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The potential role of chromosome telomere resetting consequent upon sex in the population dynamics of aphids: an hypothesis

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Models of population structure have emphasized the importance of sex in maintaining lineages. This is because, despite the well known 'two-fold cost of sex' compared with asex, it is considered that recombination rids the genome of accumulated mutations and increases its potential for adaptive variation. However, asexual lineages of eukaryotic organisms can also rapidly gain genetic variance directly by various mutational processes, thereby proving that so-called 'clones' do not have strict genetic fidelity (Lushai & Loxdale, 2002; Loxdale & Lushai, 2003a), whereas the variation so produced may well have adaptive advantage during the evolutionary process. This being so, obligated asexuals or cyclical parthenogens that occasionally indulge in sexual recombination ('rare sex') cannot be deemed as 'evolutionary dead-ends'(Lushai, Loxdale & Allen, 2003a). In addition, the persistence of asexual lineages (i.e. lineage longevity) may also involve the integrity of the telomere region, the physical end of the chromosomes (Loxdale & Lushai, 2003b). In this earlier study on this topic, we argued that the persistence and ultimate senescence of eukaryotic cell lineages (based upon the frequency of 'capped' and 'uncapped' chromosomes related to telomere functionality; Blackburn, 2000) may directly relate to the survival and persistence of lineages of whole asexual organisms. Aphids are a good model system to test this hypothesis because they show a variety of sexual/asexual reproductive strategies, whereas their mode of asexual reproduction is of the mitotic (= apomictic) type. We also suggested that many aphid lineages require occasional or even rare sexual recombination to re-set telomere length to allow lineages to persist. Ample empirical evidence from diverse taxa, lineages, and different developmental stages now reveals that the telomere states are indeed re-set by recombination (homologous or meiotic), thereby rejuvenating the lineage in question. The generational clock element of telomeric functionality has also been successfully described in artificially-induced mammalian clonal systems. It thus appears that telomere function is a central molecular mechanism instigating and promoting lineage continuity *per se*. By contrast, we hypothesized that other long-lived asexuals, or the rare category of ancient asexuals such as bdelloid rotifers, have compensatory mechanisms for maintaining chromosome functional integrity, which are somewhat different from conventional telomeric repeats. In the present study, we carry the analogy between eukaryotic cell functionality and aphid lineages a stage further. Here, we hypothesize that the changing frequency of capped and uncapped telomeres, progressing to senescence in a stochastic manner, may be an underlying factor that significantly contributes to population dynamics in asexual lineage evolution. *Biological Journal of the Linnean Society*, 2007, **90**, 719–728.

ADDITIONAL KEYWORDS: aphid – asexual – evolution – eukaryote clone – mutational load – Red Queen hypothesis – sexual – telomeres.

'An unverified hypothesis is of little or no value; but if any one should hereafter be led to make observations by which some such hypothesis could be established, I shall have done good service, as an astonishing number of isolated facts can be thus connected together and rendered intelligible' (Charles Darwin, 1876)

INTRODUCTION

At the recent joint meeting of the European Science Foundation (ESF) and Linnean Society entitled 'The Paradox of Asexuality: An Evaluation', 22–24 Septem-

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ber, 2005 (Loxdale, van Dijk & Ricci, 2005; PARTNER IV), the question was again raised as to 'why sex?' or, more appropriately in this case, 'why not sex?' During the course of the meeting, it was often posited why it is that sex is lost from some taxa, sometimes and apparently completely (e.g. the bdelloid rotifers and darwinulid ostracods; Mark-Welch & Meselson, 2000; Martens, Rossetti & Horne, 2003), but often maintained in many taxa that show cyclical parthenogenesis to a greater or lesser degree (e.g. aphids and nematodes; Loxdale & Lushai, 2003a, b; Simon *et al*., 2003). It appears that very few taxa are in fact totally obligate asexuals, with the majority retaining some degree of sexual recombination, even if only periodic (e.g. aphids with their annual sexual phases found in many species; Loxdale & Lushai, 2007). However, other organisms, including some other aphid species (e.g. the shallot aphid, *Myzus ascalonicus* Doncaster and aphids of the genus *Trama* [e.g. *Trama troglodytes* (von Heyden), in which males have never been found; Blackman, Spence & Normark, 2000; Blackman & Eastop, 2000] appear to indulge in sex much more rarely, if ever, like the bdelloids. The absence of males in such apparently obligate asexual taxa could be a sampling effect (i.e. not enough specimens are gathered from enough places to ever completely prove the absence of rare functional males and, hence, the possibility of sporadic recombination), whereas it is possible that some such 'obligate' asexual aphids such as *Trama* spp. do in fact produce males very rarely, and that these may be functional (Normark, 1999). Some aphids species [e.g. the grain aphid *Sitobion avenae* (F.)] have complex life cycles. This species displays a range of life-cycle forms, including anholocyclic (i.e. obligate asexual females), holocyclic (i.e. facultative asexual females with an annual sexual phase triggered by abiotic factors: night length and temperature), androcylic (i.e. asexual females that produce males), and 'intermediate' forms (i.e. asexual females that produce a few males and sexual females) and, at the same time, showing the phenomenon of 'sexual leakage'. Here, occasional sex occurs between androcyclically-produced males and functional sexual females from other life-cycle lineages, so that gene flow persists between these various lineages [Simon, Rispe & Sunnucks, 2002; in the case of the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), see also Delmotte *et al*., 2001]. With *R. padi*, such cross-lineage mating can lead to hybridization events between species and may be the cause of lineages or even species (Fenton, Malloch & Germa, 1998; Delmotte *et al*., 2003).

Sex appears to be important to many taxa, including those that only mate but rarely. Only a few groups, such as the darwinulid ostracods and bdelloids, have given up sex in favour of total celibacy and, interestingly, evidence is accumulating that bdelloids, which comprise some 380 species worldwide, have successfully adaptively radiated to fill a diverse range of ecological niches (Birky *et al*., 2005; Fontaneto, Melone & Ricci, 2005). With organisms that display sexual and asexual life-cycle forms, evidence has been sought for many years to show that there is a geographical basis to the demographic distributions of life-cycle forms related to abiotic factors. In aphids, such distributions have been shown in relation to climate, often latitudinally based, with holocyclic forms (i.e. that produce cold hardy overwintering eggs) mainly confined to regions with cold winters, whereas obligate asexuals occur in regions with milder winters [e.g. *Sitobion avenae*; Simon *et al*., 1999; Llewellyn *et al*., 2003; peach-potato aphid, *Myzus persicae* (Sulzer); Guillemaud, Mieuzet & Simon, 2003]. Such adaptive geographical distributions of sexual-asexual lifecycle forms have also been found in other cyclically parthenogenetic animals [e.g. nonmarine cypridoid ostracods, *Eucypris virens* (Jurine); Horne, Baltanas & Paris, 1998].

Organisms that are hermaphroditic and that often show levels of polyploidy in relation to sexual/asexual life-cycle forms, such as the planarian flatworm, *Dugesia* (*Schmidtea*) *polychroa* (Schmidt), appear to display habitat associations/demographic distributions related to both ploidy levels as well as abiotic factors (for further details, see D'Souza *et al*., 2004). Similarly, some species of freshwater snail show this type of trend, affecting the spatial distribution of the animals in lakes (i.e. depth) in relation to life-cycle form (sexual or clonal), which is in turn related to biotic factors; here, infection by parasites (Jokela *et al*., 2003). What was very apparent from the ESF–Linnean meeting is that the entire topic as to 'why sex' or 'why not sex' is still far from resolved and, as such, the debate continues on apace and with vigour.

Classically, sex is thought to have evolved long ago for the exchange of genes and increase of genetic variation within natural populations, as is evident in the conjugation performed in some strains of free-living bacteria (Kohiyama *et al*., 2003). It is also postulated by some that it perhaps evolved as a necessary means of expelling the effects of invading transposons in early recombinant molecules, where they may have behaved as 'infecting moieties' (Hickey, 1982; Arkhipova & Meselson, 2000; Schön & Martens, 2000). Nowadays, recombination may serve this function, at the same time as shedding the genome of deleterious genes or other DNA regions (Arkhipova, 2005). By so doing, the accumulation of such deleterious material within asexual lineages (the so-called Muller's ratchet; Muller, 1964) is slowed down or even prevented, and thereby lineages do not become extinct quickly by a process of 'mutational meltdown', as proposed by Lynch and coworkers and experimentally supported in clonal lineages of Cladocerans [*Daphnia*

pulex (Leydig)] after approximately 40+ generations in the wild (Lynch *et al*., 1993, 1998; Lynch & Blanchard, 1998).

Sexual recombination has its costs too. Thus, as well as expelling unfavourable genes and bringing favourable ones together, allowing adaptations to new ecological situations, it can also introduce unfavourable gene combinations. In addition, there is the much vaunted 'two-fold cost' of sex to consider: a sexual organisms has to find a mate with all that this entails (including behaviourally in higher animals), whereas asexuals singly produce offspring in clonal lineages (West, Lively & Read, 1999). In organisms such as aphids, the costs of sex may be much more than two-fold, because of the reduced likelihood of the sexes meeting and mating, a function both of their modes of aerial displacement during the autumnal sexual phase and the necessity of finding suitable host plants on which to land, perhaps a widely-dispersed and uncommon primary woody host (Loxdale *et al*., 1993; Ward *et al*., 1998). The various arguments and debates about sex have been considered at length over the years and have led to ideas of pluralism, which includes the two main hypotheses: positive benefits arise from (1) acceleration of adaptation to changing environments due to increased variance [the result of novel gene combinations (i.e. environmental models; Bell, 1982)] and (2) an enhanced ability to rid the genome of mildly deleterious mutations preventing meltdown [mutation-based models (i.e. the mutational deterministic hypothesis of Kondrashov, 1988; for further details, see West *et al*., 1999)].

Another often overlooked aspect of the evolutionary sex–asex controversy is the involvement in re-setting the chromosome telomere lengths in organisms that have these structures. The telomeres are nucleotide repeats (commonly TTAGGG in vertebrates, TTAGG in insects, and TTTAGGG in plants) in association with various proteins that occur at the end of the functional chromosomes and protect these during recombination events (McEachern, Krauskopf & Blackburn, 2000; Sykorova *et al*., 2003; Chan & Blackburn, 2004). As an individual ages (i.e. at each cell division), the telomeres become shorter in a stochastic manner (or so it is hypothesized; Blackburn, 2000) such that particular cell lineages senesce and eventually die and, when enough lines have died out, so does the whole organism. A new born organism's telomere repeats may comprise $(TTAGGG)_N$ repeats, where N may be many thousands, but these can decline in a long lifetime (as in humans) to only several hundred repeats (Jones, 1996). The telomeres are known to be associated with several bodily malfunctions (besides ageing *s.s*.), including cancers, whereas they have been shown to be re-set sometime early in embryogenesis in vertebrate embryos (see below). One of the mechanisms involved at the genome level may include homologous recombination, which is argued to be broadly analogous to normal meiotic recombination (Shibata, 2001).

Loxdale & Lushai (2003b) postulated that the telomere may be re-set by specialist mechanisms of which recombination was a very important and possibly key factor in the persistence of sexual recombination in many taxa. We came to this conclusion after consideration of the bizarre life cycles of aphids, dominated by abiotic factors of temperature and photoperiod, where there are often have 14 or so asexual generations in-between a single annual sexual phase in which the winged migrants return from the summer herbaceous host and seek out a primary woody host on which mating occurs, and overwintering eggs are laid. The life cycle is complete when an asexual 'stem mother' hatches from the egg in the spring and produces a new asexual lineage (Dixon, 1998).

In the present study, we revise our original speculations of the action of telomere re-setting in sexual recombination, with special reference to aphids as a model. Of the many orders of insects studied to date, aphids, which belong to the Homoptera, a suborder of the Order Hemiptera (Imms, 1970), have telomeric repeats at the ends of their chromosomes; others, including the related Heteroptera (also a suborder of the Hemiptera), do not (Spence *et al*., 1998; Frydrychova *et al*., 2004). Diptera also do not have telomeres and use instead retrotransposons to maintain chromosome length and integrity (Biessmann, Walter & Mason, 1997). This division into organisms with telomeres and those without may seem at first to preclude the notion that telomere length re-setting is indeed important in sexual recombination (for a discussion of the distribution of the telomeric repeat in various organisms, see Frydrykhova *et al*., 2004). On the other hand, certainly for aphids, it appears to account for many aspects of their strange biology, including the normal periodic sexual phase in holocyclic species, and the persistence of rare sex in this group of animals (Loxdale & Lushai, 2003b).

Interestingly, the individual aphid, which can have as many as 30–90 asexual progeny within asexual lineages, eventually senesces and dies. But what then happens to the lineage itself in terms of telomere length? In our earlier study on this topic, we argued that a variety of specialist mechanisms, including recombination, maintain telomeric integrity between generations in a lineage based on an aphid model (Loxdale & Lushai, 2003b).

If the decay of telomere function can be correlated with lineage persistence over historical time, as we claimed using this model, then, from another shorter-term perspective, the same suite of telomere-related mechanisms could have very interesting effects on population dynamics. Here, we discuss the potential of

the model to present a new role (which, for clarity, we term 'Hypothesis II') for sexual recombination in conjunction with the original telomere re-setting hypothesis ('Hypothesis I'; Loxdale & Lushai, 2003b). We highlight its relevance to the population trends in asexual lineages: their maintenance, evolution, population surges, and lineage proliferation. This underlying mechanism is affected by interacting mutational and selective processes.

HYPOTHESIS II

In the example of asexual aphid species presented in our earlier study (Loxdale & Lushai, 2003b), we correlated asexual lineage propagation with that of eukaryotic cell lineages. Such cells normally divide up to 15–60 times *in vitro* leading to senescence and death (Hayflick, 1965; Rawes *et al*., 1997). We thereafter equated such cellular division with finite generations before asexual lineages decline in functionality and die out. These short spans of asexual lineage persistence are presently explained by various mutation (Kondrashov, 1993) and density-dependent (Hamilton, Axelrod & Tanese, 1990) models, respectively. Furthermore, cancer cells such as He La, which are uncontrolled in terms of proliferation and are not limited by a set number of replications as far as is known, seemingly mirror the state indicated by ancient and potentially 'immortal' asexuals (Normark, Judson & Moran, 2003).

Previously, we (Loxdale & Lushai, 2003b) applied the cellular senescence model of Blackburn (2000). In mammalian and yeast cells, lineage longevity is determined primarily by a series of complex interactions involved with the function of telomeres and the associated reverse transcriptase enzyme, telomerase (McEachern *et al*., 2000; Chan & Blackburn, 2004). We correlated this with longevity of asexual aphid lineages. Blackburn's cellular model describes the stochastic processes involved in switching between 'capped' and 'uncapped' chromosomes occurring in populations of cells of differing age (Blackburn, 2000: figs 1, 2). In general, capping is necessary for preserving the physical integrity of the chromosome end, whereas regulated uncapping is a property of dividing cells. During early cell division, most cells have relatively long telomeres and are capped and therefore undergo fast proliferation (active cell cycling). As cells age towards senescence, the ratio of capped to uncapped telomeres within the cell population switches over and they tend to have shorter telomeres which become, in terms of population frequency, progressively more uncapped before concomitant senescence and death (Blackburn, 2000). Interestingly, evidence from studies from diverse taxa, including yeasts (Lundblad, 2002), Tetrahymena (Ciliate protozoan; Kirk *et al*., 1997) and birds [tree swallows, *Tachycineta bicolor* (Vieillot); Haussmann, Winkler & Vleck, 2005], reveals how instrumental functional telomeres are in the proliferation and persistence of eukaryotic individuals and lineages.

More importantly for our original hypothesis (I), this empirical evidence also indicated that the telomere states are re-set by recombination, thereby rejuvenating the lineage in question. Such an analogy was also independently inferred a decade ago by Jones (1996). However, our hypothesis is now supported by empirical evidence from artificially cloned cattle, which show that telomeres are re-set when a new individual is formed (Lanza *et al*., 2000), and by studies of early bovine developmental stages (i.e. between morula and blastocyst), which detail a similar re-setting event (Schaetzlein *et al*., 2004). Additionally, cloned mice and sheep with shortened or dysfunctional telomeres tend to age prematurely, develop cancers and die early (Sedivy, 1998; de Lange & Jacks, 1999; Shiels *et al*., 1999; Briggs, 2003; see also Pearson, 2003), tree swallows with short telomeres have reduced survival relative to birds of the same age with longer telomeres (Haussmann *et al*., 2005; for a review of the empirical evidence linking telomere dynamics with whole organism lifestyle and lifespan, see Monaghan & Haussmann, 2006), whereas the telomerase/telomeric age-related phenomenon is also passed down both generational somatic and germ lines, as seen in the case of strains of so-called 'knockout' mice lacking functional telomerase activity (Rudolph *et al*., 1999). It thus appears that telomere function is central to the general molecular causal event instigating and promoting lineage continuity, as previously proposed (Loxdale & Lushai, 2003b).

Here, we go further. We propose that sexual recombination not only re-sets telomere functionality, but also reinstates an asexual lineage's functionality, proliferation, and ability to effect surges or selective sweeps in an ecological landscape. The conceptual leap involves equating a cellular physiological state of a cell line with the functionality of eukaryotic lineages. The individual lineage event amplifies and links to the population dynamics over many generations. In other words, lineages persist or decline because of an essential factor other than that of mutational load and density-dependent selective mechanisms.

A schematic of the extended hypothesis (II) is described in Figure 1A. This shows trends in capped telomere frequency (i.e. cell population norms within assayed individuals; Lanza *et al*., 2000; Frydrychova & Marec, 2002; for details of telomere length assay techniques, see Frydrychova *et al*., 2004) that we would expect for different experimental lineages. Therefore, the schematic best-fit curves describe (1) ancient asexuals (Fig. 1Ai) and sexuals maintained over gen-

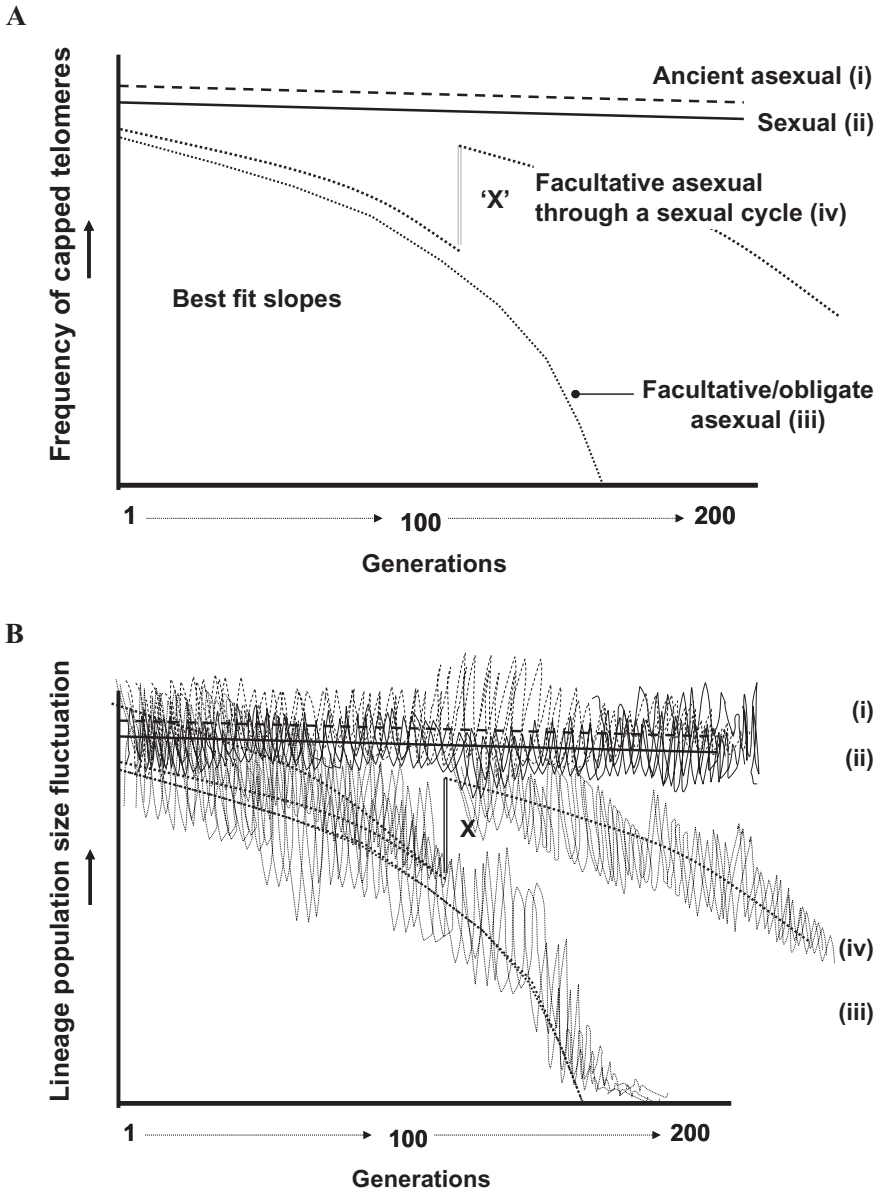

Figure 1. A, hypothetical best-fit slopes showing frequency of capped telomeres (cell population norms) measured using a molecular essay on three classes of lineage: (i) ancient asexuals; (ii) sexuals; and (iii) facultative/obligate asexuals. Measurements describe essays over generations (1–200 or as necessary). This schematic only aims to show the relative abundance of capped telomeres between the lineages, and does not suggest that ancient asexuals will necessarily have a greater capped telomere frequency than sexual lineages. A schematic of the expected curves for telomere frequency in (iii) a maturing obligate asexual lineage 'control' and (iv) a replicate facultative asexual lineage induced to produce sexuals at point 'X', mated and resulting in a new lineage after sexual egg hatch. This test will indicate if recombination can reset telomere frequency to levels within the population similar to those seen prior to the asexual decline with time. B, best-fit-slopes, schematically representing the population size fluctuation in a selective and mutative 'neutral' environment for independent ancient asexual, sexual, facultative, and obligate asexual lineages (i–iv). It is most important to note that the population sizes of both facultative and obligate asexual lineages will begin to diminish in frequency over time in correlation with a function of their telomeric activity, as seen in Figure 1A (the two slopes being superimposed in theory). Hence, when telomere function is maintained, population size will remain high. In ancient asexuals and sexuals, it is considered that, respectively, a compensatory mechanism and sexual recombination will be the causal factors maintaining population size. In the facultative asexual, we indicate that a re-proliferation of population, or what is routinely considered a 'selective sweep', is effected by a recombination event, 'X'. The asexual lineage is the 'control' if recombination does not occur. The fuzzy line around the best fit slopes shows miscellaneous fluctuations around a population mean.

erations (e.g. 1–200) (Fig. 1Aii) and (2) obligate asexual females and facultative asexual females (the latter can go through a sexual phase when induced using appropriate abiotic cues; Dixon, 1998) (Fig. 1Aiii).

It is considered that the slope for 'capped telomere frequency' in the ancient asexual lineage (Fig. 1Ai) and continuously sexual lineages (Fig. 1Aii) would be maintained over the time-frame of the hypothetical experiment whereas, for the obligate asexual females and facultative asexual females (Fig. 1Aiii), capped telomere frequency would diminish noticeably over generations 1–200. Even so, in all of the aforementioned life-cycle types, each individual within interspersed generations is born, senesces and eventually dies.

Figure 1Aiv describes a further important scenario testable using facultative asexual aphid lineages, where an ageing facultative asexual lineage with concomitant decline in the frequency of capped telomeres, will be 'rejuvenated' after sexual recombination and this will relate to a restored frequency of capped telomeres, represented by 'X' in Fig. 1A (Loxdale & Lushai, 2003b).

Figure 1B shows schematically the respective individual lineage populations described in Figure 1A through time and space. Here, lineages are shown in a given environment and going through generations with perturbations over time (the fuzzy line represents population size fluctuation over generation times with a best-fit slope going through a population mean).

Notably, these hypothetical trends indicating population fluctuations are shown to be strongly associated with the envisaged capped telomere frequency described in Figure 1A. In a simple neutral environment, the best-fit slopes represent the population trends for independent ancient asexual, sexual, facultative and obligate asexual lineages (Fig. 1Bi–iv). The most important element is that the population levels of both facultative and obligate asexual lineages will begin to diminish in mean population size over time and in correlation with the function of their telomeric activity.

By contrast, where telomere function is maintained (i.e. in ancient asexuals and sexuals), we consider that the respective compensatory mechanism(s), along with recombination which re-sets telomere function, will be the causal factors in maintaining population size, vigour, etc. Alternatively, in facultative asexuals, we suggest that a new proliferation of a population, or what is described as a 'selective sweep', would follow a 'rare' recombination event, as in 'X'. The whole premise of this model is that sex would synchronously rejuvenate the declining telomere function as well as the associated population decline. The obligate asexual lineage would be the 'control' indicating the population trend were recombination not to occur.

DISCUSSION AND CONCLUSIONS

In the present study, we argue a case for eukaryotic asexual lineage evolution that may well be explored using the multiple life-cycle strategies of aphids. If some asexual aphid populations persist for long periods, as indicated in recent studies of cereal aphids (Delmotte *et al*., 2001), a population consisting of billions of asexual females may produce quite a high proportion of males (= androcycly). In the case of *S. avenae*, this was reported to be as high as 41–54% of clones collected from Scotland, East Anglia and Hertfordshire, UK (Helden & Dixon, 2002). This allows males from otherwise asexual lineages to mate with females from other lineages (see below). Alternatively, some *S. avenae* asexual lines may occasionally produce sexual females (= rare sexual female produced in an asexual lineage = intermediates life cycle present as 0–9% of clones in the same three regions; Helden & Dixon, 2002). These can also mate with males from other lines of the same species (for further details of such complex life-cycle types, see Delmotte *et al*., 2001; Simon *et al*., 2002). If rare sex is indeed occurring in supposedly largely asexual populations, are such asexual lineages perhaps only images of genetic integrity (fidelity/longevity)? In reality, they may be frequently rejuvenated lineages resulting from sexual recombination with concomitant selective sweeps of associated re-set 'telomeric genotypes'. This thereby gives the impression of asexual genetic continuity and integrity through time.

Besides telomere re-setting with concomitant maintenance of chromosome end integrity, the fact that aphid chromosomes are holocentric (i.e. they lack a localized centromeric function) may prevent chromosome fragments from being lost during cell divisions should chromosomal break-ups occur (e.g. translocations) and hence may actually facilitate karyotypic changes (Blackman, 1980; Blackman, Spence & Normark, 2000). Furthermore, the maintenance of meiosis, even very occasionally, may be important in maintaining diploidy in eukaryotic genomes (Birky, 1996), and that its loss (as apparently found in some aphids such as *Trama* spp. and *M. ascalonicus*) leads to a decay in the structure of the chromosome (Blackman *et al*., 2000) concerning the loss of rDNA arrays in *Trama* with various genetic consequences (e.g. structural heterozygosity) in association with potential ecological consequences such as host adaptation. The latter is yet unproven empirically in this group of aphids, but there is evidence for its existence in *Sitobion* species (Sunnucks *et al*., 1998).

Linkage between asexual and sexual lineages, often involving rare males, has been suggested in a growing number of species, including aphids of the genus *Trama* (Normark, 1999) and *R. padi* (Delmotte *et al*.,

2001), water fleas, *Daphnia* spp. (Crease, Stanton & Herbert, 1989) and nonmarine ostracods (Schön *et al*., 2000; Simon *et al*., 2003). Such events need not be limited to a single species, but can occasionally occur between closely-related species, in effect, the best available match (cf. Delmotte *et al*., 2003; Lushai *et al*., 2003b). Changes in adaptive niches may also be influenced by interspecies introgression, as suggested between the predominantly asexual *S. avenae* and its holocyclic sister species, the blackberry-grain aphid, *Sitobion fragariae* (Walker) (Sunnucks *et al*., 1997; for ostracods, see Havel, Herbert & Delorme, 1990; Chaplin, Havel & Herbert, 1997). Alternatively, and even more rarely, aphids may have developed, similar to true ancient asexuals (see also below in the case of bdelloids), compensatory mechanisms that maintain telomere function, thereby allowing truly 'immortal' asexual lineages to persist and either evolve specific adaptations (e.g. host) or evolve as special 'general purpose genotypes' (Lynch, 1984; Blackman *et al*., 2000; Van Doninck *et al*., 2002).

A presumption with aphids is that sexual recombination also leads to the production of cold tolerant eggs and this has been argued as a reason for sex in aphids (Blackman, 1980), beyond the conventional proposition for the increase of population genetic variance and elimination of genome-wide deleterious mutations. Nevertheless, aphids such as *S. avenae* and *M. persicae* can become sexual and produce eggs even in regions with mild winters when egg production cannot be a survival necessity (Helden & Dixon, 2002; Guillemaud *et al*., 2003). This has been thought to be an ecological 'bet hedging' strategy (Delmotte *et al*., 2001). In our analogy, however, the sexual egg is the event allowing for lineage continuity and proliferation.

Is there more to this persistence of sex in aphids? The late W. D. Hamilton (1936–2000) described a scenario of diverse, large, migrant asexual populations colonizing extensive ecological landscapes [e.g. Aspen forests (*Populus tremula* L.)], and considered them suitable to remain apace with pathogen interactions (i.e. the Red Queen hypothesis). In later models, such asexual stability could only be maintained if suitable pockets ('islands' of genetically unique individuals, so-called 'pacemakers') were retained that continuously contributed to the required diversity of the system to keep apace of selective processes (the 'Red Queen pacemaker'; Sasaki, Hamilton & Ubeda, 2002). Molecular studies are already revealing large genetically heterogeneous asexual populations with genotypes, some rare, that are apparently maintained in unique (i.e. host adapted) niches (Haack *et al*., 2000; Lushai, Markovitch & Loxdale, 2002). We believe that asexual lineages persist because of rare recombination events (as above), and also to exchange genes with rare genotypes as described by Sasaki *et al*. (2002). Such a scenario produces both increased genetic diversity and genetically-ecologically fit individuals (in terms of biotic: predators, parasitoids, pathogens, and perhaps abiotic factors) with restored telomere function. These subsequently give rise to proliferating, regenerated asexual lineages. Meanwhile, the less fit aphid genotypes with shortened/nonfunctional telomeres decline in frequency within the population as a result of antagonistic selection.

Amongst the best candidates for a total abstinence of sex, even rare sex, and hence our hypothesized method of re-setting telomere function in asexuals, are the free-living bdelloid rotifers and darwinulid ostracods. These ancient asexuals have been celibate for aeons (Mark-Welch & Meselson, 2000; Martens *et al*., 2003). We earlier hypothesized (Loxdale & Lushai, 2003b) that these would have compensatory mechanisms to facilitate the control of their chromosomal ends and empirical evidence has indeed recently shown that bdelloids at least lack conventional telomeres, but compensate with a retroelement thought to be associated with telomeric functioning (Arkhipova & Meselson, 2000, 2005). Contrasting with this group, the protozoan *Giardia lamblia* (= Lamblia intestinalis), one of the earliest branching eukaryotes (Lloyd, Ralphs & Harris, 2002), not only retains sex, but also has reverse transcriptases (retrotransposons) that are only functionally associated with the chromosomal telomeric regions (Arkhipova & Morrison, 2001). Thus, an association between telomeres and their functional integrity, including periodic re-setting, probably during meiosis or certainly at the early stages of development following fusion of the zygotes, is already apparent from the available published studies of a range of taxa (Lanza *et al*., 2000; Lundblad, 2002; Schaetzlein *et al*., 2004). If these trends have been correctly interpreted, then survival and proliferation of re-set clonal lineages, as exemplified by aphid populations and lineages in general, appears to be plausible.

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