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Certainty versus stochasticity: cell replication biases DNA movement from endosymbionts and organelles into nuclei

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ABSTRACT

Background: Endosymbiotic bacteria such as *Wolbachia* spend their entire life histories within other organisms' cells. This close proximity of endosymbiont and host genomes allows for transfers of DNA between them. Such events are observed to be strongly biased, however, with overall DNA migration from cytoplasmic elements to host nuclei.

Question: Are DNA transfers from cytoplasmic to nuclear genomes more likely to be retained than those in the opposite direction based on how mitotic and meiotic cell division disperses nuclear and cytoplasmic DNA to daughter cells?

Mathematical model: Simulations track the survival of individual DNA intergenomic transfers in populations across 100 non-overlapping generations. Reproduction is separately modelled as either asexual in a haploid species or sexual in a diploid species.

Key assumptions: Transfers can either have no effect or increase chances of host reproduction by up to 20%. The distribution of genomes into offspring is stochastic (i.e. a given modified genome is as likely to be transmitted as an unmodified one).

Conclusions: Even when DNA transfers are equally bidirectional, transfers into host nuclei are retained more often than ones into cytoplasmic genomes. Consequently, biased migration has potential consequences for life-history evolution, whereby genes that exchange locations also switch 'sides' for intergenomic conflict. Thus, biased migration of genes is a long-term evolutionary process favouring host interests over that of their endosymbionts and organelles.

Keywords: endosymbiont, horizontal gene transfer, intergenomic conflict, mitochondria, *Wolbachia*.

INTRODUCTION

Obligate endosymbionts are organisms whose entire life history is played out within the cells of other species. Hence the fate of an endosymbiont is inextricably entwined with that of its host. If the host fails to survive or reproduce, the endosymbiont suffers the same

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catastrophic loss in fitness. This connection limits the degree to which any endosymbiont can exploit its host without causing its own extinction. Nevertheless, endosymbionts and hosts can experience a range of conflicting evolutionary interests (Burt and Trivers, 2006). Conflict can arise because host genomes are always transmitted during cell division, while endosymbiont genomes may not be. For example, sperm cells contain replicated host germ line DNA, but endosymbionts are usually absent. Hence males could be valued very differently from the perspective of DNA in nuclei versus DNA in the cytoplasm. One way such a potential conflict could be minimized or eliminated is through horizontal gene transfer (HGT) across genomes. If the same genes are found in both locations, there would be no intergenomic conflict between the two sets of DNA.

Although conflict resolution should equally favour HGT in both directions, the overwhelming majority of reported DNA movement is from endosymbiont to host. Prokaryotic DNA has been found in numerous eukaryotes (reviewed in Dunning Hotopp, 2011), but with only a couple of known instances of the reverse (e.g. Woolfit *et al.*, 2009; Duplouy *et al.*, 2013). This biased migration of genetic material occurs not only in parasitic bacteria such as *Wolbachia* (Saridaki and Bourtzis, 2010), but also in the evolution of mitochondrial and chloroplast organelles. Mitochondria have lost genes to their host's nuclei with little to no migration in the opposite direction (Adams and Palmer, 2003; Brandvain and Wade, 2009). For yeast, Berg and Kurland (2000) estimate approximately one transfer per 10^5 generations from mitochondria to nuclei and less than one transfer per 10^{10} generations in the opposite direction.

Biased migration could result from the greater certainty of vertical transmission for nuclear DNA over cytoplasmic DNA during cell division. Consider a haploid cell with an endosymbiont population. Any DNA transfer into the nuclear chromosome would thereafter be represented in all daughter cells. In contrast, a transfer into a single cytoplasmic element would not be represented in all daughters because cell division tends to distribute cytoplasmic elements randomly (Burt and Trivers, 2006). In a stable population (i.e. only one daughter cell, on average, survives), HGTs into endosymbionts risk being stochastically eliminated in every generation. In diploid species, cytoplasmic HGTs face an added 50% mortality factor as they are almost always only maternally transmitted. For endosymbionts or organelles, any transfer located in a male body has no evolutionary future. This means that even if the likelihood of DNA transfers and fitness benefits are all equal across gene location, there could still be an apparent evolutionarily bias towards DNA moving from cytoplasm to nucleus due to the dynamics of cell division. We estimate by simulation these combined effects of stochasticity in transmission and sexual reproduction in terms of producing biased migration.

MODEL

Simulated populations are 100 'hosts', imagined as either single-celled organisms (the asexual 'haploid' condition) or gamete-producing cells within multicellular organisms (the sexual 'diploid' condition). Each host is infected with 100 endosymbionts with equal fitness effects. The endosymbiont's effect on its host's fitness could be neutral, positive (as in the case of *Buchnera* in aphids), or negative (as in many infections by *Wolbachia*). The model does not differentiate between these alternative evolutionary relationships. Instead, we concentrate on the relative change in host fitness due to genetic lateral transfer. This can be viewed as negative infections becoming less harmful because transferred genes are less effective at manipulating hosts, or that a positive mutualism is proportionally enhanced.

Simulations start with one lateral transfer of genetic material within one host, into either one nuclear chromosome or one genome of one endosymbiont in the within-cell population. Simulations can run up to 100 non-overlapping generations (i.e. the population at $t + 1$ is the offspring of the population at time t), or until the transfer is lost from the population, with no additional transfers occurring during this period. Every combination is replicated 20,000 times.

Transfer events can be fitness-neutral in terms of the endosymbiont's effect on its host's relative likelihood of producing offspring. This is analogous to transferring non-coding DNA, non-functional genes, or genes whose fitness consequences are unaffected by location. Alternatively, transfer events can be fitness-positive, generating a 10–20% greater likelihood of reproducing relative to unmodified individuals. This is analogous to functional genes being better expressed in their new location. Fitness-positive effects are not dosage-dependent: one modified endosymbiont produces equal benefit as do several, and homozygotes and heterozygotes are equally fit in diploids.

In the haploid condition, 100 randomly chosen hosts divide into two cells with one cell randomly selected to survive to the next generation. Haploid chromosomes containing transfers are passed to both daughter cells. For endosymbionts, populations within hosts first replicate. Each extant endosymbiont makes one identical copy. These doubled populations are randomly divided across daughter cells. Therefore, stochastic distribution may result in daughter cells not having the same number of modified endosymbionts as parents. The model assumes that host reproductive success is not influenced by the number of endosymbionts, as seen in a commensal relationship. Clearly, if endosymbionts had positive or negative effects, then number per cell would be an important variable, but endosymbiont load is a separate evolutionary process from lateral transfer effects. We do not, therefore, consider it in this model.

In the diploid condition, 100 hosts are randomly chosen as mothers and randomly mated to another host from the population. One randomly chosen chromosome from each parent is assigned to a single offspring. Thus, offspring could gain modified nuclear chromosomes from either or both parents. For the endosymbiont population, however, offspring receive endosymbionts only from their mother. To simulate meiosis, two cell divisions produce the single, functional egg cell and three evolutionarily dead-end polar bodies. The endosymbiont population replicates as in the haploid case prior to each cell division. Thus, modified endosymbionts can pass into future generations only if they are segregated into eggs.

By chance, some hosts will be selected to produce more than one offspring (particularly if they contain a fitness-positive transfer), and others will not be selected. Therefore, transfers can be lost from populations in several ways: (1) all modified hosts fail to reproduce; (2) with diploidy, modified chromosomes are lost through stochasticity in meiosis; (3) all modified endosymbionts are lost through stochasticity inherent to mitotic or meiotic cell division.

RESULTS

Fitness-positive transfers (10% added benefit) are more likely to be retained across 100 generations than fitness-neutral ones, and transfers to nuclei are more likely to survive than transfers to endosymbionts (Fig. 1). Sexual reproduction increases this asymmetry in survival probabilities of transfers, because unlike nuclear inheritance, any transfer into an

endosymbiont is transmissible only if it occurred in a female. With a fitness increase of 10% (Fig. 1a) or a neutral effect (Fig. 1b), both asexual (haploid) and sexual (diploid) modified chromosomes are considerably more likely than modified endosymbionts to survive. The same qualitative difference across nuclear and cytoplasmic locations remains when the positive effect is doubled to 20%. Modified chromosomes survive in 31.1% and 31.0% cases for asexual and sexual reproduction, respectively. Transfers into endosymbionts survive 6.7% of the time with asexual reproduction and 4.3% with sexual reproduction.

Under positive selection host ploidy has no effect on transfer survival, but under neutral conditions haploid transfers are maintained slightly more often because genetic drift is stronger in the smaller chromosome population.

Transfers are most often lost when rare and susceptible to stochasticity in reproduction (i.e. early in simulations: Fig. 1). If not quickly lost, fitness-positive transfers spread and most individuals in populations are carriers after 100 generations (Fig. 2a: for a 10% positive benefit). Conversely, fitness-neutral transfers must spread through drift, becoming common in relatively few populations (Fig. 2b).

DISCUSSION

Simulations of DNA transfers between endosymbiont and host genomes show that the dynamics of cell replication and division can create a bias for retaining modifications in nuclei over cytoplasmic elements. Horizontal gene transfers (HGTs) into haploid chromosomes of asexually reproducing hosts transmit with certainty into all daughter cells, but a modified cytoplasmic element would be found in only one daughter. The difference is further exaggerated with diploidy and sexual reproduction because both sexes contribute genetic material to their offspring's nuclei, but only mothers pass on endosymbionts. This would create a migration bias of overall movement of DNA flowing from endosymbionts to hosts. This bias is present both when transfers are fitness-enhancing (e.g. functional genes) or without significant fitness consequences (e.g. mobile genetic elements or pseudogenes). The results may even underestimate the bias in migration for positive transfer events by assuming dosage independence for endosymbionts. One modified endosymbiont in a population of 100 may have considerably less effect than one modified nuclear chromosome in a population of one or two.

Gene migration extrapolated over evolutionary time could make endosymbiont 'bodies' redundant and subject to elimination [possibly evidenced in a currently uninfected mosquito and filarial nematodes species, but with *Wolbachia* genes in their genomes (Klasson *et al.*, 2009; McNulty *et al.*, 2010)]. Ioannidis *et al.* (2013) estimate that over 10% of a *Wolbachia* genome has moved into its nematode host's DNA, and that the entire *Wolbachia* genome is potentially transferable. In contrast to the movement of DNA into nuclei, simulated HGTs into endosymbionts had less than a 0.1% chance of being retained unless the transfer increased overall host reproductive fitness.

The mechanics of cell division are an addition to the proposed factors for biases in accumulation. For instance, endosymbionts and organelles can replicate and recycle numerous times before cell division, meaning newly incorporated DNA can be lost due to stochastic processes (Berg and Kurland, 2000), and that pools of 'escaped genes' available for transfer are likely dominated by non-nuclear sources (Adams and Palmer, 2003). Alternatively, bias in DNA distribution could reflect selection after transfer. Endosymbionts and organelles still need to compete for within-cell resources for replication, leading to strong selection to

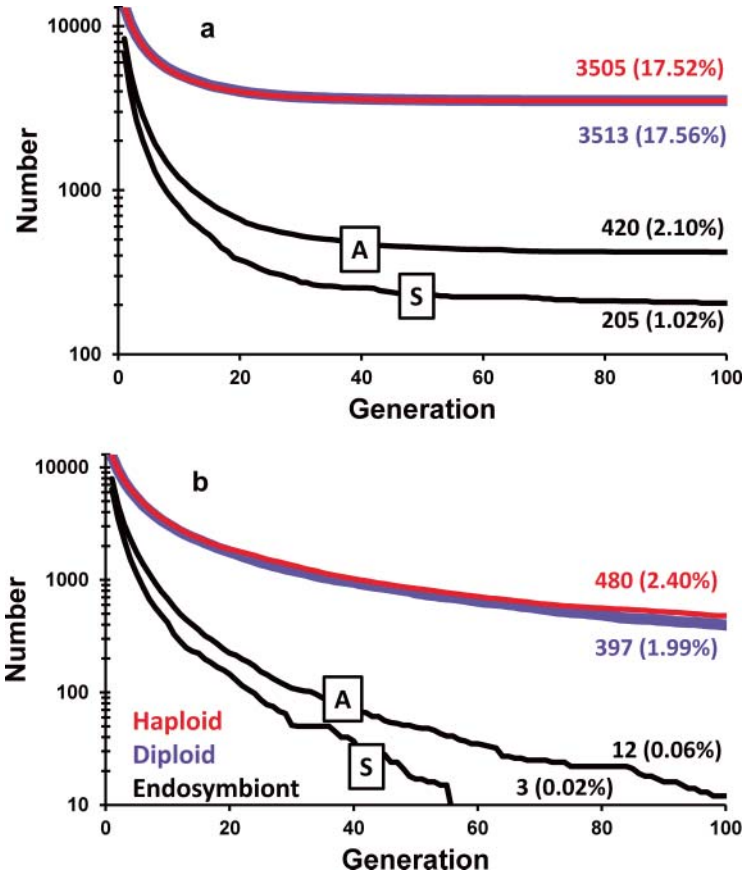

Fig. 1. Number of populations having at least one individual with a modified genome over time. All simulations begin with one transfer event in one individual and numbers denote how many populations (from 20,000) have such individuals after 100 generations. (a) Transfer events increase fitness of affected individuals by 10%. (b) Transfers are selectively neutral. Hosts are diploid, reproducing sexually and meiotically (S) or haploid, reproducing asexually and mitotically (A).

excise non-beneficial transfers to streamline genomes (Kurland, 1992). Also, if mutation rates differ between nuclear and non-nuclear DNA, replicated genes could mutate to non-viable or deleterious versions more often in organelles and endosymbionts (Berg and Kurland, 2000; Brandvain and Wade, 2009). Purifying selection, genetic drift, and replicative efficiency would then favour their loss from cytoplasmic genomes. This is a horizontal ratchet where reacquisition of lost genes is unlikely, and DNA therefore amasses in nuclei (Doolittle, 1998).

Biases created through cell division do additionally predict evolutionary outcomes that specifically correlate to transmission mode effects on HGT survival. First, HGTs are more prevalent in asexual plants than outbred species (Brandvain *et al.*, 2007). Second, the movement of DNA from endosymbionts into hosts accelerates when the endosymbiont switches from a facultative to an obligate life history. This increased movement occurs even though obligate organisms have 4–5 times fewer mobile DNA elements in their genomes (Toft and Andersson,

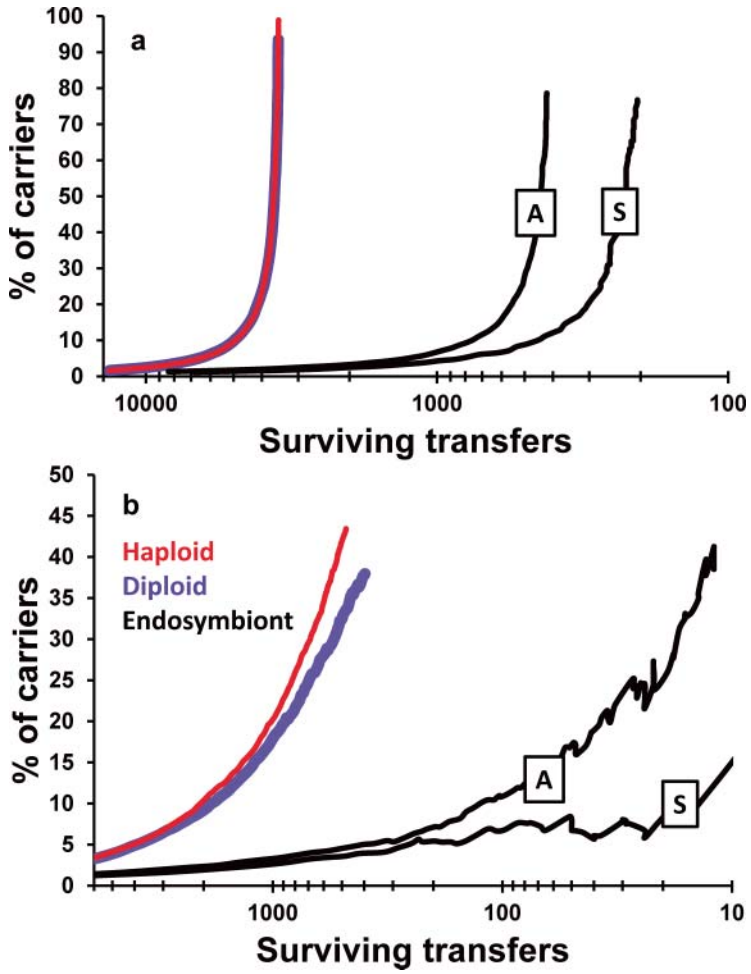

Fig. 2. Mean percentages of individuals having modified genomes in populations where modified genomes are still present. (a) Transfer events increase fitness of affected individuals by 10%. (b) Transfers are selectively neutral. Hosts are diploid, reproducing sexually and meiotically (S) or haploid, reproducing asexually and mitotically (A).

2010). In both cases, a key difference in the compared groups is the increased certainty with which an HGT would be inherited by offspring when in the nuclear genome.

The biased migration model is consistent with the aforementioned examples, but unexplained patterns of gene movement remain. For example, HGTs into hosts of functioning genes from primary or obligate mutualists such as *Buchnera* and *Tremblaya* appear to happen much less often than similar HGTs from secondary or facultative endosymbionts, such as *Wolbachia*, which often negatively affect host reproduction (Nikoh *et al.*, 2010; Husnik *et al.*, 2013; Ioannidis *et al.*, 2013). Perhaps because in mutualistic relationships endosymbionts are often segregated into special cells (e.g. bacteriocytes), this creates both isolation from germ cells and reduced transmission stochasticity (McCutcheon and Moran, 2012).

Although endosymbionts and their hosts have a mutual interest for successful host reproduction, they can be in severe conflict over the details (Burt and Trivers, 2006). If we analogize them as potentially competing 'teams', then gene migration is like trading a player. Consider a *Wolbachia* gene that creates a female-biased sex ratio because, as a maternally inherited element, males are evolutionary dead-ends. If that gene is transferred horizontally, its existing effect would become instantaneously deleterious, because from its new vantage point being in males would be of great selective advantage. Interestingly, such changes in fitness objectives may help explain why many *Wolbachia* HGTs are genes that were or have become non-functional (Ioannidis *et al.*, 2013) and why genome reduction is greater in mutualistic *Wolbachia* strains than in those that manipulate host sex ratios (Toft and Andersson, 2010). Overall, the long-term evolutionary diffusion of DNA from cytoplasmic to nuclear genomes could alter the balance of power in intergenomic conflict and resolve conflicts of interests in favour of the hosts. Many species of ants, for example, are infected with *Wolbachia*, but exhibit no colony-level deleterious effects or sex ratio irregularities (Russell, 2012). Furthermore, in many species of filarial nematodes, *Wolbachia* is an obligate mutualist, such that uninfected nematodes cannot survive (McNulty *et al.*, 2010). The degree to which the evolution of mutualistic and beneficial endosymbiotic relationships is a consequence of genomic reorganization is ripe for further exploration.

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