia (Vorburger & al. 2003a). Studies on other aphids have further demonstrated host preference in aphids, so that the phenomenon is now no longer an issue (e.g. Vanlerberghe-masutti & Chavigny 1998 and Fuller & al. 1999 in the melon-cotton aphid, *Aphis gossypii* Glover; Ruiz-Montoya & al. 2003 in the cabbage aphid, *Brevicoryne brassicae* L.). Some evidence for host-preferring or even adapted populations of the highly polyphagous *M. persicae* have also recently been documented (Vorburger 2006, Kasprowicz 2006), which points towards some kinds of cryptic, sympatric speciation taking place, perhaps as a result of clonal selection of predominantly asexual lineages (see below).

Of interest in the context of host preference are the findings of Lushai & al. (2002) who showed using RAPDs that asexual winged female foundresses coming into the crop early in the growing season already

had identifiable insect genotype-plant host preferences as seen from field trials involving four hosts (wheat, barley, *D. glomerata*, and Yorkshire fog, *Holcus lanatus* L.) arranged in a Latin square. The host-preferring genotypes could be separated into four main clades (see also Zitoudi & al. 2001 and Margaritopoulos & al. 2005 in the case of the *M. persicae* species complex). Clades of biotypes of the greenbug, *S. graminum*, a major cereal pest in the USA, have been separated into three main host-preferring clades using mtDNA and RAPD markers. Here, the clades distinguished appear to pre-date the introduction of cultivated cereals into the Americas in historical times (Anstead & al. 2002, 2003).

All these studies show that cryptic, sympatric speciation is occurring in a range of aphid species studied, sometimes possibly over short temporal and spatial scales, aided by the high reproductive rate of the insects (Loxdale & Lushai 2007). There is also evidence for rapid chromosomal changes effecting speciation events in aphids, both cereal aphids and the *M. persicae* complex, to name but a few (Blackman 1980, 1987, Blackman & al. 1989, Brown & Blackman 1988; see also Hales & al. 2000, Wilson & al. 1999, 2002).

There is certainly now some evidence for clonal selection in relation to host plant, identified using molecular markers. At a local geographic scale, Llewellyn & al. (2004) have found, using microsatellite markers, such selection in *S. avenae* infesting wheat fields of different cultivar in southern England, as have Vorburger (2006) and Kasprowicz (2006) in *M. persicae*, in Australia and Scotland respectively, infesting a range of hosts. However, in another paper, Vorburger & al. (2003b) were unable to show evidence for 'general purpose genotypes', or GPGs in *M. persicae*. Whilst clear evidence for host preference was shown in terms of mean adult weight and reproduction (colony size after 15 days) on three hosts (radish, spinach and tomato), the geometrical colony size between obligate asexuals and cyclical parthenogens was not significantly different (as it should have been according to the prevailing theory supposed to govern the evolution of genotypes tolerant of a wide range of environment conditions; see Vorburger & al. (2003b) for details). Wilson & al. (1999), using microsatellites, have provided convincing evidence of clonal selection in populations of *Sitobion* from New Zealand at a geographic scale, here related probably to climate (= latitude). Some clonal selection is undoubtedly related to plant chemicals, including secondary compounds (e.g. Dimboa; Cambier & al. 2001, Hansen 2006), although recent evidence suggests that symbiotic bacteria are also strongly implicated (Simon & al. 2003b, Alkhedir & Vidal 2007, in prep.).

Pesticide resistance and clonal selection

Resistance by aphids to pesticides was first observed in the 1950s and '60s and is now a global problem, causing massive expenditure on control (e.g. Riley & al. 1997). Because of the rapid evolution of highly resistant forms in some species, notably *M. persicae*, involving cross-resistance mechanisms (Devonshire 1989, Foster & al. 2000), this has additionally spawned major research efforts in various countries worldwide to find alternatives to chemical control, more especially biological control agents, perhaps used in integrated pest management strategies. As detailed by Foster & al. (2000), in *M. persicae* three basic mechanisms are known: elevated carboxylesterases related to gene amplification which confer resistance to organophosphates and carbamates; MACE (modified aceylcholinesterases) which also confer resistance to these poisons, and knockdown resistance or 'kdr' (and 'super-kdr, a related mutation'), which confer resistance to pyrethroids by affecting the nerve sodium channel gating system (see Field & Blackman 2003, Foster & al. 2000, Foster & Devonshire 2007 and Loxdale 2007 for overviews).

In a series of elegant experiments, Foster and co-workers have shown that the highly resistant *M. persicae* (R_2 and R_3) undergo a negative selection in the field over the winter time, the esterase and kdr mechanisms apparently having pleiotropic effects on behaviour and hence fitness and survival. Thus the frequency of the highly resistant forms, whilst positively selected in the growing season as a consequence of pesticide applications, decline in the winter as a result of antagonistic selection such that their frequency, as measured in 12.2 m high suction trap catches, falls greatly. As a consequence, the frequency of these genotypes is seen to rise and fall over the course of many field seasons studied (Foster & al. 2000, 2002). It appears that the highly resistant forms, which also have an autosomal 1,3 chromosome translocation involved in the conferment of resistance (Blackman & al. 1995), are more sluggish and liable to be rained upon and show a reduced propensity compared with other resistant strains and susceptibles to form winged morphs and to fly. Such resistant forms are also less responsive to the aphid alarm pheromone, β-(*E*)-farnesene and thereby

more likely to fall prey to primary hymenopterous parasitic wasps, *Diaeretiella rapae* (M'Intosh), which indeed actually find these aphids more attractive (Foster & al. 2005). This response constitutes a further negative fitness cost to such aphids and involves pleiotropic effects on behaviour of the host aphid (Foster & al. 2005). The rapid observed shifts in frequency of the resistant forms of *M. persicae* in the field in the UK over the course of several years is thought to be largely associated with changes in pesticide usage (Foster & al. 1998), although some direct effect of negative selection over the preceding winter in reducing local populations of the high resistance genotypes cannot be discounted (see also Kasprowicz 2006). The fact that such aphids are predominantly asexual (Fenton & al. 1998a, 2003, 2005, Kasprowicz 2006) and thus their genomes are effectively linked (so that they cannot recombine and are subject to linkage disequilibrium), must add to the large population genotype swings noted for resistant *M. persicae* in the field (e.g. Foster & al. 1998, Vorburger 2006). A potentially dangerous situation for the aphid is that whilst the genes conferring resistance are positively selected during pesticide applications, because of the lack of recombination, the rest of the genome is dragged with it in a kind of mass hitchhiking event (Foster & al. 2000, Loxdale 2007). When the pesticide chemical selection pressure is reduced, the aphid may be maladapted to other ecological pressures (host plant, climate) and hence can then be negatively selected against, over and above the aforementioned apparently direct negative fitness costs described above.

That there is a cost involved in the production of high levels of the carboxylesterases – E4 and FE4 – that confer resistance in *M. persicae* (in R_2's, some 0.02% of adult aphid wet body weight, i.e. 0.1 µg per *c.* 500 µg; Devonshire & al. 1986) is seen in relation to the expression of these enzymes. As shown by Sawicki & al. (1980), the expression declines over the course of several generations following cessation of pesticide application (to produce 'revertants'), so that whilst the esterase genes responsible are apparently unaffected in number (i.e. amplicons, as demonstrated using homologous probe and PCR-based methods; Field & al. 1989, 1999), the amount of esterase declines, potentially leading to a false assessment of the frequency of highly resistant genotypes in the field if only immunoassay or PAGE (polyacrylamide gel electrophoretic) enzyme assay techniques are used (Field & al. 1999, Foster & al. 2000). The switching on and off of the esterase genes is under epigenetic control, which appears the opposite to that found in mammals (in mammals, methylation of the DNA switches genes off, whereas in *M. persicae*, the reverse is true or at least appears to be). Methylation is certainly responsible for switching on the E4/FE4 genes in highly resistant aphids; see Hick & al. 1996, Field & Blackman 2003).

Conclusions

From what has been said, although Dan Janzen's (1977) original concept was a fascinating and not implausible one, the experimental evidence obtained in the past 20 years or so, especially using molecular and chromosomal markers, seems to refute it. As outlined, even species populations are not homogeneous genetically and there are clear examples of chromosomal forms, introgression and hybridization events within otherwise 'good' species. In addition, there is indisputable evidence for photoperiodic-based differences (e.g. production of apterous males; Smith & Mackay 1989), host adaptation and clonal selection in relation to plant host, climate and pesticide resistance, the last related to intrinsic and extrinsic factors affecting gene expression and fitness, including attraction of wasp parasitoids to aphids (Foster & al., 2005). All this means that aphid asexual lineages, let alone species, cannot be considered as 'evolutionary individuals' in any sense of the term. Rather, they represent a 'plastic population' that is both opportunistic as well as a slave to ongoing selective environmental forces (Loxdale & Lushai 1999). Even the evidence for phenotypic plasticity in aphids (e.g. Wool & Hales 1997) may ultimately be shown with modern high resolution molecular markers to have a genetic basis.

The only way to get further insights into the adaptation and evolution of aphids, including the reality of the clone, is by sequencing the genome, since even when many loci are tested to produce complex MLGs (e.g. Moncada & al. 2006), this still represents only a fraction of the genome surveyed. Presently an international consortium of scientists is doing just that (Caillaud & al. 2004) and has just produced a paper relating the nature and variety of expressed sequence tags (ESTs = expressed genes) in the pea aphid to function (Sabater-muñoz & al. 2006). This work already shows that hemimetabolous insects (here represented by aphids, Order Hemiptera), which long ago (estimated 330 million years) branched off from

holometabolous insects (represented by diptera, *Drosophila*) have rather few EST sequences in common (< 34%), as may perhaps be expected considering their long evolutionary separation, different ecologies, lifestyles and physiological requirements. However, the study clearly points to the future and the power of functional genomics in elucidating the role of different (expressed) genes, although genes such as regulatory proteins in introns are not revealed by the technology and mRNAs are difficult to isolate from some cell types and tissues (www.ncbi.nlm.nih.gov/About/primer/est.html), a serious omission considering the potential importance of EST markers in studies of development, survival and adaptation (see also Behura 2006). Finally, whilst Behura (2006) presents many techniques in his recent overview of molecular markers in entomology, and whilst many of these have very useful and important applications (e.g. genetic linkage mapping using AFLPs, transposon display, SSCPs, SNPs, etc.), ultimately for assessing levels of population genetic polymorphism, sequencing is surely the final arbiter and as the technology yearly becomes cheaper, faster and easier to perform, including the length of sequences obtained, then maybe within a few years, as Behura emphasizes, partial or even full sequencing of the genome will provide all the evidence to assess primary gene sequence (although not necessarily function of either exons or introns) and will certainly tell us what constitutes a clone at this level.

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