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Hugh D Loxdale, Adalbert Balog, David Biron. Aphids in focus: unravelling their complex ecology and evolution using genetic and molecular approaches. *Biological Journal of the Linnean Society*, 2020, 129 (3), pp.507-531. 10.1093/biolinnean/blz194 . hal-03018009

HAL Id: hal-03018009

<https://hal.science/hal-03018009>

Submitted on 21 Nov 2020

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Aphids in focus: unravelling their complex ecology and evolution using genetic and molecular approaches

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Aphids are renowned plant parasites of agriculture, horticulture and forestry, causing direct physical damage by sucking phloem and especially by transmission of plant pathogenic viruses. The huge yield loss they cause amounts to 100s of millions \$US *p.a.* globally, and because of this damage and the intense efforts expended on control, some 20 species are now resistant to pesticides world-wide. Aphids represent an ancient, mainly northern temperate group, although some species occur in the tropics, often as obligate asexual lineages or even asexual ‘species’. However, besides their notoriety as enemies of plant growers, aphids are also extremely interesting scientifically, especially at the molecular and genetic level. They reproduce mainly asexually, one female producing 10-90 offspring in 7-10 days and therefore, theoretically, could produce billions of offspring in one growing season in the absence of mortality factors (i.e. climate-weather and antagonists). In this overview, we provide examples of what molecular and genetic studies of aphids have revealed concerning a range of topics, especially fine-grained ecological processes. Aphids, despite their apparently limited behavioural repertoire, are in fact masters (or perhaps more accurately, mistresses) of adaptation and evolutionary flexibility and continue to flourish in a variety of ecosystems, including the agro-ecosystem, regardless of our best efforts to combat them. **206 words**

ADDITIONAL KEYWORDS: Adaptation, antagonists, aphid, asexual reproduction, ecology and evolution, pests, molecular DNA markers, predators, parasitoids

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'In the fields they seem to go off gradually one by one in the heat of the day, heedless of a destination, and like a packet vessel freighted with passengers for different ports; touching and discharging the cargo at intervals, and then anew buoyantly resuming the voyage.' (1850a)

'It thus appears to be impelled through a circuit, and to be ever striving to regain the source whence first it drew the vital stream; like a bird, that, with uncertain aim, has wandered from its home at day-dawn, but directs its way back unerringly, when the tempest lowers, and the shadows of evening thicken.' (1850b)

James Hardy (1815-98) describing, respectively, the aerial migration of the grain aphid, *Sitobion* (= *Aphis*) *avenae*, between its spring/summer grass and cereal hosts; and the host alternation of the rose-grain aphid, *Metopolophium* (= *Aphis*) *dirhodum*, migrating in the autumn from its grass and cereal spring/summer hosts to its primary overwintering woody host, rose, and *vice versa* the following spring.

INTRODUCTION

Aphids (Order Hemiptera: Suborder Homoptera) are small, sap sucking parasites of plants worldwide, especially in the temperate zones (Dixon *et al.*, 1987) and are renowned as major pests of a variety of crops – forestry, horticultural and agricultural, not only causing damage by physical means but also by transmission of plant pathogenic viruses (van Emden & Harrington, 2007, 2017). On a more positive note, they are well known as a major food source for small Passerine breeding birds, such as tits, warblers and finches (Bibby & Green, 1983; Cowie & Hinsley, 1988; Wilson *et al.*, 1999) and many arthropod predators and parasitoids such as spiders, ladybird beetles and hymenopterous wasp parasitoids (Brodeur *et al.*, 2017; Bálint *et al.*, 2018). In addition, they have also proved extremely valuable as experimental animals in a wide variety of fundamental studies in key areas of biology, especially genetics and molecular ecology, the interface between ecology and evolution (e.g. Loxdale, Lushai & Harvey, 2011a; Loxdale *et al.*, 2011b; Loxdale & Balog, 2018). And such usage goes back a long way. For example, the 18th century Genevan naturalist and philosophical writer Charles Bonnet (1720-93) was the first person to demonstrate parthenogenetic reproduction in animals following study of aphids in 1740 (Bonnet, 1745; Lawrence, 2009).

Since then, aphids have been extensively studied in terms of their behaviour, especially migratory flight behaviour, including landing and sap sucking behaviour on plants; physiology, especially developmental related to juvenile hormones; virus transmission; reproduction and fecundity, especially including, but not exclusively, by asexual means; ecology in terms of predator-prey relationships, including colour polymorphisms; symbionts; crop damage; host plant choice and plant resistance; aphid polyphenisms and epigenetic programming; chromosomes (karyotype and chromosomal structure and function); insecticide resistance; and last but by no means least, our own speciality, molecular ecology, studied using high resolution molecular markers, initially allozymes, later DNA markers of various kinds, and now including genomics approaches involving large-scale sequencing of the aphid genome (*cf.* van Emden & Harrington, 2007, 2017 and Vilcinskis, 2016a and references therein).

In the present paper, we provide a brief overview of some of the important insights that aphids have provided in terms of their fundamental genetics and ecology, and by extension, ecology and evolution. This is by no means meant to be a comprehensive review, rather a broad 'brush-stroke' introduction to some of the main discoveries made in these areas, including our own contributions to this topic. In this way, and without being anthropomorphic, it may be described as "What aphids have taught us about themselves". We provide only a small number of

examples of aphid molecular ecology relevant to what we consider to be the main theme of the paper, as this large topic area has recently been reviewed by Loxdale *et al.* (2017).

BACKGROUND

Aphid, sap sucking plant parasites of the superfamily Aphidoidea, seemingly evolved in the Triassic, some 220-210 MYA, initially on Gymnosperms, but later in the Cretaceous, adaptively radiating onto Angiosperms (von Dohlen & Moran, 2000; Grimaldi & Engel, 2005; Podsiadlowski, 2016). They are predominantly a temperate group, although asexual species and strains are found in the tropics (Dixon *et al.*, 1987; Foottit, 1997). In all, around 4,000 species are presently known (Dixon *et al.*, 1987; Eastop, 1986), some major pests of agriculture, horticulture and forestry, whilst around 1,600 species and subspecies occur in Europe (Nieto Nafria *et al.*, 2014-19). In the UK, ~ 620 species are known of which 305 species and subspecies (49%) belong to the subfamily Aphidinae (Macrosiphini) (Blackman, 2010). The biology of many of the species attacking crops, trees and ornamental plants globally are described by Blackman (2010) and Blackman & Eastop (1994, 2000, 2006), along with their status as global pests of agriculture, horticulture and forestry (Dedryver, Le Ralec & Fabre, 2010; van Emden & Harrington, 2007, 2017). The damage that aphids do derives not only from physical damage by sucking plant phloem but also because they are notorious vectors of pathogenic plant viruses of one kind and another, often specific to an individual plant species or family (Katis *et al.*, 2007; Stevens & Lacomme, 2017).

PATTERNS OF DIVERSITY AND ECOLOGICAL-EVOLUTIONARY CHANGE

SPECIALISM *VERSUS* GENERALISM

Most species and sub-spp. (~ 99%) are specialist, attacking one species of plant or members of the same plant family (Eastop, 1986). The remaining ~ 1% include many of the polyphagous pest species, some apparently highly so (Tatchell, Parker & Woiwod, 1983). This *apparent* level of polyphagy may however be the result of relaxed abiotic and biotic selection in manicured and cossetted agro-ecosystems (*cf.* Loxdale, Balog & Harvey, 2019 for further details).

That aphids appear to be mainly specialist in their plant host associations relates to the fact that aphids have co-evolved with their plant hosts probably over vast swathes of time, maybe many millions of years. Such fine-tuning in relation to plant chemical antifeedants and antagonists (predators, hymenopterous parasitoids and pathogens) and climatic-weather factors, is not only a driving force of such specialism, but most probably constrains species populations from host switching, as would be expected with any organism filling a precisely defined ecological niche with multivariate biotic and abiotic governing factors, as originally expounded by G. Evelyn Hutchinson (1903-91) (Slobodkin, 1993; Colwell & Rangel, 2009). The fact that even highly polyphagous aphid species are possibly not as ‘generalist’ as hitherto thought (Loxdale, Balog & Harvey, 2019), is evidence in support of notions that the term ‘generalism’ is probably a misnomer and instead, the more precise terms *mono-*, *oligo* and *polyphagous* should be adopted to describe their diet breadth (*cf.* also Loxdale, Lushai & Harvey, 2011a and Loxdale & Harvey, 2016). That certain pest species, more especially the peach-potato aphid, *Myzus persicae* (Sulzer) (Fig. 1), are apparently capable of infesting plants in 40+ families (Tatchell, Parker & Woiwod, 1983; Blackman & Eastop, 2000; Blackman, 2010) and hence appear to be highly polyphagous (Mathers *et al.*, 2017), may be seen as some

kind of rather unique specialism in its own right (Loxdale & Balog, 2018), since most aphids do not show such an adaptation. Another possibility is that *M. persicae sensu lato* comprises an array of morphologically similar-identical, host adapted cryptic species (Loxdale & Balog, 2018).

In addition to species, host adapted subspecies are known, e.g. the tobacco feeding form of *M. persicae nicotianae* (Blackman) (Blackman, 1987a), which shows differences in timing of winged males in response to sex pheromone cues from sexual females (oviparae), thereby reinforcing assortative mating and host related divergence from populations of *M. persicae sensu stricto* (Margaritopoulos *et al.*, 2007). Some such host-adapted forms are known to be related to karyotypic differences in chromosome number (see section *Chromosomes* below).

LIFECYCLES

Aphids have complex lifecycles (reviewed in Dixon, 1977, 1985a, 1998; Moran, 1992), involving in many species an alternation of generations (sexual and asexual), in turn often involving migration of winged forms from a summer herbaceous host on which asexual propagation continues throughout the spring and summer plant growing season, to a primary host, usually a woody host, on which the sexual forms (winged males and wingless sexual females, oviparae) mate, the latter laying cold hardy winter eggs (Dixon, 1998). The production of the winged males and winged pre-sexual females (gynoparae), which migrate to the woody host, the latter giving rise to oviparae, is governed by environmental cues (i.e. holocyclic lifecycle; Dixon, 1998). Eggs hatch in the spring and after a few asexual generations, including the original asexual stem foundress or fundatrix morph, on the fresh growth of the primary host, winged asexual migrants to herbaceous hosts to continue asexual propagation, often as wingless individuals (apterous virginoparae) until induced by environmental conditions (ambient light and temperature and food plant quality) in the autumn to produce a further generation of winged migrants that return to the primary host in host alternating species (Dixon, 1998). Some aphids, for example two species, *Macrosiphoniella tanacetaria* (Kaltenbach) and *Metopeurum fuscoviride* Stroyan (Figs. 2a & b) found on tansy, *Tanacetum vulgare* L., undergo an alternation of generations but without host alternation (Massonnet, Simon & Weisser, 2002; Massonnet & Weisser, 2004; Blackman, 2010). A very few species are obligate asexuals or produce very few sexual forms or males only (i.e. androcyclic forms; Blackman, 1971), or are facultative asexuals (i.e. anholocyclic lifecycle), producing sexual forms when climatic conditions allow, i.e. low temperature and light conditions (Dixon, 1998; Llewellyn, 2000; Simon, Rispe & Sunnucks, 2002). Some aphid species show polymorphisms in colour related to climatic factors and bacterial endosymbionts (Jenkins, 1991; Jenkins *et al.*, 1999; Alkhedir, Karlovsky & Vidal, 2010; Tsuchida *et al.*, 2010), plant host adaptation associated with such symbionts, more especially facultative or secondary symbionts (Alkhedir *et al.*, 2016; Tsuchida, Koga & Fukatsu, 2004), and polyphenism (changes in morphology, e.g. from wingless (apterous) to winged (alate) individuals) related to juvenile hormone titre (Braendle *et al.*, 2006; Schmidtberg & Vilcinskis, 2016), the last probably under epigenetic control (Srinivasan & Brisson, 2012). Thus, there are seen to be variations of all these strategies within and between species and they may truly be said to be ‘Insects for all Seasons.’

Undoubtedly, a main feature of the life cycle of aphids is the production of large number of asexual offspring by parthenogenetic females (virginoparae) in the spring and summer months, and in some obligate asexual genotypes and species all year round, usually in regions with mild

winters, e.g. the shallot aphid, *Myzus ascalonicus* Doncaster, in which males have never been found (Blackman, 2010). It has been estimated that theoretically, and in the absence of abiotic and biotic mortality factors (climate and antagonists like predators, wasp parasitoids and pathogens, especially Entomophthoralean fungi) a single aphid could produce countless billions of offspring in a single growing season (Harrington, 1994). That aphids are capable of such prodigious feats of reproduction has proven a great boon in study of these and indeed other cyclic parthenogenetic organisms, especially using molecular genetic markers, studies which have included the discovery that such lineages undergo rapid mutational changes of one form and another, including single point mutations, gene duplications and transposon-related changes (e.g. Loxdale & Lushai, 2007, Loxdale *et al.*, 2017; Mittapalli *et al.*, 2011; Mandrioli *et al.*, 2019a,b; see section below, *Clones, Clans and Clines*).

The observation that only around 10% of aphids undergo seasonal host alternation between different species of plants (Eastop, 1973a, 1986) maybe a legacy from their origins on gymnosperms to fast growing, potentially more nutritious herbaceous hosts. In addition, such an escape into ‘enemy free space’ (Jeffries & Lawton, 1984), may well have had fitness advantages. This is by potentially placing them at a distance from their co-evolved antagonists, factors that constrain their ecological-evolutionary flexibility in a Hutchinsonian sense (Loxdale, Balog & Harvey, 2019), despite the inherent dangers of leaving their original natal host in search of other suitable hosts – perhaps widely scattered and hence difficult to locate – on which to propagate asexually. In other words, such alternation of hosts may also have fitness trade-off (benefits vs. disbenefits), but which have seemingly largely benefited the aphid species concerned over evolutionary time in the sense that they still indulge in this practice.

As Eastop (1986) commented, aphids are much more specialist on their primary woody hosts compared with their secondary herbaceous hosts. Even so, perhaps the actual number (rather than *apparent* number) of co-evolved secondary hosts to which the aphids migrate in the spring and summer is lower than hitherto suspected (see Table 1 in Loxdale, Balog & Harvey, 2019 and section below, *Plant resistance and resistance against hymenopterous parasitoids*).

CHROMOSOMES

In terms of the genetics of reproduction, sex is determined chromosomally, i.e. XX (female), XO (male) (Blackman, 1980a; Wilson, Sunnucks & Hales, 1997; Hales *et al.*, 2002; Wilson & Sunnucks, 2006; Schmidtberg & Vilcinskas, 2016), probably governed by epigenetic factors, i.e. DNA methylation (Srinivasan & Brisson, 2012), involving differential expression between autosomes and the sex chromosomes and indeed the sexes (Mathers *et al.*, 2019).

Aphid chromosomes are of the holocentric kind, with no determined centromere, but rather with centromeric activity spread along the entire chromosome (Blackman, 1980a). Chromosome number varies greatly between species, ranging from 4-72 (Blackman, 1980b). The high number of small chromosomes can lead to non-disjunctions on the metaphase plate during meiosis (anaphase) and can in turn lead to new strains and indeed species of aphids being produced, some related to adaptive radiation onto new plant hosts (e.g. Blackman, Eastop & Hills, 1977; *Amphorophora* spp.; see also section below, *Immortal aphids*). Blackman & Eastop (2007, 2017) review taxonomic issues related to aphid karyotype. It is not clear why some species have very few chromosomes and appear chromosomally stable and other species, very many (Blackman, 2019). As Blackman also discusses (2019), it is also not clear why some

aphids like birch aphids, *Euceraaphis betulae* (Koch) have B chromosomes, whilst other species do not.

Of interest when discussing sex determination in aphids is the fact that it is unlike the situation in, for example, mammals where two X's denote a female, and one a male in association with the paternally-associated and male determining Y-chromosome (McLaren, 1988). Sex determination in aphids is separate from sexual reproduction in the sense that males do not carry male determining chromosomes/genes, females being XX, males XO. A female can therefore potentially 'decide' (be selected) to produce any sex ratio that is selectively advantageous - all XX or all XO, although there is no clear evidence at present that they can do so. Probably the only other animal group that can do this are haplodiploid hymenopterous insects like bees, wasps and ants, although in these animals the males are haploid, whereas in aphids, they are diploid, only lacking one X-chromosome.

Wilson *et al.* (1997), using microsatellite markers, have shown that loss of one X chromosome during determination of male aphids (*Sitobion* near *fragariae*) is random. Furthermore, the authors found one out of three microsatellite loci tested to be X-linked, whilst they also posit, based on earlier work by Blackman & Spence (1996), that '*since an X chromosome passes into males independently of its ancestry, 50% of male embryos will be deficient in rDNA and should therefore be non-viable. Male-producing aphids are frequently observed to carry 'aborted' embryos amongst their developing embryos (reviewed in Blackman, 1987b)*'. Hence, this scenario is likely to select against the viability of males during development of the embryos in the female ovariole and distort the balance away from an expected 1:1 sex ratio (*cf.* Wilson *et al.*, 1997 for further details and also in this light, Foster & Benton, 1992 who found that the sex ratio of the Poplar spiral gall aphid, *Pemphigus spyrothecae* Passerini was distorted roughly in the ratio 2:5). Wilson *et al.* (1997) also found no evidence of imprinting of aphid chromosomes in terms of male determination, as they state '*inasmuch as neither the maternal nor the paternal X chromosome was selectively eliminated*', whilst Hales *et al.* (2002), again using microsatellites, found no evidence of recombination as such on the aphid X-chromosome during the production of both sexes.

Very interestingly in terms of understanding sex determination in these animals, Hales & Mittler (1987) showed that the number of X-chromosomes passing to either sex is determined by the titre of juvenile hormone, a high titre resulting in a female (XX), a low titre, a male (XO), and is seemingly involved in the expression – via DNA methylation – of the genes determining sexual morphology of the embryo during embryogenesis (Srinivasan & Brisson, 2012). Interestingly, Mathers *et al.* (2019), throwing further light on this matter, state that '*Gene ontology (GO) term enrichment analysis showed that asexual female-biased methylation and male-biased methylation genes were both enriched for GO terms relating to core biological processes, including metabolism and regulation of gene expression.*' This includes the protein SUMOylation, essential for dosage compensation of the nematode, *Caenorhabditis elegans* sex chromosome. Furthermore, '*Changes in methylation appear to be associated with core processes in aphid polyphenism and sex determination.*'

Lastly, recent work involving quantitative genetic and genomic approaches has revealed that permanent asexuality in aphids is determined as a recessive trait involving a single locus identified as a 2.5 Mbp genomic region located on the X chromosome (Jacquiéry *et al.*, 2014; Jacquiéry, 2019). These authors also showed using population genetic data that substantial gene exchange occurred between reproductively distinct lineages (obligate and facultative) such that some sexual lineages (i.e. with a facultative low light/temperature-induced autumnal sexual

phase) may be transformed into obligate asexual ones in a ‘contagious manner’. Hence aphids have hitherto unsuspected dynamic genetic mechanisms allowing adaptation in the face of seasonal climatic changes, presumably because sexuality, if not required, carries some fitness cost/s in terms of expression of the genes involved, as no doubt reflected in the case of sexual-asexual clinal variation, as noted below (see section, *Clones, Clans and Clines*).

REPRODUCTION AND FECUNDITY

Asexual reproduction is mitotic (ameiotic) (Blackman, 1980a, 1981), although at least one researcher claimed the existence of what he termed *endomeiosis* in aphids (Cognetti, 1961) and it would certainly be worth exploring this area using more extensive high-resolution molecular (DNA) markers, i.e. SNPs (single nucleotide polymorphisms) and sequencing [Nb. Certainly Tomiuk & Wöhrmann (1982) and Hales *et al.* (2002), respectively using allozyme and microsatellite markers, found no evidence of recombination in any of the aphid asexual lineages they tested in this respect]. During asexual propagation, asexual females (virginoparae) show the phenomenon of telescoping of generations, that is to say, each female not only has her own offspring developing within her, but also her grandchildren within each of the embryos she carries in her oviduct (Dixon, 1998). In this way, aphids are capable of amazing feats of reproduction, a single aphid producing 10-90 offspring in 7-10 days and 15-20 asexual generations a year (Blackman, 1971; Dixon, 1998). Thus in a single growing season, one aphid can potentially produce billions of offspring, depending on pertaining mortality factors, i.e. weather-climate and antagonists (Dixon, 1989; Harrington, 1994).

It appears that telescoping of generations and the huge reproductive output of asexual female aphids (virginoparae) is inexorably linked to the breeding strategy of these cyclic parthenogens, *K*-selected (‘cheaply produced’) and *r*-selected (‘expensively produced’), i.e. in this case, parthenogenetic *vs.* sexual modes of reproduction. Dixon (1985b, 1998) discusses this theory in relation to aphids and cites Stearns (1976) arguing that ‘*a single genotype, with seasonally expressed adaptations has no place within the framework of life-history theory, centred as it is on the idea of r- and K-selection*’. [The pros and cons of the theory are reviewed in en.wikipedia.org/wiki/R/K_selection_theory]. Nevertheless, it is clear that asexual organisms indulging in rapid modes of reproduction leading to mass numbers of offspring are ‘hedging their bets’ in an ecological sense, such that at least a high proportion of these offspring will survive and in turn reproduce. There are trade-offs in such a strategy in terms of the advantages for asexuals with regard to the two-fold cost of sex (Maynard Smith, 1978; Simon *et al.*, 2002), the disadvantages for the sexual forms in terms of the likelihood of finding a mate (enhanced in aphids by the use of sex pheromones by the wingless female sexual form (ovipara) to attract winged males; Birkett & Pickett, 2003), as well as the disadvantages in asexual forms due to a lack of sexual recombination enhancing population genetic variability (reviewed in Simon *et al.*, 2002). But on the other hand, because of the high reproductive output of asexual aphids, even if the mutation rate is very low, new mutations, perhaps adaptive in an ecological sense, will arise and potentially thrive. But whether such largely genetically identical offspring are really an ‘Evolutionary Individual’ as such is debatable (see section below, *Widely distributed genotypes in space and time*).

CLONES, CLANS AND CLINES

Even so-called mammalian clones do not display exact genetic fidelity; this is because the donor nucleus is implanted into an enucleate ovum of an individual bearing different mtDNA from the donor (Martens, Loxdale & Schön, 2009). Research on aphids by several different groups has demonstrated using high-resolution molecular (DNA) markers (especially random amplified polymorphic DNA, RAPDs and amplified fragment length polymorphisms, AFLPs and sequencing), that clones, or more correctly asexual lineages, show mutational changes, sometimes very quickly (i.e. within a few generations), mostly within somatic cells, but sometimes in the germ line, and mostly, but not exclusively, in non-coding regions of the genome (Lushai *et al.*, 1998; Vorwerk & Forneck, 2007; Loxdale, Vorwerk & Forneck, 2013; Fazalova & Nevado, 2019). The last authors estimate the spontaneous mutation (μ) between host-adapted races of the pea aphid, *Acyrtosiphon pisum* (Harris) (Fig. 3) (12 parthenogenetic lines from different host races, 28 generations each, 38 x whole-genome re-sequencing) as $\mu \approx 2.7 \times 10^{-10}$ per site per haploid genome per generation. Hence, such asexual lineages are clearly not genetically identical and therefore a better term is required, for example *clan*, as suggested by Loxdale *et al.* (2017). Whilst this mutation rate is extremely low, it has to be considered in the light of the number of genes in the haploid genome of aphids [IAGC (2010) records 2,459 gene families in the 464.3 Mbp genome of the pea aphid, *A. pisum*] multiplied by the huge reproduction rate of asexual aphids involving potentially billions of individuals produced in a single growing season from an asexual stem mother of any particular lineage, i.e. so-called ‘genetic inflation’ (Loxdale *et al.*, 2017). In this scenario, the frequency of spontaneous mutations occurring within a given population may be considerably greater.

In addition to these mutations, Lushai *et al.* (1997) discovered, using RAPD analysis of asexual lineages of two cereal aphid species kept under of strict hygiene, not only genetic differences between different morphs (winged and wingless) but also between the sexes, males and oviparae. These changes may relate to transposon effects (Loxdale, 2008a; Loxdale & Balog, 2018), or less probably, to some kind of yet unconfirmed endomeiotic process involving crossing over of the chromatids (i.e. Cognetti, 1961). Aphids are certainly known to display transposons (transposable elements, TEs) (e.g. Mittapalli *et al.*, 2011; according to the IACG, 2010 ‘Approximately 38% of the assembled genome is composed of TEs’), and these are in turn known in other insects like mosquitoes to be involved in inversion polymorphisms at ‘hot spots’ along the chromosomes (Cáceres *et al.*, 1999) and indeed have been found in aphids, e.g. *M. persicae*, including on the X-chromosome (Monti *et al.*, 2012a). Such polymorphisms may allow sympatric speciation to occur (Noor *et al.*, 2007; Loxdale, 2016a).

Besides the published available sequence of the pea aphid, to date three other global pest species have been examined following extensive sequencing of their genomes, i.e. the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Nicholson *et al.*, 2015), the soybean aphid, *Aphis glycines* Matsumura (Wenger *et al.*, 2017) and *M. persicae* (Mathers *et al.*, 2017). Comparative analysis of the genomes of these various species by Wegner *et al.* (2017) reveals some interesting things, including the fact that *A. glycines* is the smallest of the 19 genomes tested to date using flow cytometry estimates (see their Table 1), i.e. 302.9 Mbp vs. 393.0 for *D. noxia* (Nicholson *et al.*, 2015), 464.3 Mbp for *A. pisum* (IAGC, 2010) and 347-355 Mbp for *M. persicae* (Mathers *et al.*, 2017), and has fewer TE’s than *A. pisum*, which the authors assume contributes to the smaller comparative size of this species.

Wenger *et al.* (2017) also tentatively relate the overall sizes, composition and content of these genomes to the life histories of the respective species: thus compared with *A. pisum* and *M. persicae*, the latter species reputedly highly polyphagous, *A. glycines* which is highly specialised on Poaceae, did not display, despite earlier predictions by the authors, ‘a reduction of genes related to feeding on host detoxification and plants such [as] detoxification and salivary gland effectors’, nor was genome size related to phylogenetic relatedness. From this, it seems that significant differences in genome size exist despite the fact that all these aphids belong to the family Aphididae and often have widely differing host ranges (diet breadth).

In their comparative study using published sequences of the genomes of two *M. persicae* clones in contrast with that of *A. pisum*, Mandrioli *et al.* (2019b) examined the synteny between the genomes and conclude that (a) *M. persicae* clones display more widespread *intra-* and *interclonal* differences than hitherto assumed, especially including deletions and rearrangements; and (b), that the holocentric nature of the aphid chromosome favours genetic rearrangements that can be passed on transgenerationally via the parthenogenetic mode of reproduction.

In the case of the notorious pest of cereals, *D. noxia*, Nicholson *et al.* (2015) showed the genome to display ‘a widespread, extensive reduction in the number of genes per ortholog group, including defensive, detoxification, chemosensory, and sugar transporter groups in comparison to the *Acyrtosiphon pisum* genome, including a 65% reduction in chemoreceptor genes. Thirty of 34 known *D. noxia* salivary genes were found in this assembly’, genes that show less homology with the salivary genes commonly expressed in insect saliva, e.g. glucose dehydrogenase and trehalase. They also detected genes involved in insecticide resistance and endosymbiont-derived genes and those involved in virus transmission, even though, interestingly, *D. noxia* is apparently not a viral vector.

In a very recent paper (2019), Quan *et al.* (2019), examining the 294 Mbp genome sequence of the cotton-melon aphid, *Aphis gossypii* Glover, an *apparently* highly polyphagous species attacking 500+ host plants (barring unidentified cryptic species, sub-species, etc.) add further to the understanding of the functional genomics of such serious pest aphids. Thus for example, they reveal metabolic pathways under rapid evolution, including 3 of 9 involved with detoxification of xenobiotics. In addition, 34 ORs (olfactory receptors) were detected, along with 50 GRs (gustatory receptors), 23 IRs (ionotropic receptors) and 12 OBPs (odorant-binding proteins); the authors claim that the number of GRs, IRs and ORs is the smallest number to be found only after *D. noxia*. They also claim there to be a ‘possible relationship between gene family size in these two chemoreceptor receptors (GRs and IRs) and the host range of aphids. In contrast, there is little concordance between the family size of detoxification related genes and plant host range in aphids.’ As they further suggest, ‘the structural and functional divergence of the detoxification proteins or salivary proteins may influence the process of aphid-plant interactions and hence determine the host range of an aphid species.’ As they show, *A. gossypii* apparently codes for an incomplete immune system in comparison with specialist and oligophagous aphids, i.e. *A. glycines* and *A. pisum*, respectively. In discussing this fact, they cite the IAGC (2010) syndicate’s analysis of the *A. pisum* genome, who remark that such contraction of the immune system may relate to the acquisition of [secondary] endosymbiotic bacteria, which in turn allow adaptation to a greater host plant range (*cf.* Quan *et al.*, 2019 for further details).

Lastly, it is known that aphid populations show clinal variation. Hence, aphid genotypes and genotype frequencies, besides displaying spatial and temporal changes in gene frequency due to random stochastic processes, including selection and genetic drift, are also subject to direct influences of climate, probably also via selection, and in this case, on the lifecycle morph itself (anholocyclic vs. holocyclic). A good example concerns the bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Fig. 4). In this species, mitochondrial DNA (mtDNA) haplotypes exist in a north-south cline in France correlated with life-cycle mode, i.e. obligate asexual (anholocyclic) or facultative (holocyclic) depending on the severity of the winter, anholocyclic forms being common in regions with milder climates, i.e. south of France, holocyclic forms involving sexual reproduction with laying of cold hardy eggs being more abundant in areas with more severe winters, i.e. north of France (Martínez-Torres *et al.*, 1997; reviewed in Loxdale & Lushai, 2007, Loxdale *et al.*, 2017).

INTROGRESSION-HYBRIDISATION

Aphids are also known to undergo introgression events, including in terms of ribosomal gene sequences (ITS region) (Fenton, Malloch & Germa, 1998a) and microsatellites and mtDNA (Sunnucks *et al.*, 1997; Wilson, Sunnucks & Hales, 1999). Indeed, some species, e.g. the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), may be the product of a hybridization event (Delmotte *et al.*, 2001), possibly a mutational event occurring but once during its entire ecological-evolutionary history (*cf.* also Footitt, 1997 and in the case of Cladocera, i.e. *Daphnia* spp., Tucker *et al.*, 2013). The fact that some races of aphids, e.g. pea aphid host adapted forms (pink and green) have undergone sympatric divergence is seemingly related to unique non-recombining regions of the genome (Via & West, 2008; Fazalova *et al.*, 2018), as it is in some moths (Lepidoptera; Emelianov, Marec & Mallet, 2004). It is also well established that *A. pisum sensu lato* have undergone extensive adaptive radiation onto a variety of plant hosts within the Fabaceae (Frantz *et al.*, 2006, 2009; Peccoud *et al.*, 2009), reinforced by the existence of morphological differences between forms, especially wingless males (Knäbe, 1999), and intrinsic post-mating pre-zygotic isolation (Fazalova *et al.*, 2018), a process which may have taken at least half a million years (Fazalova & Nevado, 2019).

INSECTICIDE RESISTANCE

Aphids have been controlled using pesticides since the 19th and early 20th centuries (i.e. nicotine) and especially since the 1950s, when synthetic pesticides were first developed and became widely available globally (Dedryver, Le Ralec & Fabre, 2010). In the UK, instances of chemical control failure in *M. persicae* were recorded in greenhouses as early as the mid-1960s (Gould, 1966), the aphid showing resistance to both carbamate and organophosphate insecticides (OPs), the molecular basis of resistance to the latter due to multi-function oxidases (Needham & Sawicki, 1971). Later, such resistant forms were found in the field in many countries worldwide, and some 20 species are now known to show resistance (Devonshire, 1989; Foster, Devine & Devonshire, 2007a, 2017). Initially, this was found to be conferred via elevated carboxylesterase activity, although today several mechanisms are known in relation to a number of synthetic compounds, including carbamates, OPs, pyrethroids and most recently, neonicotinoids. These mechanisms include detoxification of the poisons, as found in the case of carboxylesterases and cytochrome P450 CYP6CY3 enzymes, or modification (mutation/s) of target binding sites in the insect nervous system, as with modified acetylcholinesterase (MACE) against carbamates and OPs, knockdown resistance against pyrethroids (*kdr*), and

R81T against neonicotinoids, the last due to a mutation of the $\beta 1$ subunit in the nicotinic acetylcholine receptor (nAChR) (reviewed by Foster *et al.*, 2007a, 2017 and Bass *et al.*, 2014).

In the case of *M. persicae*, it now displays four main resistant strains, S (susceptible), R₁, R₂ and R₃, the last having 80 x more carboxylesterase genes (E4) than S strains (Field *et al.*, 1999). This species also shows MACE, *kdr* and neonicotinoid resistance and some individuals are even cross-resistant to one or more pesticides via the aforementioned mechanisms. Some *M. persicae* resistance strains also show a variant of the esterase enzyme, known as FE4 (Bass *et al.*, 2014; Foster *et al.*, 2007a, 2017). In some highly resistant strains of *M. persicae*, an autosomal (A1, A3) translocation is associated with the expression of elevated levels of carboxylesterase (Bass *et al.*, 2014; but see also Rivi *et al.*, 2013, where this appears not to be so).

In relation to the theme of this paper, the elevation of the E4 gene (amplicon) in the higher resistance strains of *M. persicae* is an example of evolution via gene duplication* (Devonshire & Sawicki, 1979). Some years after the discovery of the mechanism of carboxylesterase resistance in *M. persicae*, it was discovered that the highly resistance forms displayed a lower resistance status when not actively selected against with pesticide (Sawicki *et al.*, 1980; ffrench-Constant, Devonshire & White, 1988). Later research revealed that this was not due to a loss of the E4 amplicons *per se*, but rather to a switching off of the expression of the E4 genes themselves (Field *et al.*, 1989a). Indeed, use of homologous probes showed that in fact the number of genes was unchanged, only their expression (Field *et al.*, 1989b; *cf.* also Field & Blackman, 2003 for an overview). It was also shown that expression is governed by epigenetic programming (IAGC, 2010); methylation of the DNA turns the genes on, the opposite of the situation found in vertebrates like mammals (Hick, Field & Devonshire, 1996). That the expression of amplified E4 genes is switched off is probably because of a fitness cost incurred by aphids no longer actively selected against with pesticide, more especially since the expressed enzyme amounts to some 0.1% of an adult aphid's entire body weight of *ca.* 400-500 μg , i.e. \sim 400-500 ng E4 (Devonshire, Moores & ffrench-Constant., 1986). As aphids may take up to several asexual generations to revert to a lower resistance status (Sawicki *et al.*, 1980), this response may be equated with a kind of 'near-Lamarckian' phenomenon (Lushai, Loxdale & Allen, 2003; Loxdale, 2018). But then again, it could be argued that as an asexual aphid's offspring and grandchildren are actually within her body when exposed to chemical treatment, in this sense all three generations are directly influenced (and the E4 genes epigenetically modified) as soon as chemical treatment is halted.

Another fitness cost is incurred as a result of the pleiotropic effect that highly resistant *M. persicae* are subject to, more especially by possession of mechanisms that influence the

*Sequencing of the pea aphid genome (IAGC, 2010) has revealed the presence of extensive duplications. Thus 'Analysis of the pea aphid phylome revealed 2,459 gene families that appear to have undergone aphid lineage-specific duplications, a number greater than that of any other sequenced insect genome. Only the genome of the crustacean *Daphnia pulex* appears to have experienced a similar level of lineage-specific duplications.' More recently, Fernández *et al.* (2019) in studies of the pea aphid phylome ('the complete collection of phylogenetic trees for each gene in its genome') have discovered gene duplications at various regions of the genome, some negatively, a majority positively selected, the latter involving neofunctionalization and affecting a number of important biological functions, including resistance to insecticides. These changes are claimed by the authors to be 'a main driving force reshaping the pea aphid genome.'

functioning of the nervous system. Such aphids tend to be more sluggish and also less responsive to aphid alarm pheromone, E- β -farnesene (Foster et al., 2007b) than susceptible aphids or aphids of a lower resistance status, and are hence more likely to be predated by **birds** and especially invertebrate predators and hymenopterous parasitoid wasps (braconids). They are also less likely to fly, which seemingly partly accounts for the periodic reduction of winged forms of these genotypes in 12.2 m high Rothamsted suction trap samples, including during the winter months (Foster et al., 2002; Foster et al., 2007a, 2017; Loxdale, 2018). That highly resistant/cross resistant *M. persicae* show fluctuating patterns of abundance when captured in such traps, sometimes dying out after only a relatively few years, may well be due to the multiple fitness costs borne by such aphids, especially in the absence of chemical selective pressure (Fenton et al., 2010). Even some lower resistance genotypes such as the once abundant ‘O’ genotype of *M. persicae*, can disappear without any obvious reason after years of relative abundance, perhaps again due to fitness costs (Foster, Oliphant & Williamson, 2019) or perhaps due to the accumulation of deleterious mutations (see *Immortal aphids* section below).

Resistant populations may perhaps arise from a single mutated individual. Even at a mutation rate as low as 10^{-10} – 10^{-9} per gene per generation, as aforementioned, because of the huge reproductive potential of aphids, one in a billion individuals may not seem an unreasonable probability allowing mutated individuals to spontaneously arise, including those resistant to insecticides. Until the early years of the 21st century, resistance was unknown in UK populations of the grain aphid, *Sitobion avenae* (F.) (Fig. 5). Then in 2014, *kdr*-resistant winged individuals were captured in 12.2. m high suction traps run by the Rothamsted Insect Survey (RIS) (Harrington, 2014). Since then, the genotype has been found commonly across southern Britain (Dewar & Foster, 2017). It has even evolved a homozygous super-resistant form in recent years at the *kdr* locus (Foster et al., 2014). Examination of stored suction traps samples of aphids collected in previous years via the RIS network of survey traps actually revealed the first appearance of the *kdr*-resistant mutant genotype of this aphid species as early as 2009, although it had hitherto not been sampled directly in field-collected aphids sampled by hand (Foster et al., 2014; Foster et al., 2017). In this way, study of resistance aphids provides clues not only to the evolution of the various molecular mechanisms themselves (Bass et al., 2014), but also to the population genetics and dynamics of aphids bearing these genes (Margitopoulos et al., 2009; Kasprócz et al., 2008; Loxdale, 2009; Fenton et al., 2010).

IMMORTAL APHIDS?

That certain aphid genotypes can persist in the field for many years seems clear. However, whether these truly derive from a single asexual foundress is debatable. This is highlighted in the case of insecticide resistance as generally, only one or a few loci are examined in population surveys and therefore, it is an assumption that samples of the genotype in question, especially if collected from geographically widespread locations, represent the same or independently evolved lineages. Assuming it is the same genotype/lineage, as a result of Muller’s ratchet and the likelihood of a genetic load of deleterious mutations accumulating, the lineage is likely to die out sooner or later (mutational meltdown) (Lynch & Blanchard, 1998; Lynch et al., 1993). In the case of cladocerans, *Daphnia pulex* Leydig maintained under strict laboratory conditions, this was experimentally determined to be about 40 asexual generations (Lynch et al., 1998). More recent empirical studies using genome wide sequencing of *Daphnia* populations do not

support the mutational meltdown theory (Tucker *et al.*, 2013). As the authors state ‘*Our results suggest that the exposure of preexisting, deleterious recessive alleles by loss of heterozygosity may be a much more substantial contributor to the genetic deterioration of obligately asexual lineages than the gradual accumulation of new mutations.*’ On the other hand, since mutation is the basis of evolution, then it is also possible that new lineages evolve and adaptively radiate according to novel ecological niches arising, i.e. host switching (Loxdale, 2016a). Such changes are known in aphids, and are often associated with large-scale karyotypic changes, due to fission and fusions. Thus for example, the corn leaf aphid, *R. maidis* has host adapted forms infesting sorghum, *Sorghum bicolor* (L.) Moench. and maize, *Zea mays* L., depending on chromosome number (Brown & Blackman, 1988). In *M. persicae*, laboratory experiments have shown that rapid chromosomal changes do indeed occur as a result of fission and fusions, such that variations of the normal karyotype (2n=12) are seen, not only within asexual lineages, but even between embryos within the same mother (Monti *et al.*, 2012a,b). It has long been known that there is a clear relationship between chromosome number/karyotype and host plant adaptations in some aphid species, including *M. persicae* (Blackman, 1980b; Blackman & Eastop, 2007, 2017).

As well as such large scale genetic changes leading to rapid ecological-evolutionary divergence, the structure and presumably function of the chromosomes undergo *intra*-chromosomal mutational changes due to the presence of labile ‘hot spots’ (Pennisi, 1998). These are the sites of transposon activity that not only lead to inversion polymorphisms (Cáceras *et al.*, 1999) – hence perhaps the mechanism responsible for the maintenance of host adapted genotypes within holocyclic lineages of aphids (Loxdale & Brookes, 1990; Loxdale, 2016a) – but are also weak points allowing chromosomes to cleave and re-join during translocation. Furthermore, in a recent comparative study of the extent of synteny and conservation in the gene order of two pest aphids, *M. persicae* and *A. glycines*, involving specific probes and fluorescent *in situ* hybridization of the chromosomes following sequencing of specific sections of the genomes of the two species, Mandrioli *et al.* (2019b) suggest that the holocentric nature of aphid chromosomes may favour genome changes, whilst in their recent study using published sequences of the synteny between *M. persicae* clones vs. the *A. pisum* genome, they demonstrate the existence of gene rearrangements, including deletions.

Whilst hitherto it has not been possible to determine with certainty the persistence of particular aphid genotypes in the field, the widespread and much cheaper availability of sequencing techniques in recent years renders this no longer a pipe dream. Despite the empirically proven spontaneous presence of large and smaller scale mutations (i.e. karyotypic and genomic) found within and between lineages of given aphid species, it is now possible to determine the phylogeny and possible long term persistence of certain aphid host adapted biotypes using genome-wide sequencing approaches (e.g. Fazalova & Nevado, 2019). However, this approach has yet to be achieved using asexual lineages collected at random directly from the field.

The question may thus be asked whether such asexual lineages live for more than 40 generations in the field without meltdown? Fazalova & Nevado (2019) studied 12 asexual lines (24 genome) of different *A. pisum* host races reared over 28 generations in the laboratory and discovered a low level of spontaneous mutations. This evidence alone shows the error of assuming that such asexual lineages reared long term under laboratory conditions, even if founded from a single asexual female, are genetically identical. On the contrary, since 28

generations represents less than two years' worth of generations (at – say – 15 to 20 generations per plant growing season, and of course, dependent upon climatic conditions), then it is highly unlikely that lineages are unaffected by spontaneous mutational events over the course of a decade or more. In addition, unless asexual lineages are maintained in scrupulously exact ambient spring/summer conditions (i.e. > 15°C ambient temperature and a light/dark (L:D) regime of > 16 hrs. light; Dixon, 1985b, 1998), then sexual morphs may well be produced under such conditions. These morphs can mate and the oviparae produce viable eggs, even on a secondary herbaceous host, as found in the case of *M. persicae* reared on radish (*Raphanus raphanistrum* subsp. *sativus* (L.) Domin) in a greenhouse (R.L. Blackman, pers. comm.).

Where aphids have been kept long term in culture on artificial diet (van Emden, 2009), for example the *M. persicae* lineage started at Silwood Park, Imperial College, UK in the mid-1980s, and with sub-cultures reared from the same original lineage at Reading University, UK, both lineages were seen to die out almost simultaneously after 33 years (van Emden, 2019). van Emden (2019) posits the question “Do the populations of what are believed to be permanently parthenogenetic aphid genotypes have a limited life span?”

In this light, telomere length at the ends of the chromosomes of eukaryotes, as also found in aphids (IAGC, 2010), and essential for their proper functioning (Blackburn, 2000; McEachern, Krauskopf & Blackburn, 2000), declines over generations in many animals studied to date, potentially leading to the extinction of the species lineage in question (Blasco, 2005) – that is, unless this is periodically re-set by sexual reproduction, as earlier hypothesised by Loxdale & Lushai in the case of aphids (Loxdale & Lushai, 2003a; Lushai & Loxdale, 2007 and references therein). Of course, because of the distinct possibility that over a period as long as three decades, ambient environmental conditions below the critical low light and temperature thresholds known to induce sexuals may not have been accurately maintained, then sexual forms (males and oviparae) could have occasionally been produced, thereby allowing the aphid telomeres to be re-set, in turn rejuvenating the lineage/s. However, this seemingly did not happen, suggesting that (a) the theory is incorrect or (b), the spring-summer ambient L:D conditions for these colonies were indeed accurately maintained over the time period involved (>3 decades), thereby preventing the induction of sexual forms.

Monti *et al.* (2011) dispute the idea of telomere re-setting in aphids. Thus in laboratory experiments using fluorescent *in situ* hybridization (FISH) probes against the telomere (TTAGG)_n repeat of *R. padi* asexual lineages that had been in culture for 40 years, they found no differences in fluoresce binding intensity between generations within such lineages. However, again, unless strict ambient conditions are maintained, then the possibility of sexual reproduction cannot be entirely eliminated, along with re-setting of telomere lengths. Only further empirical work can positively support or deny this contention.

As things currently stand, we simply do not know how long given aphid genotypes persist in the field. But from what has been outlined above, it is improbable that they last for more than a few years, decades at most, simply because the world is a dynamic place and environmental conditions are likely to change rapidly, especially in the intensively cultivated agroecosystem with reliance of the development and use of novel insecticides (Loxdale, 2016b). Such chemicals are likely to eliminate the older, less insecticide resistant lineages, and anyway, as here briefly discussed (*Insecticide Resistance* section), the higher insecticide resistant

genotypes carrying fitness costs, which seemingly cause their demise, perhaps sooner than later.

Whatever happens in the laboratory is of course not the real world in terms of what aphid lineages are actually exposed to vis-à-vis a plethora of abiotic and biotic selective factors, including competitive forces, i.e. *intra*- and *inter*clonal, as well as *interspecific* due to different aphid species competing for resource on the same plant/s. To further explore the topic of the survival and longevity of aphid asexual lineages in the field, it has proved instructive to plot aphid microsatellite multilocus genotype (MLG) data in the form of a Willis curve – named after the famous and long-lived English botanist, John Christopher Willis, FRS (1868-1958), in which the frequency of occurrence of MLGs (Y-axis) is plotted against the number of sets of different MLGs (X-axis). Thus each MLG represents a single individual genotype within a population sample collected from the field or if detected as multiple copies, an asexual lineage representing this particular genotype. When this was done in the case of local field populations of the two tansy aphid species, *Macrosiphoniella tanacetaria* and *Metopeurum fuscoviride* (Figs. 2a & b) and a cereal aphid, *S. avenae* (Fig. 5), the study revealed the presence of a high frequencies of single repeat MLGs, and thereafter, an exponential decline leading to very few multi-copy MLGs or clones *sensu lato* (*cf.* Figures 2a-c in Loxdale, Massonnet & Weisser, 2010, and Loxdale, 2016a and references therein).

This process is seemingly akin to some kind of ‘molecular-evolutionary machine’, with new genotypes being produced in the spring, either by recombination of existing genotypes during autumnal sexual reproduction and subsequent hatching of overwinter eggs, or by spontaneous mutations, some as mentioned earlier, involving major genomic-karyotypic re-arrangements (like the ‘hopeful monsters’ envisaged by Hugo de Vries (1848-1935); *cf.* Gould, 1980a). The new genotypes rapidly face a host of environmental challenges, probably including *intra*-asexual lineage and *inter*-lineage competition, and either flourish and survive through the plant growing season to produce a relatively few successful lineages or, as seems to be the case, rapidly decline and die out at an early stage in their evolution. In the autumn, the surviving lineages again mate to produce overwintering eggs or undergo spontaneous mutations and the process effectively repeats itself, except that new genotypes now compete with existing genotypes and ultimately, may replace these, partially or completely.

WIDELY DISTRIBUTED GENOTYPES IN SPACE AND TIME

The ecologist Dan Janzen in a famous paper (Janzen, 1977) entitled ‘What are dandelions and aphids?’ proposed the concept of the evolutionary individuals (EI), that is a parthenogenetic lineage (here dandelions and aphids) that because of their assumed close genetic similarity/identity, was able to spread out and successfully exploit resources over a wide geographical area. However, because of the known mutational ability of aphids amplified by their prodigious reproductive capabilities, this was deemed unlikely by Loxdale (2008b). Even so, this is not to say that certain successful aphid genotypes do not exist or even appear to be so-called ‘superclones’, dominating the environments, especially agroecosystems, where they exist, at least for a while (e.g. Fenton, Woodford & Malloch, 1998b; Figueroa *et al.*, 2005; Harrison and Mondor, 2011; Vorburger, Lancaster & Sunnucks, 2003). An argument against the long term persistence of such genotypes is that usually only a small number of loci are tested to establish genetic identity (e.g. Haack *et al.*, 2000), whilst the remainder of the genome remains untested, unless extensively DNA sequenced, as is now becoming possible (e.g.

Fazalova & Navado, 2019). Hence we generally do not know to what extent such lineages are undergoing adaptive changes nor over what timescales.

Whilst different aphid genotypes wax and wane, especially including insecticide resistant ones (Fenton *et al.*, 2010; Foster *et al.*, 2007, 2017; Foster, Oliphant & Williamson, 2019), because of ongoing ecological pressures due to a range of abiotic and biotic factors, now including global warming (Harrington *et al.*, 2007), probably no aphid lineage can persist in a long term evolutionary sense (i.e. over historical time scales, let alone geologic ones) without undergoing adaptive changes. Not to do so is to court rapid extinction. Thus many aphid species, more especially those invasive species entering new territories or hosts, after a surge in numbers and expansion of geographic range, either soon die out or become less of an economic problem as they ‘equilibrate’ in an ecological sense with the plethora of antagonists, adapting to them. Good examples of this latter scenario include the arrival of the lupin aphid, *Macrosiphon albifrons* (Essig) into the UK and other European countries from North America in the early 1980s (Carter, Fourn & Bartlett, 1984), and the Russian wheat aphid, *D. noxia* into the wheat belts of South Africa and North America in the late 1970s and mid-1980s, respectively, from its original range of the Caucasus region (*cf.* Loxdale, 2018 and references therein).

Presumably both species had, over the long course of co-evolution in their natal range and on their original plant hosts, become ‘acclimatised’ in an ecological sense to the prevailing abiotic and biotic environmental factors. However, on arriving in their new range/s and habitats, possibly aided by human transport (vehicles, ships, aeroplanes), they were subjected to *relaxed selection* (Loxdale, Balog & Harvey, 2019), and were thus able to thrive accordingly, at least for a while. As time has gone on (decades), both species are no longer the economic problem that they once were during the initial phase of their invasions and seemingly their antagonists, especially including hymenopterous parasitoids and entomopathogenic fungi, have adapted to them and controlled their numbers (*cf.* Loxdale, 2018 and references therein).

PLANT RESISTANCE AND RESISTANCE AGAINST HYMENOPTEROUS PARASITOIDS

Because aphids and the offspring that they produce, either asexually or following mating and laying of overwintering eggs, are struggling as individuals to survive, be that in the ‘real world’ or the agro-ecosystem with its reduced biodiversity and thus reduced selective pressures, host switching is always a possibility. Yet this is fraught with problems, especially adaptation to the new secondary plant antifeedants that a species may encounter on changing hosts, especially if these are in a different plant family (Loxdale, Lushai & Harvey, 2011a; Loxdale & Harvey, 2016; Loxdale, Balog & Harvey, 2019). Hence, most such host switches occur within plant families, which share, due to their common evolutionary origins, the same or similar arrays of antifeedants. Attacking aphids must have the biochemical/genetic/molecular abilities to deal with such antifeedants, suggesting that in the case of detoxifying enzyme systems, like carboxylesterases and P450 enzymes, they must have allosteric binding sites capable of dealing with diverse chemical structures. Some aphids like *M. persicae* do seem to be polyphagous, but these are the exception rather than the rule (see section, *Generalism vs. Specialism*). Most aphids in contrast are adapted to single plant families or single species, i.e. are oligo- or monophagous. Even assumed highly polyphagous pest aphid species may not be so polyphagous as once thought due to *relaxed selection* in such manicured and cossetted

environments (Loxdale, Balog & Harvey, 2019), and the topic clearly needs re-appraisal involving further empirical investigation, especially using high resolution molecular markers.

Of course, because most terrestrial plants are sessile, they can only defend themselves by anatomical (e.g. spikes, trichomes and waxy surfaces) and chemical means, or with assistance from invertebrate predators and parasitoids which they summon using volatile kairomones like jasmonate (e.g. Takabayashi & Shiojiri, 2019). As such, they have been locked in an arms race with aphids and other damaging herbivores for aeons, certainly since the Jurassic epoch and probably before (Grimaldi & Engel, 2005; Podsiadlowski, 2016), and especially including their adaptive radiation onto Angiosperms in the Cretaceous (von Dohlen & Moran, 2000). Aphids have had to adapt to the array of defences elicited by the plants they attack, and when selective pressures, especially including competitive pressures, are great enough, host switching becomes an option. One positive benefit is that, assuming the aphid species in question can thus switch, mostly to a related species in the same plant family, it potentially enters ‘enemy free space’, at least for a while, during which time the same co-evolved or new antagonists may combat it, as seems to have happened with the aforementioned invasive aphid species, *M. albifrons* and *D. noxia* (see section below, *Host plant influence on preference and escape into ‘enemy free space’*).

Because this arms race has been going on so long, long before human agriculture led to the growing of crop varieties over large areas, often displaced in a geographical sense from their original land of origin, aphids have evolved – not unsurprisingly – resistance against plants. This is more especially so for those plants that we have cultivated *en masse*, e.g. cereals, and which often have, as a consequence of selected breeding for traits such as high yield, a reduced titre of defending antifeedant/s (*cf.* Loxdale, Balog & Harvey, 2019 and references therein). The evolution of plant resistance by aphids is exemplified in the case of the greenbug, *Schizaphis graminum* (Rondani), a major pest of cereals in North America and to a lesser extent Europe. Since 1882, eleven host-adapted biotypes (Eastop, 1973b) have been discovered (Saldúa *et al.*, 2011). These can break the resistance mechanisms of specific hosts, for example sorghum, but evidence from mtDNA analysis reveals some of these associations are ancient, pre-dating agriculture in the Americas, which arose at least 8,000 years BP (Porter *et al.*, 1997; Shufran *et al.*, 2000; Anstead, Burd & Shufran, 2002; Piperno *et al.*, 2009). van Emden (2017) reviews the mechanisms associated with the breaking of plant resistance (antixenosis & antibiosis) by aphids (*cf.* also Jaouannet *et al.*, 2014).

In addition to aphids breaking the resistance of plants during their long co-evolution, aphids have in turn evolved immunological mechanisms to combat hymenopterous parasitoid wasp (braconids) attacking them. Interestingly, this resistance is assisted by the presence of secondary (facultative) endosymbiotic bacteria within the bacteriocytes (mycetomes) of aphids, leading to encapsulation of the egg or early stage larvae of the attacking parasitoid (Vilcinskas, 2016b). More interesting still is the finding that different aphid species show plant host associations correlated with the different known species of secondary bacteria (Skaljac, 2016), which reinforces tightly co-evolved associations between plant, aphid, parasitoid and bacteria in a seemingly *tetra*-trophic feeding cascade. A further reinforcement of the act of specialisation comes from the parasitoids themselves, which, on emerging from the aphid mummy as an adult wasp, seek out the plant species on which the host aphid from which they derived originated (Storeck *et al.*, 2000). So in light of all these aspects, it cannot be said that aphids are ‘free agents’ in terms of the plants they attack. Rather they are highly constrained

ecologically, surviving against all the challenges that their particular unique multidimensional niche expose them to, abiotic and biotic.

KIN SELECTION

The reality of Hamiltonian kin selection as first proposed by William D. Hamilton (1936-2000) (inclusive fitness theory) in 1964a,b, i.e. that living organisms show a genetically-mediated altruistic response towards closely-related individuals such as offspring and siblings even at the cost of the individual's own survival, has been widely advocated in the literature, more especially in relation to eusocial haplo-diploid insects like bees, wasps and ants (Hamilton, 1964a,b; Foster, Wenseleers & Ratnieks, 2006). However, there are detractors from the theory, even amongst specialists of these insects (Wilson, 2005; Wilson & Hölldobler, 2005; cf. also Foster *et al.*, 2006).

Aphids with their very close *intra*-asexual lineal genetic identity (with so-called clone mates perhaps sharing >99.9% of their genomes in common, that is with the exception of differences due to mutational changes of one kind or another), may be seen to be excellent exemplars in this respect, dedicating much effort in protecting their relatives – clone mates of the same and different generations. However, with the exception of gall aphids, they do not (Stern & Foster, 1997; Foster, 2002). Most aphids release alarm pheromone, E- β -farnesene, when attacked by arthropod predators and hymenopterous parasitoids (Pickett & Glinwood, 2007). However, whilst this is most likely to affect members of a colony, which are most often be derived from the same asexual foundress or stem-mother (fundatrix), other less genetically closely related individuals could also be involved. If so, the pheromone works across different genotypes and has a broader effect than directly influencing individual genetic lineage fitness, both *intra*- and *inter*generational. Again, it is tantamount to a group selection phenomenon, whereby the fitness of the group is enhanced in a positive, but probably indirect way.

Aphids can also defend themselves in a direct manner, by kicking out at would be attackers (Döring *et al.*, 2008), but that this is not that effective can be witnessed on seeing the colour films of aphids and their antagonists, especially invertebrate predators and hymenopterous parasitoids, by Prof. Urs Wyss of Kiel University in Germany (<http://www.entofilm.com/>). Even so, gall aphids, for example, the poplar-lettuce gall aphid, *Pemphigus bursarius* (L.) (Fig. 6a & b), a pest of lettuce, *Lactuca sativa* L. (Family Asteraceae) and poplar spiral gall aphid, *P. spyrothecae* Passerini, have special so-called first instar nymphal soldier morphs with thickened hind legs (Aoki & Kurosu, 1986), and which display apparent altruistic behaviour, as first described by Aoki in 1977. The legs of such soldier morphs indeed seems to be more effective than the legs of the normal nymphs in deterring attacks by arthropod predators (Foster, 1990, Foster & Rhoden, 1998, cf. also en.wikipedia.org/wiki/Pemphigus_spyrothecae and Siddiqui *et al.*, 2019). Gall aphids, which are eusocial, are known from molecular genetic studies to be highly inbred (Hebert *et al.*, 1991; Johnson *et al.*, 2002), often with rather little *inter*-gall aerial migration/gene flow in various species examined (although there is more *inter*-colony mixing in *Pemphigus obesinymphae* Aoki, i.e. > 41%; Abbot *et al.*, 2001), and this general lack of mixing may be evidence of kin selection (Foster, 1990). But if so, then again it is of a group selection type whereby the whole colony benefits from the actions of a few, so that their loss 'in action' (Foster, 1990) is unlikely to directly influence the long-term genetics of the colony and indeed local population. Having said that, in *Pemphigus*, clonal nest mates can potentially develop into winged sexuparae which fly off in the autumn from the secondary

hosts and produce sexuals which mate and lay overwintering eggs on the primary host (cf. Osiadacz & Halaj, 2014 in the case of the lifecycle of the club-shaped poplar gall aphid, *P. populi* Courcelet).

So what does this all mean in the end? Certainly gall aphids defend their colonies (Alton, 1999), but even if – say – 5-10% of the many hundreds of individuals in a gall (Alton, 1999) are consumed by predator/s (arthropod and avian), is this a disaster for the colony? Probably not, but perhaps it would be more of a problem if >95% were predated, so that in this circumstance defence would be a selectively advantageous behaviour. If this is true, then there is a probably a link with density dependence (Boyd, 1982; Alton, 1999; Abbot, 2009). Furthermore, as shown by Benton & Foster (1992), the soldier morphs have a house keeping role in term of removing the old skins, dead aphids and honeydew droplets, the latter especially likely to have a detrimental effect on the colony's health and hence viability due to fungal infections. (cf. also Foster & Rhoden, 1998; Alton, 1999). Another aspect of this scenario is that whilst the clone mates within galls are closely related genetically (barring 'sneaky' intruding genotypes due to inter-gall migration), then there may be a selective advantage in defending clone mates **which ultimately produce winged sexuparae which leave the gall and in turn produce sexuals which mate** with genetically diverse individuals from other galls, thereby increasing population genetic variation by outbreeding. In other words, colony defence effectively enhances the genetic contribution of a particular gall colony to the overall gene frequency in the local population.

But then again we have to come back to the central point: why don't all aphids show such a behaviour...other than kicking out at arthropod predators/wasp parasitoids to deter their attentions and using alarm pheromones to alert direct clone mates as well as other more genetically diverse members in a colony of would-be attackers? It is also apparent that the contained populations of gall aphids locked within their 'fortress' prior to the urge of sexuparae to leave, **migrate and produce sexual forms which mate** with genetically diverse individuals of their species on the chosen specific primary host has in itself survival advantages, as originally shown by Whitham (1979).

According to Abbot *et al.*'s (2001) molecular genetic study of *P. obesinymphae*: '*The gall-dwelling colonies of a social aphid species (Pemphigus obesinymphae) are not pure clones, but are invaded by large numbers of aphids from other clones. Intruders behave and develop selfishly once they have invaded a colony of non-kin. They refrain from risky defensive behaviours and accelerate their own development into reproductive rather than defensive stages. This conditionality in the social life of P. obesinymphae reveals complex dynamics and a degree of behavioural plasticity not previously known in aphid societies.*' In a later overview (Abbot, 2009) in which the dynamics of aphid and especially gall aphid clonality, fitness and inter-colony migration are discussed in detail, the author concludes: '*Between-group dispersal in gall-forming aphids is ubiquitous, implying that factors acting ultimately to increase between-clone interactions and decrease within-group relatedness were present in aphids prior to the origins of sociality.*' As for the evolution of soldier morphs *per se*, he further states: '*The origins of soldiers in aphids therefore may be closely tied to their role in fostering clonal growth and expansion, by virtue of the positive effect they have on the longevity of the reproductive gall foundress.*'

Clearly, our knowledge at the present time is far from complete and more research is required to throw further light on this intriguing topic in relation to the trade-offs of individual and group fitness and benefits à la the realities – or not – of kin/group selection along with the evolution of sociality (Wynne-Edwards, 1962; Gould, 1980b; Alonso & Schuck-Paim, 2002; Foster *et al.*, 2006; Okasha, 2015), including soldier morphs and defensive altruism in the case of gall aphids (reviewed by Abbot & Chapman, 2017).

LOCAL INTERPLANT DISTRIBUTION IN RELATION TO HOST PLANT CHEMOTYPE

Only relatively recently has the level of fine-grained ecological complexity begun to be appreciated in different species of aphids. This complexity has been greatly enhanced by the employment of high-resolution molecular markers, especially DNA markers (*cf.* Simon *et al.*, 2002; Loxdale & Lushai, 2007; Loxdale *et al.*, 2017 & Loxdale & Balog, 2018 for overviews). For example, Lushai, Markovitch & Loxdale (2002) demonstrated, using RAPD markers, that *S. avenae* asexual winged morphs migrating into the crop in spring (here a Latin square arrangement of four host plants, wheat, barley, Yorkshire fog, *Holcus lanatus* L. and cocksfoot grass, *Dactylis glomerata* L.) showed clear preferences at the level of host species. More recent research using a monophagous, ant-attended species of aphid, *Metopeurum fuscoviride* feeding on tansy, *Tanacetum vulgare* L., has revealed a preference for chemotypes of the same host. Tansy plants contain a range of terpene/terpenoid antifeedants, especially Camphor, Borneol and β -Thujone. Using a range of polymorphic microsatellite markers, evidence was produced that different *M. fuscoviride* genotypes show a preference for different tansy plant chemotypes (Benedek *et al.*, 2015; *cf.* also Zytynska *et al.*, 2019). This association also has effects on the ‘context-dependency’ in nature and strength of predator attacks on the aphids, despite the presence of guard ants. Thus Borneol had significant positive effects on *M. fuscoviride* in relation to spider density and significant negative effects on ladybird beetles density (Benedek *et al.*, 2015). But this may not be the complete story, and only further research can reveal this.

It is known from direct observation that the three main species attacking tansy, namely *M. tanacetaria*, *M. fuscoviride* and the aposomatically coloured crimson tansy aphid, *Uroleucon tanacetii* (L.), also monophagous (Blackman, 2010), show different feeding preferences on the host plant, *M. tanacetaria* in the flower heads, *M. fuscoviride* on the stems and *U. tanacetii* on the lower leaves (Loxdale *et al.*, 2011b). Recent analysis, including chemical analysis of the phloem of an individual tansy plant, has revealed different trichome densities at different parts of the plant as well as different terpene/terpenoid profiles (Jakobs & Müller, 2019). This, the authors claim, is largely responsible for the preferred feeding sites of two of the monophagous tansy aphids, *M. tanacetaria* and *U. tanacetii*. Thus the anatomical and chemical cues lead to differential niche partitioning and resource allocation by the two species, and thereby prevents competitive exclusion, assuming that is, the aphids co-exist simultaneously on the same plant, something that may not occur, certainly in the case of *M. fuscoviride*, since the guard ants (e.g. black garden ant, *Lasius niger* L.) tend to kill the other aphid species present, especially *M. tanacetaria*, which is not aposomatically coloured, but rather, pale green (Blackman, 2010), or occasionally light brown (B. Massonnet, pers comm.).

What the results do show is that concepts of some generalism are very far from the truth. Rather, what is observed is *finer and finer-grained levels of ecological sub-division and complexity*, until, ultimately, even specialist feeding sites are recorded within an individual plant, surely the last word in ecological specialism.

DISCUSSION

From what has been said it is clear that aphids show mutational changes, often large scale and rapid, including duplications and deletions, including in the mtDNA (IAGC, 2010). Hence clones *sensu stricto* cannot and do not exist, whilst these lineages are probably not immortal, but rather are under complex abiotic and abiotic selective pressures, especially insecticide resistant strains. If so, this means that their transgenerational ‘lifespan’ is probably measurable in years at most rather than longer time scales. Furthermore, asexual lineages, because of the various ecological constraints that impinge on them, are not widespread in terms of the concept of an ‘Evolutionary Individual, EI’, as originally proposed by Dan Janzen (1977).

Furthermore, whilst aphids do show widespread host adaptation, this is likely to be limited because of the constraints imposed by one or more co-evolved plant antifeedants, such that species tend to switch to closely related plants, usually within the same plant family (Loxdale, Balog & Harvey, 2019). Interestingly, the large number of multilocus genotypes produced at the beginning of the plant-growing season, as seen in several aphid species using polymorphic microsatellite markers, soon decline in number, to leave a few dominant ones, probably the effect of the combined influences of abiotic and biotic selecting factors (Loxdale, Kigathi & Weisser, 2009, Loxdale, Massonnet & Weisser, 2010). Aphids certainly do adapt to the onslaughts by braconid wasps, encapsulating the eggs or young larvae, seemingly aided by one or more secondary or facultative species of bacterial endosymbiont (Vilcinskas, 2016b).

As for kin selection, there seems to be limited evidence for this in aphids, although the defence by specialised soldier morphs in gall aphids may be accepted in this light. Even so, what benefit the soldiers gets out of such a scenario in terms of genetic input into the colony, the individual ultimately being the unit of selection (Gould, 1980b), is debatable. Is this altruism? Perhaps, but then again, to our minds, it falls within the sphere of the much-criticised concept of group selection. Beyond that, only more empirical data can throw light on the topic in relation to aphids, one way or another.

Aphids appear to show finer and finer-grained specialisation in relation to their plant hosts. This is not only in terms of the range of plant species infested, but within species, seemingly even with an individual plant, so that site-specific ecological preference is apparent, certainly in the case of tansy-feeding aphids (Jakobs & Müller, 2019; Loxdale, Balog & Harvey, 2019). Lastly, pea aphids also reveal a context dependency in relation to arthropod predators – spiders and ladybird beetles – a trait which potentially has selective advantage as they invade enemy free space to avoid them. As such, they gain positive fitness benefits in terms of reproductive success, but often at the expense of reduced fecundity on a less than ideal host (Via, 1999; *cf.* also McLean, Ferrari & Godfray, 2009) so that trade-offs must exist in such a strategy.

CONCLUDING REMARKS

In conclusion, in this overview we describe, using a number of examples, how and why aphids have proven very useful as exemplars of a range of phenomena of special interest to molecular ecologists, and indeed, biologists in general. We are sure that this is not the end of the story in this respect. Indeed, we expect that using high definition molecular markers, more especially sequencing, other fascinating phenomena will be described in the near future, perhaps wholly unexpected, as some of the aforementioned phenomena have been in their own right. We live in a golden age of discovery in this respect, simply because the modern

technology allows us to probe and investigate areas and topics in aphidology undreamt of by earlier generations of aphid researchers, e.g. the genomics data on members of the Aphididae tested to date, as well as in relation to new molecular findings concerning closely related Grape Phylloxera (Hemiptera: Phylloxeridae) (Tello & Forneck, 2019)

In the pea aphid, *A. pisum*, the IAGC syndicate's findings, following the first sequencing of the entire genome of an aphid, highlighted genes associated with basic metabolism, mitochondrial DNA, telomeres, TEs, duplications, deletions, and influences of the genome in relation to endosymbiotic bacteria. In relation to the last aspect, the IAGC (2010) states '*Our findings indicate that overall aphids have acquired few functional genes via lateral gene transfer from bacteria. However, these few genes may be critical in the maintenance of the symbioses exhibited by aphids.*' The report also suggests that '*This analysis highlighted several noteworthy features of pea aphid metabolism. First, the genetic capacities of pea aphids and of Buchnera [the primary symbiont of aphids] for amino acid biosynthesis are broadly complementary, an effect that can be attributed principally to gene loss from Buchnera. This complementarity results in several apparent instances of metabolic pathways shared between the pea aphid and Buchnera.*' Hence, over the long course of symbiotic mutualism, aphids (here the pea aphid) have become intimately associated with their symbionts, to the extent that their metabolisms are inexorably intertwined and neither partner is any longer a free agent as such, the symbiont usually having a reduced genome in terms of functionally expressed metabolic genes (Shigenobu *et al.*, 2000; Moran & Plague, 2004; Moran, 2007). Ditto the aphid, which lacks the ability to synthesise certain essential amino acids (Shigenobu *et al.*, 2000; Akman Gündüz & Douglas, 2009). Furthermore, uniquely amongst animals, the red colour pigments of aphids such as the pea aphid, which shows a red-green colour polymorphism, is seemingly due to the horizontal gene transfer of the enzyme gene (carotenoid desaturase) necessary for the biosynthetic production of the red pigments torulene and dehydro- γ , ψ -carotene (lacking in the green form of the aphid) from a fungus at some time in the aphid's ancient ancestry (Moran & Jarvik, 2010). The colour polymorphism of the pea aphid has proportionately different selective advantages in terms of surviving attacks by arthropod predators such as ladybird beetles (Coleoptera: Coccinellidae) and parasitoid wasps (Hymenoptera: Braconidae) (Losey *et al.*, 1997; Bálint *et al.*, 2018). In light of these various factors, aphids are clearly amazingly specialised animals, even at the metabolic level, being, as they are, dependent upon all kinds of genomic plasticity for their survival and success.

ACKNOWLEDGEMENTS

We thank the two anonymous reviewers for their wise and insightful comments which have helped us improve the manuscript of this paper, and Drs. Mohsen Mehrparvar and Bob Dransfield & Bob Brightwell for kindly allowing us to use their excellent colour photos of aphids.

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Figures 1-6

Fig. 1. Peach-potato aphid, *Myzus persicae*, a cosmopolitan species displaying a green-pink colour polymorphism and widely reported as being highly polyphagous. The species has a range of lifecycle strategies, including host alternation involving alternation of generations, obligate asexuality as well as androcyclic lineages that predominantly produce males. Adult body length = 1.2-2.1 mm.; photo (not to scale) showing adult asexual pink form of the aphid with nymphs plus larvae of one of their antagonists, the aphid midge *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) (© R.D. Dransfield & R. Brightwell, influentialpoints.com; see their website and Blackman & Eastop (2000) for further details of life history, including distribution).

Figs. 2a & b. Tansy aphids, (a) *Macrosiphoniella tanacetaria* and (b) the ant-attended *Metopeurum fuscoviride* on their host, *Tanacetum vulgare* L. Both species display a green-brown colour polymorphism and an alternation of generations between asexual spring and summer forms and the production of sexual forms in the autumn, the sexual females (oviparae) laying cold hardy overwintering eggs. Adult body length = 3.2-4.1 and 2.2-2.9 mm., respectively; photos (not to scale) showing apterous (wingless) asexual adult females (virginoparae) with nymphs (© M. Mehrparvar; see Blackman (2010) for further details of life history, including distribution).

Fig. 3. Pea aphid *Acyrtosiphon pisum*, a cosmopolitan species, a major pest of members of the Leguminosae (Fabaceae). Displays a green-pink colour polymorphism related to host preference, and reproduces seemingly predominantly sexually. According to Blackman (2010), males are usually wingless in the U.K., but winged in other parts of its range. The species is known to have host-related races or **biotypes/subspp.**, so is actually a species-complex. Wingless males on some of its hosts may have initially triggered and indeed reinforced sympatric speciation. Adult body length = 2.5-4.3 mm.; photo (not to scale) shows green apterous virginopara (© R.D. Dransfield & R. Brightwell, influentialpoints.com; see their website and Blackman & Eastop (2000) for further details of life history, including distribution).

Fig. 4. Bird-cherry-oat aphid, *Rhopalosiphum padi*. The species host alternates grasses and cereals and a primary woody host, principally bird cherry, *Prunus padus*, on which the sexual forms mate and oviparae lay cold-hardy overwinter eggs, although facultative asexual forms are also known, depending on climate. Adult body length = 1.2-2.4 mm.; photo (not to scale) showing apterous virginoparae with nymphs (© R.D. Dransfield & R. Brightwell, influentialpoints.com; see their website and Blackman & Eastop (2000) for further details of life history, including distribution).

Fig. 5. Grain aphid, *Sitobion avenae*, another cosmopolitan species, oligophagous on grasses and cereals, and indeed a major pest of the latter. It displays a range of colour forms from apple green to dark green and salmon pink to dark brown. Whilst also showing a range of reproductive modes, it is predominantly asexual in temperate climates, but produces sexual forms leading to the laying of overwintering eggs when induced below the critical thresholds of temperature and light (< 15°C; < 16 hrs light). Adult body length = 1.3-3.3 mm.; photo (not to scale) shows alate (winged) asexual migrant with nymphs (© R.D. Dransfield & R. Brightwell, influentialpoints.com; see their website and Blackman & Eastop (2000) for further details of life history, including distribution).

Figs. 6a, & b. Poplar-lettuce gall aphid, *Pemphigus bursarius*, a host alternating species displaying alternation of generations, migrating between a spring/summer host (members of the daisy family (Family Asteraceae), where they feed on the roots, and can be a pest, especially of lettuce), and a primary woody host, poplar, e.g. *Populus nigra* L. Adult body length = ~ 1.5–2.4 mm.; photo (not to scale) shows (a) gall and (b) alate migrant on *Populus nigra* (© R.D. Dransfield & R. Brightwell, influentialpoints.com; see their website for further details of life history, including distribution).



Figure 1.



Figures 2a, 2b.



Figure 3



Figure 4



Figure 5



Figure 6a, 6b