

ARTICLE

Fig. 1a, Winged and wingless morphs of the Grain aphid, *Sitobion avenae*; b, *S. avenae* colony on panicle of oats; c, Giant Willow aphids, *Tuberolachmus salignus*

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Big issues in population biology: lessons from a greenfly

Presidential Address

Hugh D. Loxdale †

Royal Entomological Society, The
Mansion House, Chiswell Green Lane,
Chiswell Green, St. Albans,
Hertfordshire AL2 3NS, U.K.

† Address for correspondence:
Institute of Ecology, Friedrich Schiller
University, Dornburger Str. 159, 07743
Jena, Germany (e-mail:
Hugh.Loxdale@uni-jena.de)



Introduction

According to Creationists and proponents of so-called Intelligent Design (ID), living organisms came into the world fully formed and perfectly adapted to the world around them (see Jones, 2006 concerning the arguments against these doctrines, and also Quammen, 2004). However, it is evident since the findings and theories of Darwin and Wallace in the mid-19th Century and from data collected and theoretical constructs advanced subsequently that this cannot be (Dobzhansky *et al.*, 1977; White, 1978; Ridley, 1993; Claridge *et al.*, 1997; Mayr, 2002). The world is a

dynamic place and environments change as a result of both abiotic and biotic factors. Meanwhile, living organisms mutate, mutation being a fundamental property of the DNA itself. Most of these mutations are neutral or mildly deleterious (Lynch *et al.*, 1999), some more so and thus negatively selected (e.g. Vorburger, 2001); others are favourable mutations and are positively selected. In this way, the organism concerned is subject to the effects of natural selection and adapts to fill novel niches, often by a process of radiation, or perhaps perishes as a consequence of one or more maladaptive features. The gene may be the ultimate unit of selection (Dawkins, 1989), but the phenotype, and indeed the whole organism, is surely the true winner – or loser – in the great race of life on Earth (Thompson, 1994). Darwinian evolution is often seen as a very slow process. However modern studies, especially using a plethora of molecular (DNA) and chromosomal markers, show that population divergence events – not necessarily producing new species, although they may do so – can also be rather fast, including as a result of chromosomal rearrangements, hybridisation and sympatric modes of speciation (Fredga, 1977; Blackman, 1980; Blackman *et al.*, 1989 and references therein; Hawthorne & Via, 2001; Rieseberg, 2001; Vorburger, 2001; Scali *et al.*, 2003; Emelianov *et al.*, 2004).

Aphids too are of course not immune to the effects of mutation, selection/drift, adaptation, and extinction. On the contrary, due to their fast rates of asexual (parthenogenetic, apomictic) reproduction and short generation times (~10 days), and involving ‘telescoping of generations’ (Dixon, 1998), a single virgin female can typically give rise to between 30-90 offspring (Blackman, 1971; Johnson & Lyon, 1988). This can result under ideal conditions in countless billions of individuals in a single growing season (Harrington, 1994; Dixon, 1989), with perhaps a significant number bearing mutations. With this

in mind, it is thus strange that when scientists discuss the so-called aphid clone this is still generally seen to be ‘identical’ to its stem mother, with little genetic/functional plasticity to be able to adapt in the face of changing ecological circumstances (Lushai *et al.*, 2003).

Even at typical mutation rates of 10^{-9} to 10^{-6} per gene per generation, a large number of mutant aphids are likely to be produced with such an astronomical rate of reproduction. For example, in one large alfalfa-growing region in California alone, it has been calculated that there were some 1.7×10^{11} Spotted Alfalfa aphids, *Therioaphis trifolii* forma *maculata* (Buckton) produced in a couple of growing seasons (Dickson, 1962). At a conservative mutation rate of 10^{-7} , this means that at a given locus, around 17,000 mutations might arise, including some conferring resistance to insecticides, i.e. or e.g.? organophosphates (Dickson, 1962; see also Loxdale & Lushai, 2003a; Vorwerk & Forneck, 2007 and ‘Clonal selection’ section). The large majority of these are mildly deleterious and at non-coding regions of the genome; however, others are probably important, i.e. selectable. If the aphid ‘clone’ *sensu stricto* did indeed exist (and here I do not mean just the offspring of an asexual lineage but rather the assumed – but *not* empirically proven – genetically identical entity within the lineage started from a single stem mother), a natural population of these organisms would be seen to be represented by a population mean with no variance, a bizarre state of affairs for sure (see Fig. 1 in Loxdale & Lushai, 2003a). This is surely an incorrect view of the real world that we know from studies of numerous other organisms (see the collection of papers in Loxdale & Lushai, 2003b). Furthermore, a population founded from a single asexual female individual is the ultimate in population bottlenecks. Because of this, it appears that asexual aphids are pre-adapted to undergo very significant levels of ‘in-breeding’ and, as such, are seemingly immune – partially at least – to the evils of in-breeding

depression (Kristensen & Sørensen, 2005; but see also Vorwerk & Forneck, 2007 and ‘Images of immortality’ section). With sexual aphids, there is some empirical evidence that *intra*-clonal genetic crosses (i.e. crossing of sexual morphs of particular lineages) leads to a decrease of egg-hatching success compared with *inter*-clonal crosses (Helden & Dixon, 1997; see their Fig. 1).

In 1837, Darwin stated in his ‘B’ notebook that ‘If all organisms merely replicated their kind by vegetative budding or splitting, history would show a succession of identical individuals holding no potential for alterations of any kind’ (Browne, 1996). In this century, Simon *et al.* (2003) in a review of parthenogenetic organisms further argued that ‘It appears that most unisexual taxa occupy terminal nodes of phylogenetic trees. This suggests that, even if initially successful, they are evolutionary ‘dead-ends’. However, there are certainly exceptions to this pattern. Thus for example, bdelloid rotifers and some ostracod species have existed totally asexually for aeons (40 – 200 million years), whilst bdelloids are very species ‘rich’ with some 350 spp. and appear to be adaptively radiating and speciating (Mark-Welch *et al.*, 2000; Schön *et al.*, 2003; Birky *et al.*, 2005).

In relation to aphids, Dan Janzen in a short article in 1977 entitled ‘What are dandelions and aphids?’ argued that because both have asexual phases during which the offspring produced are ‘genetically identical’, the members of such a clone are in effect a super-organism, i.e. a single ‘evolutionary individual’, and are thereby able to exploit a much larger geographic region and its resources.

In the present article, I wish to show how the use of chemical and biochemical techniques, particularly protein-based genetic markers (allozymes) and more recently, DNA-based markers (see Loxdale & Lushai, 1998 for an overview and Behura, 2006 for other more recent techniques) have transformed our understanding of what constitutes a clone (more correctly an asexual

lineage) as well as higher levels of evolutionary divergence, whilst even what constitutes an aphid population is now under scrutiny and re-assessment. This has occurred as new-found molecular knowledge has changed once well established notions concerning morphology, genetics and evolution. Since I have spent most of my career studying cereal aphids, I shall tend to concentrate on these during my deliberations on the true nature of the beast. For general background information concerning the biology of aphids refer to **Boxes 1-3 in this issue and 4-6 in following editions.**

Is the greenfly really green?

Besides so-called greenfly, blackfly are well known to gardeners and professional plant growers. Much work has been done to elucidate the nature of aphid colouration over the years. In brief, colour in aphids, which may range widely within and between species (**Fig 3a-e**), results from waxy exudates, melanism of the cuticle (surface colour) and from pigmentation of the haemolymph, termed ground colour. The ground colour is produced by two distinct groups of pigments – aphins and carotenoids. The former are glucosidic pigments, principally protoaphin and aphinin, variation of the proportion of which is responsible for *interspecific* colouration. This group of pigments is unique to the Aphididae. The other main type of pigments, the carotenoids, is a group of C₄₀ polyenes showing a high level of conjugation and is commonly associated with microorganisms and higher plants (see Jenkins, 1991 and Jenkins *et al.*, 1999 and references therein).

In the Grain aphid, *Sitobion avenae* (F.), a wide range of colours are found, ranging from a dark green with a black dorsal abdominal patch, to apple green, pink, reddish-chestnut to dark brown and rarely, almost black (**Fig 3e**). Mechanisms of colour expression are both complex and varied and include environmental (i.e. nutritional, crowding, day length and ambient temperature), genetic factors, endosymbiotic bacteria or a

Box 1 *Aphid origins*

Aphids are an ancient group, the fossil remains of which have been found in the Triassic, some 220-210 MYA (Grimaldi & Engel, 2005). Even by this time, they were winged, indicating that they were already flying plant parasites (Heie, 1987; Grimaldi & Engel, 2005 and references therein). They probably originally evolved on gymnosperms (conifers) in temperate regions of the Earth and later adaptively radiated in the Cretaceous to angiosperms, that is to secondary herbaceous host plants, i.e. flowering plants, including trees and monocotyledons (Heie, 1994). This switching of host was often associated with an alternation of generations, the sexual forms returning to the woody host (often nowadays a deciduous host) in the autumn as a function of abiotic cues (especially day length and temperature), whilst asexual propagation was continued for a number of generations on the secondary host/s in the spring and summer months prior to this return migration (Dixon, 1977; Moran, 1992).

Box 2 *Aphid size*

Of those commonly met within Britain, most adult aphids range between 1.5 and 3.5mm in length and some 100 – 1500µg (0.1-1.5 mg) wet weight and as such are very small creatures (Dixon, 1998; Helden & Dixon, 1998; **Fig. 1a,b**). Aphids of the genus *Cinara*, which feed on various conifers, are especially large as adults, 3-5mm in length (Eastop, 1972), whilst the Giant Willow aphid, *Tuberolachnus salignus* (Gmellin) grows to 5-6mm long (Blackman & Eastop, 1994) (**Fig 1c**).

combination of these factors. In laboratory experiments involving artificial diets and antibiotics, Jenkins (1991) showed that colour variation in these aphids was related to *intra*- and *inter*-morph as well as *inter*-lineage, i.e. lifecycle-based differences. *Intra*-morphic changes are caused by environmental stress factors, e.g. crowding, poor nutrition and high temperature, resulting in quantitative changes in the carotenoid content and are reversible. In contrast, *inter*-morphic variation, which may be genetically controlled, is related to qualitative differences and associated with production of sexual forms and the fundatrix morph (first parthenogenetic stem foundress hatching from the egg) and her immediate asexual offspring. This variation is sex-linked and controlled by a gene on the X chromosome, females being XX and males XO. For example, in the Rose-grain aphid, *Metopolophium dirhodum* (Walker), the females are apple green whilst the males are always pink (Stroyan, 1949; Muller, 1964; Jenkins, 1991; Wilson *et al.*, 1997; Hales *et al.*, 2002).

Brown aphids were found to have four main carotenoids, β-carotene, lyopene, torulene and 3,4-didehydrolycopene, whereas green morphs had predominantly β-carotene. The brown lineages tested were holocyclic. There is clearly a genetic basis to some observed changes. For example, oviparae are green, as are the F1 and F2 generations, whereas the F3 generation is intermediate in colour and the subsequent generations are brown! The green lineages examined were often anholocyclic and showed no such colour changes. Jenkins (1991) concluded that colour changes are associated with holocycle. When aphids were treated with antibiotics (chlortetracycline), colouration was little affected, suggesting that the symbionts known to occur in aphids, e.g. *Buchnera* (Douglas *et al.*, 2003), are not directly involved in the synthesis of the carotenoid pigments *de novo*. Rather, they probably use the precursors acetate and mevalonic acid derivatives to synthesise the required compounds (see also Douglas, 1988).

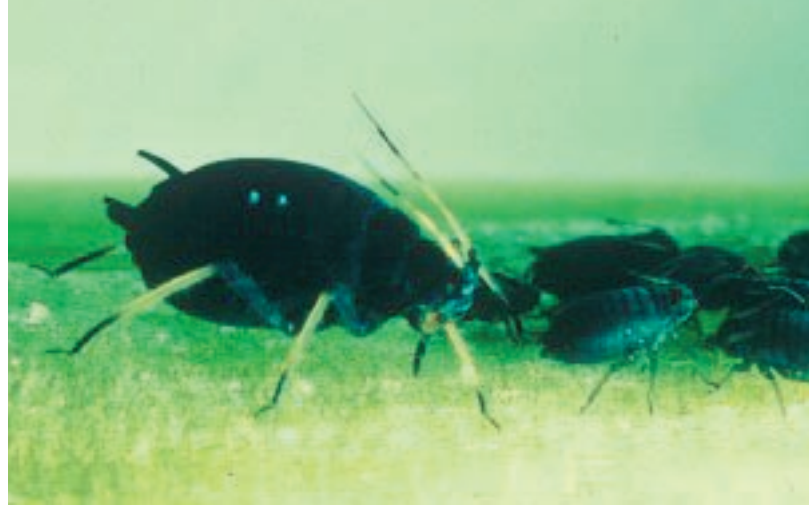


Fig. 3a-e. Colourful aphids. Left to right clockwise:

- a, red Goldenrod aphids, *Uroleucon nigrotuberculatum* (Olive);
- b, Black bean aphid, *Aphis fabae* Scopoli;
- c, pink and green Peach-potato aphids, *Myzus persicae*;
- d, red form of the Rose aphid, *Macrosiphum rosae* L.;
- e, green and brown Grain aphids, *Sitona avenae*;
- f, yellow Oleander aphids, *Aphis nerii* Boyer de Fonscolombe;
- g, pink and red forms of the Pea aphid, *Acyrthosiphon pisum*.

Box 3 *Aphid life cycles*

Aphids show a wide spectrum of lifecycle types and in many instances may be said to be 'insects for all seasons', different species undergoing different life cycle scenarios depending on prevailing environmental conditions (Eastop, 1986). Some species, such as the aforementioned giant willow aphid and the shallot aphid, *Myzus ascalonicus* Doncaster appear to be obligate asexual, i.e. anholocyclic, producing only parthenogenetic females. At least one species, *Acyrtosiphon svalbardicum* Heikinheimo, which lives on Eight-petal Mountain-avens, *Dryas octopetala* L. (Rosaceae) on Spitsbergen (Svalbard) in the high Arctic, has no winged individuals (Strathdee *et al.*, 1993). In this species, the fundatrix which hatches from the egg gives rise directly to sexual morphs (males and oviparae). These then mate and produce eggs and a few viviparae, which thence also produce sexuals in a third generation, an adaptive response to the generally unfavourable and fluctuating prevailing environmental conditions there, including a short growing season (Strathdee *et al.*, 1993). A majority of species remain on a single host (autoecious), sometimes a woody host, or a secondary host, and undergo a holocyclic lifecycle which includes an annual sexual phase, triggered by autumnal environmental factors of reduced daylight and temperature and probably host quality too (Eastop, 1986; Lushai *et al.*, 1996; Dixon, 1998). They produce numerous asexual generations during the spring and summer months. Some species, e.g. Sycamore aphid, *Drepanosiphum platanoidis* (Schrank), may undergo periods of aestivation as a direct result of poor host plant quality (Dixon, 1998).

Around 10 per cent of aphid species involve host alternation to complete the holocycle. Thus they return as winged males and gynoparae (pre-sexual females) to a primary woody host in the autumn on which the latter morph subsequently produces wingless oviparae which mate with the males and then lay cold-hardy overwintering eggs (Eastop, 1986; Dixon, 1998; Field & Blackman, 2003; Fig. 2). Of cereal aphids, the Blackberry-grain aphid, *Sitobion fragariae* (Walker) is of this type, alternating between the primary host blackberry, *Rubus fruticosus* agg., and Poaceae (cereals and grasses). In contrast, the closely related major pest species, the Grain aphid, *S. avenae* (F.) is autoecious on Poaceae, although some holocyclic (= with sexual phase) lineages will become sexual and lay overwintering eggs if climatic conditions are suitably cold (Hand, 1989). *S. avenae* and the host alternating Bird Cherry-oat aphid, *Rhopalosiphum padi* (L.), are examples of the ultimate in 'bet hedging', with lifecycle forms able to respond to prevailing climate, itself often a function of latitude/geography (see 'Airborne aphids' and 'Clonal selection' sections). Thus if conditions are generally mild, obligate anholocyclic forms, which overwinter as live parthenogenetic virginoparae, persist. In contrast, if suitable low light-cold temperature-inducing conditions prevail, facultative holocyclic forms produce autumn migrants (i.e. gynoparae and sexual morphs in *R. padi*), whilst in *S. avenae*, androcyclic forms produce parthenogenetic females and winged males but no sexual females, and intermediate forms produce parthenogenetic females and some sexual females and winged males (Simon *et al.*, 2002; Llewellyn, 2000, p. 98). Interestingly, the males of the various lifecycle lineages can interbreed with sexual females of other lifecycle lineages so that 'sexual leakage' (gene flow) occurs between lineages. As a consequence, such lineages may not be as genetically isolated as hitherto assumed (Delmotte *et al.*, 2001, 2002, 2003; Simon *et al.*, 2002; see also the 'The myth of the clone' section).

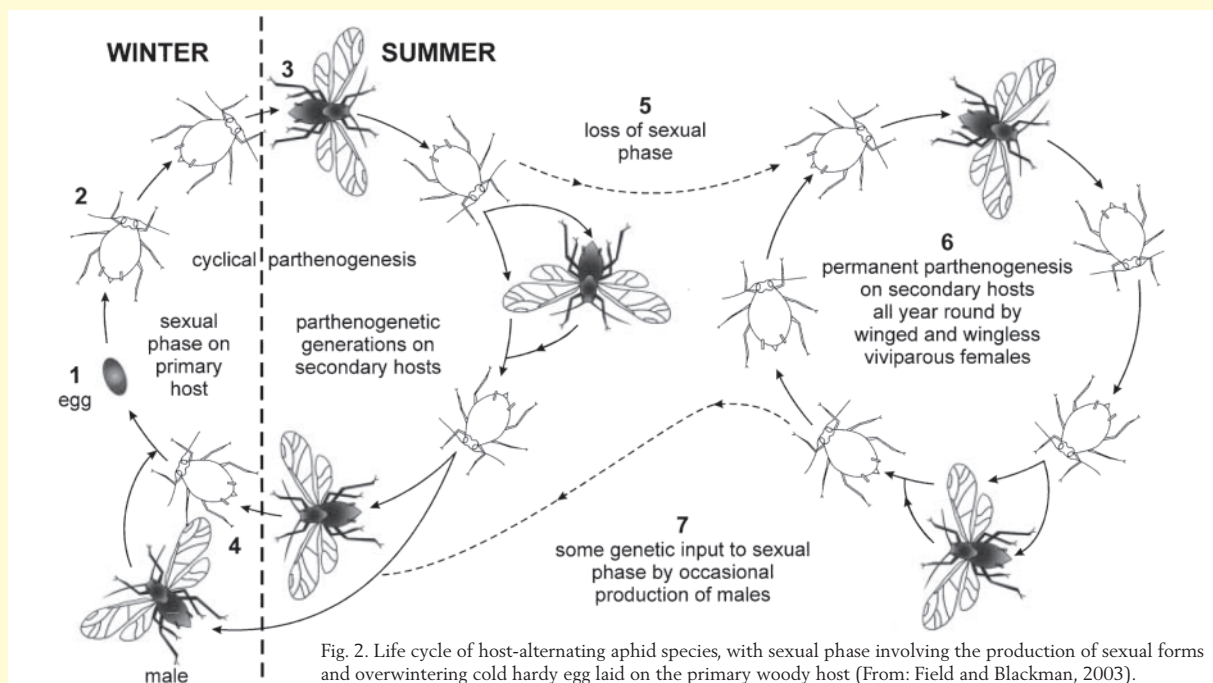


Fig. 2. Life cycle of host-alternating aphid species, with sexual phase involving the production of sexual forms and overwintering cold hardy egg laid on the primary woody host (From: Field and Blackman, 2003).

Adaptive significance

Aphid colouration apparently has adaptive significance. Chroston (1983) showed that the proportion of brown forms of *S. avenae* increased through the summer in wheat fields in southern England. Of *S. avenae* life cycle lineages sampled between 1990-92 in Scotland, East Anglia and Hertfordshire by Helden (1993), holocyclic forms were found to be abundant in Scotland (>30%), intermediates occurred in Scotland and East Anglia (< 22%), androcylic forms were abundant at all sampling sites (>40%), as were anholocyclic forms, although the latter predominated in the south (> 28%; Helden & Dixon (2002); see also Newton, 1986; Newton & Dixon

1988; Llewellyn, 2000). Because of their delayed development due to egg hatch (relative to live aphids overwintering as virginoparae), the holocyclic forms of *S. avenae* tend to colonise cereals later than anholocyclic forms. Therefore, brown aphids may not contribute greatly to the initial population, especially in areas where holocyclic forms are rare, and only do so later in the season. Genotypically brown forms (i.e. phenotypically green) regain their dark pigmentation in the third generation and so colour switching occurs in the crop (Jenkins, 1991). Chroston (1983) concluded that colour change along with the asynchronous pattern of alate colonisation (Newton, 1986) due to

differing overwintering strategies (i.e. holocyclic or anholocyclic), were the mechanisms controlling the changes in abundance of the green and brown forms of this aphid. The brown forms may also have a selective significance in terms of hymenopterous parasitism (advantageous) and predation (disadvantageous; Ankersmit *et al.*, 1986; Losey *et al.*, 1997), although such selection presumably occurs mainly later in the field season, and the brown pigment is probably costly to maintain anyway. Carotenoids may also protect against damaging solar radiation (Jenkins, 1991; Jenkins *et al.*, 1999).

To be continued in the next issue...

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