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Selfish DNA as a method of pest control

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SUMMARY

The inheritance of most genes is tightly controlled, governed by the rules of mendelian inheritance if nuclear or uniparental inheritance if cytoplasmic. A few notable genes and cytoplasmic genomes have escaped this regulation. Such genes may spread by increasing their own rate of transmission despite reducing host fitness and may be regarded as 'selfish'. Their population genetics are described and it appears they may impose a significant genetic load on the host population. Modern molecular techniques may enable similar loads to be imposed on pest species either by transferring selfish genes between species, or by linking deleterious genes to a selfish locus. Alternatively, 'modifier' genes that eliminate the virulent, or disease vectorial capacity, of the pest population may be introduced by linkage to a selfish locus. Selfish elements present in multiple copies may be preferable to single-copy elements as the former are capable of a larger reduction in host fitness. The practical application of these agents depends on five factors: (i) the rate of 'reversion' to a non-selfish form; (ii) the evolution of host repressor systems; (iii) their effect on host fitness, which determines their rate of invasion; (iv) the mechanism regulating host population size in the field; and (v) their ease of manipulation in the laboratory. The first two factors are the most uncertain in most systems, but should be amenable to experimental analysis. It is proposed that the development of such techniques may result in powerful new methods of population control which may be applied to both agricultural pests and disease vectors.

1. INTRODUCTION

Considerable human effort and ingenuity have been invested in attempts to eradicate or reduce the populations of undesirable species, chiefly agricultural pests or disease vectors. These attempts at control have frequently been expensive or ineffective. The most common forms of control are the application of external agents such as insecticides, the use of exotic species as a method of biological control (see, for example, Luck 1990; Newsome 1990), or the mass release of sterilized males. This article describes an alternative form of potential control: genetic control by 'selfish' DNA. There were attempts at this form of control in the mid-1970s and it was shown that cage populations of mosquitos could be invaded by a semi-sterilizing translocation linked to a meiotic drive factor (Curtis *et al.* 1976), and cage populations of *Drosophila* eliminated by sex-linked meiotic drive genes (Lyttle 1977). Two subsequent developments have increased the potential power of this methodology. Firstly, because several additional non-mendelian systems have been identified. Secondly, advances in molecular biology enable selfish DNA to be studied in more detail, allowing its manipulation in the laboratory, and in particular enable selfish DNA to be transferred between species.

A large body of theoretical population genetics has been developed based on Mendel's first law; that in a diploid sexual individual each allele at a locus has a probability 0.5 of being inherited by any of its offspring. Many genes have been identified which do not obey this law and are commonly known as 'non-Mendelian'; the best known examples are transposable elements and meiotic drive loci. In principle, an allele can invade a sexual population provided:

$$(w'/\bar{w})r > 0.5 \qquad (1)$$

where w' is the fitness of an individual containing the allele (assumed to be heterozygous in the early stages of invasion), $\bar{w}$ is the mean fitness of the population ($\bar{w} \simeq 1.0$ in the initial stages of invasion) and r is the frequency with which it is passed onto the offspring. If alleles obey Mendel's first law, $r = 0.5$ and they may spread only by increasing the fitness of the individual w'. In non-mendelian systems, r is not constrained to equal 0.5 but can vary over the range 0 (never inherited) to 1.0 (always inherited). Thus the fitness of a non-mendelian gene can be enhanced by increasing either w' or r. Specifically, we are interested in alleles which increase their own rate of transmission, r, at the cost of reduced fitness of the individual w', i.e. 'selfish' genes. A similar argument applies to cytoplasmic genes such as mitochondria, chloroplasts or intracellular symbionts. Inheritance is typically uniparental and will subsequently be regarded as 'maternal' (although cases of paternal inheritance are known). In this case half the population transmit the cytoplasmic genes (this half are the daughters, sons

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are genetic dead ends) so, in sexually reproducing species with a 1 : 1 sex ratio, $r = 0.5$ as in nuclear genes. This value of r can be altered: if a variant arises which is also transmitted through the sons, $r = 1.0$, alternatively (as will be described later) the number of female offspring may be increased by altering the sex ratio and in this case r equals the proportion of daughters produced.

A subset of selfish genes exist which do not alter r. They obey the normal rules of mendelian or uniparental inheritance and spread by decreasing the fitness of individuals which lack the selfish allele, thereby reducing $\bar{w}$; this subset may be regarded as 'spiteful' rather than 'selfish'. Expression (1) shows they can only spread when $w' > \bar{w}$ (since $r = 0.5$). In most cases possession of the selfish gene imposes some fitness reduction on the carrier (i.e. $w' < 1.0$) and because $\bar{w}$ depends on the frequency of the spiteful genes, they have to exceed a critical threshold frequency before invasion can occur. The more deleterious the effect on host fitness (w'), the higher this critical frequency becomes. Examples of spiteful genes are cytoplasmic incompatibility and '*M*' factors (see later) which are cytoplasmic and nuclear respectively.

It is important to note that equation (1) applies only when a selfish element can be classified as either present or absent, for example when alleles at a single locus are considered or when infection by a cytoplasmic agent rapidly reproduces to a maximum capacity such that individuals (whose fitness may be represented as w) may be classified as either 'infected' ($w = w'$) or 'uninfected' ($w = 1.0$) with no intermediate levels of infection. When intermediate levels of infection are considered, the situation is more complex; for example when the number of transposable elements, cytoplasmic agents, B chromosomes, or proportion of 'selfish' alleles in a multigene family is less than the maximum level, the fitness of an individual will depend on the level of infection and will lie in the range $1.0 > w > w'$. In these circumstances selfish elements may spread in apparent violation of equation (1) and the simple models of, for example Ginzburg *et al.* (1984) and Law & Hutson (1992). In the early stages of invasion, $w > 0.5$ and the mean level of infection increases, gradually eroding the value of $\bar{w}$ and thus maintaining the inequality of equation (1). This is illustrated on figure 1 where a selfish element may have up to 100 copies. At time $t = 1$, the element is starting to invade a population and is subject to two forces: natural selection which tends to decrease the frequency, and transmission bias which tends to increase it. Note that the force of natural selection depends not on the magnitude of mean fitness $\bar{w}$, but on its variance (Fisher's fundamental theorem of natural selection; Fisher 1930). Thus, providing that the variance in fitness does not substantially increase as mean infection levels rise (for example due to a nonlinear fitness function), the magnitude of the two forces remain unbalanced, the mean fitness $\bar{w}$ gradually declines and fixation of the element occurs even if $w' < 0.5$); this has been confirmed for selfish intracellular symbionts (Hastings 1992*a*). These are extremely encouraging results for the purposes of pest control as it demonstrates that very low values of $\bar{w}$ can be achieved (as low as $\bar{w} = w' < 0.05$). It also demonstrates that the use of elements present in

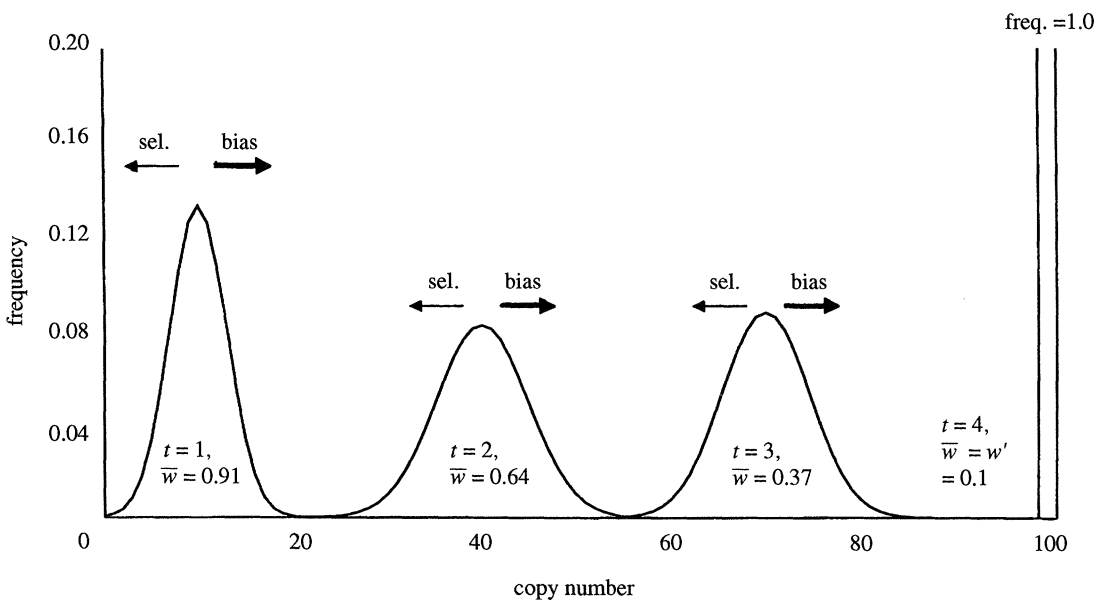

Figure 1. An illustration of the invasion of a hypothetical selfish element whose maximum copy number is 100. Copy number gradually increases over the arbitrary time intervals $t = 1,2,3$ resulting in fixation at $t = 4$. The dynamics of the system depends on two forces, natural selection (which tends to decrease copy number) and transmission bias (which tends to increase it). Mean fitness $\bar{w}$ is given by the linear function $1 - p + pw'$ where p is the copy number as a proportion of the maximum, and w' is the fitness of an individual which contains the maximum number of selfish elements; in this example $w' = 0.1$. Changes in the variance of copy number may alter over the course of fixation: in this case the variance is assumed to follow the binomial distribution.

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multiple copies may be more effective in reducing $\bar{w}$ than elements present in single copies.

The most common forms of selfish elements will be briefly described, but the main part of the text will be concerned with identifying common features and the description of a general model of non-mendelian genetics. Application of this model allows investigation of specific systems whose complexity precludes exact analytic analyses. It also identifies critical parameters necessary to predict the efficacy of selfish elements in population control. Specifically, for each type of element we need to assess: (i) its rate of invasion; (ii) the potential magnitude of its effect on host population fitness; and (iii) its 'lifespan', i.e. the likely time before its invasive ability is destroyed by mutation or negated by modifiers. They could be used to control pest populations in three ways. First, by their inherent deleterious effects on host fitness; secondly by using them as a vector to spread deleterious linked genes into the population (discussed in more detail later); and thirdly by using them to spread genes which reduce or eliminate the undesirable effects of the pest species (for example genes which reduce their virulence or their ability to transmit disease).

2. NUCLEAR SELFISH DNA

(*a*) *Transposable elements*

These are autonomously replicating pieces of DNA which, when introduced into a susceptible population, may cause a series of phenomena known as dysgenesis. These phenomena include mutations caused by insertion and by the non-disjunction that result at meiosis. Their dynamics have been the subject of several studies (e.g. Charlesworth & Charlesworth 1983; Langley *et al.* 1988; Charlesworth & Langley 1989; Charlesworth 1991; Brookfield 1991). Brookfield's simulation models showed large decreases in host fitness as a result of initial invasion but that fitness reverted to its original level as 'repressor systems' evolved, a result in agreement with laboratory studies. The rapid evolution of repression and the fact that many types of elements only reach a low copy number (with presumably only a small effect on host fitness) makes them unlikely candidates for direct control of populations. Their value lies as a potential vector to spread genes through a population (see Kidwell & Ribeiro 1993; and later discussion); this is made possible by the relative ease with which they can be manipulated in the laboratory.

(*b*) *Meiotic drive alleles*

These alleles are transmitted from heterozygotes with frequency greater than 50% so will spread through the population if they have no deleterious effects. Alternatively, if they do have deleterious effects which are frequency dependent (if, for example, the effect is recessive) an equilibrium may be reached. In the simplest case where they are recessive lethals and drive occurs in only one sex (e.g. the *t* locus in mice) the mean fitness of the host population at equilibrium is $\bar{w} = 0.5 + \sqrt{r(1-r)}$ (Crow & Kimura 1970, p. 312). For example when $r = 1.0$, $\bar{w} = 0.5$, when $r = 0.9$, $\bar{w} = 0.8$ and when $r = 0.8$, $\bar{w} = 0.9$. They constitute a powerful means of reducing population fitness. Their mechanism is currently attracting much attention, particularly the segregation disorder (SD) system in *Drosophila melanogaster* (e.g. Termin *et al.* 1991) and the *t* system in mice (e.g. Lyon 1991; Silver 1993). If modifier loci do not already exist, they may evolve to prevent the transmission bias (e.g. Lyttle 1979) although the timescale of their evolution remains unknown. A mutation in the drive 'locus' which eliminates the deleterious effects ($w' = 1.0$ in equation (1)) will be favoured by natural selection and go to fixation thus removing the load. Apparently, the reason why this does not occur is that, at least in the *t* system of mice, the 'locus' is actually an inversion containing several genes. Because of deleterious effects present in the original inversion or accumulated subsequently, the inversion is likely to be many mutational steps away from non-lethality and the chances of a single mutation restoring w' to unity is vanishingly small.

A potentially powerful method of control is to construct a sex-linked meiotic drive system. In species where no crossing over occurs between sex chromosomes their spread will result in a population consisting predominantly of one sex. If such loci become fixed the population will obviously become extinct. Several sex-linked meiotic drive systems are known (e.g. James & Jaenike 1990). In natural populations their frequency may be limited either by the evolution of repressor systems, or by deleterious effects in the homogametic sex. A prepoderance of females may result in increased population size because there are generally more than enough males to inseminate all the females in a normal wild population. Conversely, a preponderance of males may result in decreased size. In the case of mosquitos and some other disease vectors only the females bite and hence transmit the disease. In these cases distortion of the sex ratio towards males decreases the vectorial capacity of the population (Curtis *et al.* 1976).

Lyttle (1977) constructed an artificial sex-linked drive system in *Drosophila melanogaster* by linking the Y chromosome to the SD meiotic drive locus. As predicted, this resulted in populations consisting predominantly of males and cage populations were driven to extinction. Repressor systems arose which mitigated the effect of the drive construct and by comparing inbred with outbred populations it was shown that such repression arose by mutation and had a polygenic basis (Lyttle 1979). Thus they may be used either as a direct method of control (if they have inherent deleterious effects or cause a significant sex bias) or as a vector to spread linked deleterious genes, or genes which reduce the virulence or disease vectorial capacity of the pest. They may be manipulated within species but the molecular basis of their action remains obscure which curtails their usage outside the natural host.

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(*c*) *M factors*

These maternal effect loci are an example of 'spiteful' DNA and were recently discovered in *Tribolium castaneum* and *T. confusum* (Beeman *et al.* 1992; Bull *et al.* 1992). The spiteful M allele is dominant in females and acts by killing all offspring which lack the M allele. All offspring of MM parents are unaffected (as they must inherit at least a single copy of the M gene) but in crosses between a $M+$ female and a $M+$ or $++$ male the $++$ offspring are killed.

The dynamics of this system are complex as there may be two possible equilibrium frequencies apart from fixation and loss. First, there is an unstable 'threshold' frequency which must be exceeded before invasion can occur (because $\bar{w}$ decreases as their frequency increases so that eventually $w' > \bar{w}$ thus satisfying expression (1); see § 1). If there is no inherent deleterious effect of the M allele it will spread to fixation. There will be a transient reduction in population fitness as $++$ individuals are eliminated but at fixation population fitness is restored to $\bar{w} = 1.0$. As shown on figure 2, the dynamics are more complex if the M allele has a deleterious effect on the fitness of its host as, providing this effect in not dominant, a higher, stable equilibrium frequency will also exist. This equilibrium is best explained by considering the two forces of selection acting on the wild-type + gene. If the frequency of + increases, so does the frequency of $++$ zygotes, the incidence of M-mediated killing will increase and M will be favoured over +. Alternately, at lower frequencies the + allele will mainly occur in heterozygotes and because the fitness of MM is less than that of $M+$, the + allele will be favoured over the M. The stable equilibrium occurs when these two forces are balanced. Exact analytic formulae for these equilibria have not yet been obtained but the dynamics are easily investigated by simulation. The method of action is unknown but if it is due to a poison/antidote system (as in an analogous system in low copy number plasmids) and if the poison is a general insecticide the system may be effective if introduced into other insect species. Their application in population control is hampered by their lack of an endogenous deleterious effect (except for transient mortality of sensitive forms during invasion) presumably because they have evolved to maximize their efficacy in killing sensitive forms while minimizing their effect on host fitness. They may be better employed as a vector but the threshold effect means that if w' is decreased, the initial frequency to initiate invasion would have to increase.

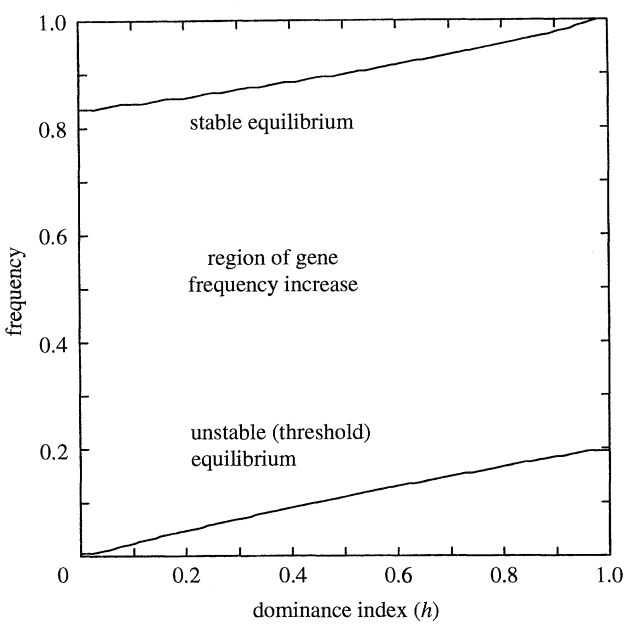

Figure 2. Changes in gene frequency of M factors according to their frequency and dominance index (h) when the fitnesses of the genotypes are as follows $++ = 1$, $M+ = 1 - hs$, $MM = 1 - s$. In this example $s = 0.2$ in both sexes. The region in which M factors increase in frequency is bounded by a lower unstable (threshold) frequency and by an upper stable equilibrium.

(*d*) *Biased gene conversion*

This may occur in single copy genes or in multigene families. A selfish allele biasing conversion in its favour (i.e. $r > 0.5$ in expression (1)) can invade a population despite deleterious effects on its host. The population dynamics of biased conversion in multigene families has been investigated by Slatkin (1986) and Walsh (1985, 1986). The model of Slatkin (1986) predicts the change in copy number of the selfish gene over a generation to be

$$[(2\alpha - 1)g/n - s](1 - i/n)i/n, \qquad (2)$$

where s is the selection coefficient against the selfish form, g is the conversion rate per gamete, α is a measure of the extent of conversion bias, i is the mean number of selfish alleles and n is the haploid copy number (from his equation (24) rearranging and removing the last two terms describing recurrent mutation). This predicts that invasion will occur provided

$$(2\alpha - 1)g/n > s. \qquad (3)$$

Fitness of an individual with i copies of the selfish gene was assumed to be $(1 - s)^i$ but leads to the position that a gamete with ten copies of a selfish gene which encodes a defective product has the same fitness irrespective of whether this is all the available genes ($n = 10$) or only 10% of them ($n = 100$). An alternative is to define $s = (1 - a)/n$ which has the advantage that a may be an explicit property of the gene, such as the metabolic activity of its product (expressed relative to wild-type activity of unity), whose effects are incremented depending on copy number. In which case the inequality in expression (3) becomes:

$$(2\alpha - 1)g > (1 - a). \qquad (4)$$

The rate of spread is determined by the factor $(2\alpha - 1)g - s$ showing that, in common with many other forms of selfish DNA, the rate of spread is inversely proportional to their effect (see later discussion of equation (5)).

The model makes several restrictive assumptions,

chiefly that g and $g(2\alpha - 1)$ are both much less than unity, and that selection acts on gametes. A general matrix model of non-mendelian genetics has been developed and used to model this system under less restrictive assumptions (see later); the results from these simulations suggest these analytic solutions are robust. Of all the types of selfish DNA considered here, biased gene conversion in multigene families is potentially the most effective. Its presence in multiple copies means that it can invade even when $w' < 0.5$. If manipulated in the laboratory so that it retains its conversion bias property but is several mutational steps away from its original sequence the chance of reversion to an active metabolic form may be negligible. The physical basis by which conversion bias is achieved is unknown but is an area of active research; once elucidated its application will constitute a powerful technique for population control.

(*e*) *B chromosomes*

These 'supernumerary' chromosomes occur in the nucleus but do not obey the normal rules of mendelian segregation. They occur in many groups of organisms, including tsetse flies (Itard 1966, 1971; Southern & Pell 1973). Their dynamics may be complex, for example they may be driven in one sex but 'drag' in the other, or may be driven in both sexes (Jones & Rees 1982; Shaw & Hewitt 1990). A simple model is developed later which allows Bs to have deleterious effects and assumes simple stochastic inheritance and truncation selection. The results shown on figure 3 demonstrate that B chromosomes may impose a substantial genetic load on a population and that the rate of invasion may be rapid once present at a moderate frequency. This suggests that a rapid decrease in host fitness may be achieved by the mass release of members of the pest species carrying B chromosomes.

The efficacy of B chromosomes as a method of control may be enhanced by constructing one encoding a deleterious metabolic poison, and to use a B chromosome as a 'vector' as discussed later. A practical problem is the expression of such a gene: DNA in the B chromosome is typically, but not invariably, heterochromatic (Jones & Rees 1982; Jones 1991), a state not conducive to efficient gene expression (although a B chromosome encoding rRNA has been reported in rye; Flavell & Rimpau 1975). Nuclear modifier genes are known which reduce the level of drive in plants (Carlson 1969) and animals (Nur & Brett 1985). However, the widespread occurrence of B systems and the observation that the modifiers mentioned above appeared to be segregating within populations, suggest that the rapid suppression of the driving of an introduced B chromosome is unlikely. B chromosomes appear to have little effect on host fitness (in fact it is not clear what limits their copy number and most models assume threshold selection) so their most likely role in pest control may be as a vector of deleterious genes.

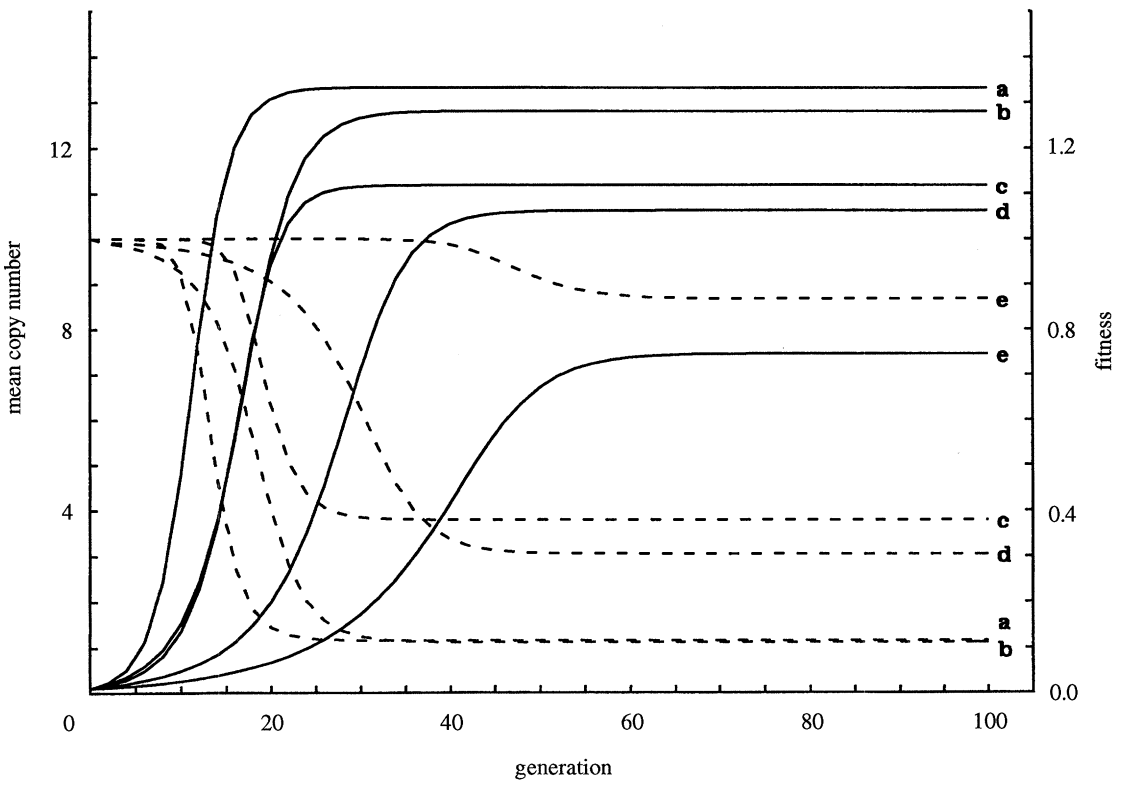

Figure 3. Changes in mean number of B chromosomes per individual (solid lines) and mean fitness (dashed lines) with generation. Both parameters prior to selection (truncation at $n = 10$), drive is assumed to occur only in one sex, and initial mean number = 0.1. a, $r = 1.0$, $w' = 1.0$; b, $r = 1.0$, $w' = 0.95$; c, $r = 0.8$, $w' = 1.0$; d, $r = 0.8$, $w' = 0.95$; e, $r = 0.6$, $w' = 1.0$.

3. CYTOPLASMIC SELFISH DNA

These genes are carried on intra-cellular genomes (ICGs). Such genomes may exist as organelles (e.g. mitochondria or chloroplasts) or as intracellular symbionts such as bacteria of the genus *Wolbachia*. If the inheritance of such genomes is biparental, $r = 1.0$ in the parameters of equation (1), a selfish form may spread provided $w' > 0.5$. Such organisms may rapidly invade a population and greatly reduce the fitness of the host population (Hastings 1992*a*). However, in most species uniparental inheritance of cytoplasm ensures that $r = 0.5$ so selfish forms cannot invade. It has been argued that uniparental inheritance arose as a defence against such genomes (Cosmides & Tooby 1981; Hastings 1992*a*, and references therein).

The restriction of ICGs to transmission through one sex (generally the female) means that several genomes have evolved mechanisms to increase their transmission. Three main strategies have been identified, two of them selfish and one spiteful.

(*a*) *Biased sex-ratio of offspring*

Intra-cellular genomes are transmitted through females so males may be regarded as an evolutionary 'dead end' for intracellular genomes. Dioecious plants contain female structures which produce seeds and male parts which produce pollen. The former transmit ICGs whereas the latter do not. ICGs are known to bias the allocation of reproductive energy away from pollen to seed (Couvet *et al.* 1990) so $r > 0.5$ although interactions with nuclear modifiers complicate their dynamics (Gouyon *et al.* 1991).

An analogous system is known in some animals (e.g. Rigaud *et al.* 1992). A cytoplasmic agent causes eggs which would normally develop as males, to develop as females (and hence transmit the ICG to their offspring). Other systems have been identified in which ICGs kill off sons, presumably reducing intra-brood competition and increasing the number of daughters which survive (Hurst 1991). In both cases the population then becomes predominantly female. The system is analogous to an X-linked meiotic drive system and, as before, there are two outcomes: a male determining mechanism resistant to the ICG (if present in the population) will be selected, or more desirable for present purposes, extinction will occur (Taylor 1990). Much effort is being invested into studying the molecular biology of sex determination and it may become possible to develop a suitable ICG; such an approach may be technically easier than constructing a sex-linked meiotic drive system.

(*b*) *Asexuality*

The above strategy may be taken to the extreme of asexuality. In some hymenopteran species ICGs can change sexual individuals to asexual, i.e. females produce only females. Asexual females have a two fold reproductive advantage over sexual females, at least in the short term, and asexuality will spread. However, such lineages lack the ability to recombine genes and therefore cannot respond rapidly to a changing environment caused, for example, by the application of insecticides.

There are two problems is using these ICGs in other organisms. First, Hymenoptera are unusual in that unfertlized eggs develop normally (but as males) so the challenge to a hymenopteran ICG was merely to change the sex of the unfertilized egg. In most purely sexual species an ICG would have to prevent meiosis in females so that eggs contain a diploid genotype (reversion to a viable haploid genotype would be unlikely given the number of recessive lethals in such populations). Secondly, asexuality would have to be complete as even occasional sexual recombination may provide most of the advantages of a fully sexual lifecycle (Hastings 1991, 1992*b*, and references therein). A comprehensive review of ICGs which bias sex allocation (including their deleterious effects on host fitness) may be found in Hurst (1993).

(*c*) *Cytoplasmic incompatibility (CI)*

This is spiteful rather than selfish DNA. This syndrome has been observed in several insect species including the mosquito *Culex pipiens* (Laven 1959; Rousset & Raymond 1991). In these cases males are not an evolutionary dead end for the ICG. Although not transmitted by sperm, males with a given ICG give rise to inviable eggs when mated with a female lacking the same ICG (or a different but compatible one); some factor produced by the ICG must enter with the sperm and kill the egg if the same ICG is not present. In effect females with the ICG can (via their male offspring) sterilize females without them. ICGs encoding cytoplasmic incompatibility can spread through a population by frequency-dependent selection provided their frequency exceeds a critical frequency f_{crit} given by

$$f_{crit} = s_f/s_h,$$

where s_f is the reduction in fitness (eggs laid) by females with the ICG and s_h is the reduction in eggs hatched in incompatible crosses (Turelli & Hoffmann 1991). After this critical frequency is exceeded, their change in frequency per generation (Δp) is:

$$\Delta p = \frac{w'p}{1 - p(1-p) - p(1-w')} - p,$$

from equation (1) of Turelli *et al.* (1992). The dynamics are, in theory, unstable so above the critical frequency, the ICG will become fixed and reduce the fitness of the population to $\bar{w} = w' = 1 - s_f$. Population structure is an important factor in assessing its efficacy: if a small isolated sub-population can be artificially infected (so that the frequency exceeds f_{crit}) the infection may be passed onto neighbouring sub-populations. As in other systems of selfish DNA their rate of spread is inversely proportional to the magnitude of their effect; in this case the rate of spread (based on a one-dimensional model) is proportional to $1 - 2f_{crit}$ (Turelli & Hoffmann 1991). There is one other advantage to this system: although

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the equlibrium fitness of the population will be w', the invasion of the cytoplasmic incompatibility (CI) entails a significant transitory reduction in fitness. When the frequency reaches 0.5 then, assuming random mating, 25% of zygotes will die as a consequence of CI. The effects may be apparent over a number of generations but as the frequency of CI increases the number of incompatible crosses will decrease and its effect on population fitness will diminish. The practical consequences of this reduction in fertility depends on the ecology of the species and will be discussed later (when the relationship between $\bar{w}$ and population size will be considered).

Cytoplasmic incompatibility appears to be due to endosymbiont bacteria of the *Wolbachia* genus. Recent molecular analysis has shown them to be remarkedly similar between species (O'Neill *et al.* 1992; Rousset *et al.* 1992), raising the hope that cross infection between species may be achieved in the laboratory (Curtis 1992), a technique now possible in *Drosophila* (Boyle *et al.* 1993). Its practical application suffers the same drawbacks as '*M*' factors (its nuclear equivalent): that as the magnitude of their deleterious effect on host fitness is increased, so is the initial frequency required to ensure their invasion.

4. A GENERAL MODEL OF NON-MENDELIAN GENETICS

In many non-mendelian systems the complex patterns of inheritance and fitness functions either preclude simple analytic solutions or require a number of simplifying assumptions. However, when these complex patterns are expressed in matrix form, their (deterministic) dynamics can be easily investigated by simulation. This method simply calculates the output of gametes each generation; random fusion is then assumed and the frequency of genotypes in the next generation calculated. The output of gametes each generation depends on three factors: the frequency of adult genotypes, the fitness of these genotypes, and the frequency of each type of gamete produced by each genotype, represented by matrices $\boldsymbol{F}$, $\boldsymbol{W}$ and $\boldsymbol{G}$ respectively. Taking the simple example of a mendelian system with two alleles A and a of frequency p and q respectively where the fitness of genotype $AA = 1$, $Aa = 1 - hs$ and $aa = 1 - s$:

1. $\boldsymbol{F}$ is a vector whose elements $\boldsymbol{F}_i$, where $i = 0,1,2$, represent the frequency of genotypes with 0,1 or 2 copies of allele a respectively, i.e.

$$\boldsymbol{F} = (p^2 \quad 2pq \quad q^2).$$

2. $\boldsymbol{W}$ is a 3×3 matrix whose offdiagonal elements are all zero and whose diagonal elements $\boldsymbol{W}_{i,i}$, where $i = 0,1,2$, represent the fitness of genotypes with i copies of allele a, i.e.

$$\boldsymbol{W} = \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 - hs & 0 \\ 0 & 0 & 1 - s \end{pmatrix}.$$

3. $\boldsymbol{G}$ is a 3×2 element matrix whose elements $\boldsymbol{G}_{i,j}$ (where $i = 0,1,2$ and $j = 0,1$) contain the frequency with which gametes containing j copies of a are produced by genotypes containing i copies of a, i.e.

$$\boldsymbol{G} = \begin{pmatrix} 1 & 0 \\ 0.5 & 0.5 \\ 0 & 1 \end{pmatrix}$$

The gametic output is held in vector $\boldsymbol{T}$ given by $\boldsymbol{T} = \boldsymbol{FWG}$ i.e.

$\boldsymbol{T}_0$ = output of $\boldsymbol{A}$ gametes $= p^2 + pq(1 - hs)$,

$\boldsymbol{T}_1$ = output of a gametes $= pq(1 - hs) + q^2(1 - s)$.

When divided by a normalizing factor $(p^2 + 2pq(1 - hs) + q^2(1 - s))$, the frequencies sum to unity, and are identical to those presented in standard textbooks e.g. Hartl & Clarke (1989, pp. 152).

The power of this methodology lies in its generality. The dynamics of non-mendelian selfish genes are determined by their rate of transmission (described by the $\boldsymbol{G}$ matrix), and by their effects on host fitness (described by the $\boldsymbol{W}$ matrix) making this model an explicit and general model of non-mendelian systems. By using computers it is easy to generate the matrices $\boldsymbol{F}$, $\boldsymbol{W}$ and $\boldsymbol{G}$ using simple (e.g. non-mendelian) algorithms and, by iteration, to examine both the dynamics and equilibrium properties of the system. It can be easily extended to systems with large numbers of genotypes and complicating factors such as differential gametic output of sexes incorporated into the model. It can also be used to investigate the evolutionary dynamics of repressor loci. Such complicated analyses are typically not tractable by standard analytic methods and the generation of suitable $\boldsymbol{G}$ and $\boldsymbol{W}$ reduces the problem to a trivial one of computer programming. The methodology has been used to examine non-mendelian genetics caused by selection in the germline (Hastings 1991) and of cytoplasmic genomes (Hastings 1992*a*) and is now extended to two further systems of interest.

(*a*) *Application of the model to biased gene conversion*

As described earlier, Slatkin's (1986) analytic model of biased gene conversion made several simplifying assumptions. The applicability of the model outside these bounds may be checked by simulation using the above methodology; in the following matrix notation the subscripts i or j refer to the number of selfish alleles in the genotype. A $2n \times 2n$ matrix $\boldsymbol{W}$ was generated with all offdiagonal elements set to zero and all diagonal elements given by the appropriate fitness function

$$\boldsymbol{W}_{i,i} = (1 - s)^i,$$

where $s = (1 - a)/2n$ ($2n$ is appropriate because selection occurs in the adult, diploid, phase). The probabilty of selecting a selfish gene in a diploid genotype containing i copies (p_{si}) is

$$p_{si} = x(1 - r(1 - \alpha)y) + yr\alpha x,$$

where x is the proportion of selfish alleles ($x = i/2n$), y is the proportion of wild-type alleles ($y = (n - 1)/2n$), and r is the conversion rate per locus ($r = g/n$). The

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first term represents the probability of selecting a selfish allele (scaled by the probability it has not been converted) and the second term represents the probability of selecting an allele which was originally wild-type but was subsequently converted to a selfish form (the rate of double events is assumed to be negligible). Similarly, the probability of selecting a wild-type allele is

$$p_{wi} = y(1 - r\alpha x) + xr(1 - \alpha)y$$

A $2n \times n$ $\boldsymbol{G}$ matrix describes the probability of selecting a gamete with j copies of the selfish allele from a diploid genotype containing i copies. Assuming unlinked loci, the elements are given by the binomial theorem (e.g. Grossman & Turner 1974, p. 73)

$$\boldsymbol{G}_{ij} = \binom{n}{j} p_{si}^{j} p_{wi}^{n-j},$$

with two exceptional elements $\boldsymbol{G}_{0,0} = \boldsymbol{G}_{2n,n} = 1$.

This differs from Slatkin's model in that selection is explicitly on adults rather than gametes and sampling with replacement takes account of conversion during mitosis in the germline. All combinations of the following parameter values were investigated: $2n = 100,\ 200,\ 500$; $g = 0.2,\ 0.5,\ 1.0,\ 5.0,\ 10.0$; $\alpha = 0.55,\ 0.6,\ 0.8,\ 1.0$; $a = 0,\ 0.5,\ 0.8$. The starting frequency of the selfish allele was 0.5 (when the rate of change should be maximal) and iterated for 2000 generations or until equilibrium was reached. Invasion or loss was as predicted by equation (4) and furthermore in the eight parameter combinations where equation (4) was an equality (e.g. $g = 1$, $\alpha = 0.6$, $a = 0.8$), the change in gene frequency was negligible (a maximum change of 0.05 over 2000 generations and typically much less than this). These results suggest the results of Slatkin's analytical model are extremely robust.

(*b*) *Application of the model to B chromosomes*

The dynamics of B chromosomes (Bs) are frequently complex (Jones & Rees 1982; Shaw & Hewitt 1990) and in the following example are restricted to the simple case of drive in one sex (assumed to be the male) and random segregation in the other (female) sex. A $n \times n$ $\boldsymbol{G}$ matrix is constructed on the simple assumption that a cell containing i Bs divides to form a non-generative polar cell and a gamete, each of which may inherit between 0 and i Bs. If r is the probability of an individual B entering the gamete, the probability of a gamete inheriting a portion of cytoplasm containing a B is $p = ir/n$, and n portions will be inherited corresponding to the n potential B chromosome 'sites'. The elements of the G matrix are:

$$\boldsymbol{G}_{ij} = \binom{n}{j} (p)^{j} (1 - p)^{n-j}.$$

There is one exceptional element $\boldsymbol{G}_{0,0} = 1$ and a conditional exception $\boldsymbol{G}_{n,n} = 1$ if $r = 1$. In the cases investigated here, $r > 0.5$ in the male $\boldsymbol{G}$ matrix and $r = 0.5$ in the female. Drive or drag in the female can be readily investigated as $r > 0.5$ or $r < 0.5$ respectively. This assumes sampling with replacement which is mathematically simple and formally allows for diversity arising in the mitotic divisions prior to gametogenesis. More complex $\boldsymbol{G}$ matrices can be generated for specific systems (e.g. Matthews & Jones 1982) but the above serves as a useful general model. One problem of investigating the population genetics of Bs is to determine the factor(s) which eventually limit their number (Jones & Rees 1982), a similar problem to that of transposable elements, which typically reach very high copy number. The method used here (and previously by Matthews & Jones (1982)) is simply to assume that all adults with copy number greater than n are sterile, i.e. truncation selection. The $\boldsymbol{W}$ matrix therefore has dimensions $2n \times 2n$ (as gametes may contain n Bs and hence zygotes up to $2n$) with all off diagonal elements set to zero and

$$\boldsymbol{W}_{ii} = w'^{i}, \qquad \text{when } i \leqslant n, \text{ and}$$

$$\boldsymbol{W}_{ii} = 0, \qquad \text{when } i > n,$$

where w' is the selective disadvantage associated with the B chromosome. The results of several parameter combination are shown on figure 3 and confirm two previous assertions: that their rate of invasion depends inversely on the magnitude of their deleterious effects and that the ultimate effect of fitness may be substantial in elements which are present in multiple copies.

(*c*) *Application to other systems*

The model is sufficiently general to allow investigation of most selfish systems. The main practical (and indeed theoretical) problem is in determining what limits the maximum copy number, and hence to determine the maximum size of the matrices. There are three ways in which the maximum copy number may be limited. Firstly, there may be a physical limit such as the number of loci in a multigene family, or the number of mitochondria may be regulated by cellular mechanisms. Secondly, there may be threshold selection such that all individuals with more than a critical number of elements are inviable or infertile (as was assumed to be the case in B chromosomes). Thirdly, the variance in fitness may increase with copy number until the forces of natural selection and biased transmission are exactly balanced. This may occur because variance in copy number increases with mean copy number, or because a nonlinear fitness function increases variance at high copy number (the latter scenario is the one typically invoked to limit the number of transposable elements; e.g. Charlesworth & Langley 1989). In the first two cases, the maximum number of elements are known (and hence the required size of the matrices) while in the third case, the maximum size of matrices may need to be determined empirically by trial and error.

5. DISCUSSION

To assess the potential use of selfish DNA to control populations we need to consider five factors: (i)

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whether the DNA is inherently selfish or whether it can mutate to a more benign form; (ii) to determine if repressor systems already exist and whether they are likely to evolve; (iii) to estimate its optimum level of fitness: a large reduction in host fitness will slow its rate of invasion; (iv) to determine whether the host population is strongly density regulated as this determines the extent to which the theoretical reduction in host fitness is translated into real reductions in population size; and (v) the ease with which selfish DNA can be manipulated in the laboratory.

Selfish elements are defined as those which increase r but reduce w' (see expression 1), or in the case of spiteful systems, alleles which reduce $\bar{w}$. Obviously alleles which can increase both r and w' will be fitter than selfish alleles, so for our purposes we must consider DNA with an inherent trade-off between host fitness and its own rate of transmission so that mutations to a more benign form ('reversions') are not selected. Selfish organelles such as mitochondria or chloroplasts fall into this category as they may encounter a conflict of interests between exporting their produced energy for general cell metabolism or sequestering it to increase their own rate of replication. Alternatively selfish DNA could be artificially created to minimise the chance of reversions. For example, naturally occurring alleles causing biased gene conversion in multigene families are likely to be only one or two mutational steps from a fully active gene and reversions may arise which embody both properties of biased conversion and metabolic activity. However, if alleles with the required property of biased conversion could be artificially synthesized they need bear little similarity to the original 'active' sequence and the probability of reversions would be vanishingly small.

The above systems all rely on 'genetic load' to reduce the fitness of the host population, i.e. that selfish elements have an inherently deleterious effect. A related strategy is to spread deleterious genes through a population by linkage to a selfish gene, in other words to use selfish DNA as a 'vector'. The term 'linkage' is used broadly and may involve conventional linkage, for example being physically adjacent to a meiotic drive locus, or 'linkage' achieved by inserting the desired gene into a transposable element, B chromosome, or cytoplasmic element. The use of transposable elements as a vector is a routine technique in molecular biology (e.g. Rubin & Spradling 1982; Spradling 1986) and its use in population control was recently discussed by Kidwell & Ribeiro (1992). There are, however, two problems with this strategy of using selfish alleles as vectors. Firstly, a gene with a specific function is likely to be disrupted by mutation; provided the mutation does not affect the rate of transmission, the harmless mutant form will be favoured by selection and replace the active form. Secondly, linkage may break down, either by recombination if encoded in nuclear DNA, or by internal deletions if encoded in transposable elements, B chromosomes, or cytoplasmic element. Such deletions result in shorter DNA sequences which may replicate faster and hence be favoured by selection as postulated for transposable elements, and mitochondria (Rand & Harrison 1986). More encouragingly, Wood *et al.* (1977) succeeded in driving a gene linked to sex-linked meiotic drive resistance factors through a cage population and Miao *et al.* (1991) suggested that two genes encoding antibiotic resistance are encoded by a B chromosome in the fungus *Nectria haematococa* (although their persistence in the face of mutation may be attributed to their being actively selected). The use of selfish DNA as a vector system is important given the progress in identifying the gene(s) which confer resistance of *Anopheles* mosquitos to *Plasmodium falciparum* (see Collins *et al.* 1986). This strategy genetically alters the genotype of the host to reduce or eliminate an undesired property, in this case the ability to transmit malaria. It need not necessarily reduce host fitness and therefore has the advantage of leaving the host population density intact; it effectively circumvents the problem of density-dependent regulation (Curtis & Graves 1988; Curtis 1992). In cases where host fitness is decreased, an appropriate mode of gene action and fitness function needs to be developed. For example multiple copies of a gene encoding susceptibility to an insecticide may be no better than a single copy of the gene. In this case the predicted advantage of multi-copy elements is lost and a single copy spread, for example, by linkage to a meiotic drive allele will be just as effective. In cases where susceptibility is a function of copy number, multi-copy elements would be the vector of choice.

The likely evolution of modifiers must be considered. Modifiers evolve because of a conflict of interest between individual selfish genes and the other genes in an individual. The genome therefore evolves to constrain all genes to obey, as closely as possible, the mendelian paradigm (Crow 1979; Dawkins 1982). The rate of mutation to modifers may vary between systems from comparatively rare in meiotic drive systems to relatively frequent in transposable elements (Kidwell *et al.* 1982, 1988) where they may arise frequently by internal deletions. Several genes are known which alter the degree of gene conversion bias (e.g. Lamb & Helmi 1989) so suppressor systems are also likely to evolve in natural populations although the rate at which they arise is uncertain. The likely evolution of repressor systems remains largely unpredictable and constitutes the single most important unknown parameter in the application of selfish DNA in population control.

The speed of invasion and hence the rate of fitness reduction should be optimized for each system. The rate of invasion and the reduction in fitness have an inverse relationship. The change in gene frequency (Δp) in the initial stages of inversion (i.e. when all copies are present in heterozygotes) from equation (1) is

$$\Delta p = p\left(2\frac{w'}{\bar{w}}r - 1\right) \qquad (5)$$

where p is the frequency of the gene. There are two important points. First, decreasing the fitness of

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carriers (w') reduces the rate of spread. Secondly, the rate of increase in gene frequency is geometric. This latter point is of great practical importance as even if gene frequency doubles each generation (the maximum rate when $w' < \bar{w}$), the arithmetic rate of increase, and hence the reduction in host fitness, will be slow until the gene is present at an appreciable frequency. For example, in figure 3*d* population mean fitness only starts to fall significantly after around generation 20 when the frequency of B chromosomes becomes appreciable. The starting mean number in this simulation was 0.1. If more realistic starting values are chosen the lag before fitness starts to decline becomes longer: fitness only starts to fall after around generation 50 if the starting mean number is 0.001 and after generation 75 if the starting mean number is 0.0001. The most effective strategy will therefore depend on ecological factors: it may be feasible to introduce a selfish gene at an appreciable frequency in tsetse fly populations (which have a relatively low population density) but impractical in a swarm of locusts with up to 10^9 individuals. The optimal strategy in tsetse fly would be to introduce a highly deleterious gene at relatively high frequency whereas in locusts it may be better to introduce several mildly deleterious forms at a low frequency. The most efficient strategy may also be partly determined by the practicalities of captive breeding: tsetse flies in particular are not very fecund so there may be problems rearing enough flies carrying highly deleterious genetic constructs.

In the preceding discussion, the parameter of most importance has been w', a measure of the relative fitness. The relationship between this measure and its ecological equivalent (the change in population size) depends on two factors. Firstly whether selection is 'hard' or 'soft' (Wallace 1968) and secondly, whether population regulation is density dependent or independent. 'Hard' selection depends on the absolute value of w', for example if w' is a measure of cold tolerance, or all individuals with a 'susceptibility' gene are killed by insecticide. 'Soft' selection occurs when only a certain number of individuals are able to reproduce irrespective of the absolute value of w'; for example if only a certain number can defend a territory or occupy an egg laying site. The practical consequences of using selfish DNA to control populations ultimately depend on the type of population regulation present in the field. This sets a 'critical load' which must be exceeded before an actual reduction in population size is achieved. For example if 50% of a mosquito generation is killed by a selfish DNA ('hard' selection), but the population is limited by density-dependent factors such that there are only sufficient egg-laying sites for 40% of the population, the theoretical reduction in fitness will have no effect on population size; the selfish DNA would have to eliminate more than 40% of a generation to achieve any reduction in population size. Thus both the mode of gene action and the ecological factors which determine population density need to be considered. In addition, the rate of migration will need to be considered if using 'spiteful' DNA. Such DNA needs to be maintained above a critical 'threshold' frequency and large-scale inward migration may reduce their frequency below this level.

Advances in molecular biology have made it possible to transfer selfish DNA between species and the evolutionary conservation of their basic molecular biology make it likely that the selfish properties will be retained. This conclusion is supported by several observations. The transposable element 'P' appears to have spread between *Drosophila* species (on the basis of sequence homology) and has been artificially transferred between species (Daniels *et al.* 1990). Spore killers have been transferred between species of *Neurospora* (Turner & Perkins 1991) and B chromosomes from rye to wheat (Flavell & Rimpau 1975). The bacteria *Wolbachia* appears to be responsible for cytoplasmic incompatibility and other selfish effects in insects and is found in many species. The results of O'Neill *et al.* (1992) and Rousset *et al.* (1992) suggest they are closely related and diverged more recently than their host species, a conclusion which implies horizontal transmission of *Wolbachia* between host species. The artificial transfer of *Wolbachia* between species has been proposed as a method of population control (Curtis 1992). Advances in molecular biology which allow selfish DNA to be used as 'vectors' or transferred between species have important practical implications for population control.

The results discussed above suggest that selfish genetic elements may be an effective method of population control. The most uncertain factors being the evolution of repressor systems and the frequency of reversion to non-selfish forms. Their use has become feasible by the improved understanding and technology associated with recent advances in molecular biology. Their application needs to be optimized in terms of fitness and initial frequency, by population genetic techniques (both analytic and by simulation) and require an understanding of the ecology of the host species. Once optimized they may be a highly effective method of population control.

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REFERENCES

Beeman, R.W., Friesen, K.S. & Denell, R.E. 1992 Maternal-effect selfish genes in flour beetles. *Science, Wash.* **256**, 89–92.

Boyle, L., O'Neill, S.L., Robertson, H.M. & Karr, T.L. 1993 Interspecific and intraspecific horizontal transfer of *Wolbachia* in *Drosophila*. *Science, Wash.* **260**, 1796–1799.

Brookfield, J.F.Y. 1991 Models of transposition in P-M hybrid dysgenesis by P cytotype and zygotically encoded repressor proteins. *Genetics* **128**, 471–486.

Bull, J.J., Molineux, I.J. & Werren, J.H. 1992 Selfish genes. *Science, Wash.* **256**, 65.

Carlson, W.R. 1965 Factors affecting preferential fertilization in maize. *Genetics* 62, 543–554.

Charlesworth, B. 1991 Transposable elements in natural populations with a mixture of selected and neutral insertion sites. *Genet. Res.* **57**, 127–134.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Charlesworth, B. & Charlesworth, D. 1983 Population dynamics of transposable elements. *Genet. Res.* **42**, 1–27.
Charlesworth, B. & Langley, C.H. 1989 The popuation genetics of *Drosophila* transposable elements. *A. Rev. Genet.* **23**, 251–287.
Collins, F.H., Sakai, R.K., Vernick, K.D., Paskewitz, S., Seely, D.C., Miller, L.H., Collins, W.E., Campbell, C.C. & Gwadz, R.W. 1986 Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambia*. *Science, Wash.* **234**, 607–610.
Cosmides, L.M. & Tooby, J. 1981 Cytoplasmic inheritance and intragenomic conflict. *J. theor. Biol.* **89**, 83–129.
Couvet, D., Atlan, A., Belhassen, E., Gliddon, C., Gouyon, P. & Kjellberg, F. 1990 Co-evolution between two symbionts: the case of cytoplasmic male-sterility in higher plants. In *Oxford surveys in evolutionary biology*, vol. 7 (ed. D. Futuyama & J. Antonovics), pp. 225–249. Oxford University Press.
Crow, J.F. 1979 Genes that violate Mendel's rules. *Scient. Am.* **240**, 104–113.
Crow, J.F. & Kimura, M. 1970 *An introduction to population genetics*. New York: Harper and Row.
Curtis, C.F. 1992 Making mosquitos harmless. *Parasitol. Today* **8**, 305.
Curtis, C.F. & Graves, P.M. 1988 Methods for replacement of malaria vector populations. *J. trop. Med. Hyg.* **91**, 43–48.
Curtis, C.F., Grover, K.K., Sugana, S.G., Uppal, D.K., Dietz, K., Agarwal, H.V. & Kazmi, S.J. 1976 Comparative field cage tests of the population suppressing efficiency of three genetic control systems for *Aedes aegypti*. *Heredity* **36**, 11–29.
Daniels, S.B., Peterson, K.R., Strausbaugh, L.D., Kidwell, M.G. & Chovnick, A. 1990 Evidence for horizontal transmission of the P transposable element between *Drosophila* species. *Genetics* **124**, 339–355.
Dawkins, R. 1982 *The extended phenotype*. Oxford: W. H. Freeman.
Fisher, R.A. 1930 *The genetical theory of natural selection*. Oxford: Clarendon.
Flavell, R.B. & Rimpau, J. 1975 Ribosomal RNA genes and supernumerary B chromosomes of rye. *Heredity* **35**, 127–131.
Ginzberg, L.R., Bingham, P.M. & Yoo, S. 1984 On the theory of speciation induced by transposable elements. *Genetics* **107**, 331–341.
Gouyon, P.H., Vichot, F. & Van Damme, J.M.M. 1991 Nuclear-cytoplasmic male sterility: single point equilibria versus limit cycles. *Am. Nat.* **137**, 498–514.
Grossman, S.I. & Turner, J.E. 1974 *Mathematics for the biological sciences*. London: Collier Macmillan.
Hartl, D.L. & Clark, A.G. 1989 *Principles of population genetics*. New York: Sinauer Associates.
Hastings, I.M. 1991 Germline selection: population genetic aspects of the sexual/asexual lifecycle. *Genetics* **129**, 1167–1176.
Hastings, I.M. 1992*a* Population genetic aspects of deleterious cytoplasmic genomes and their effects on the evolution of sexual reproduction. *Genet. Res.* **59**, 215–225.
Hastings, I.M. 1992*b* Why is sex so frequent? *Trends Ecol. Evol.* **7**, 278–279.
Hurst, L.D. 1991 The incidences and evolution of cytoplasmic male killers. *Proc. R. Soc. Lond.* B **244**, 91–99.
Hurst, L.D. 1993 The incidences, mechanisms and evolution of cytoplasmic sex ratio distorters in animals. *Biol. Rev.* **68**, 121–193.
Itard, J. 1967 Chromosomes de Glossines (Giptera-Muscidae). *C. r. Acad. Sci.*, Paris **263**, 1395–1397.
Itard, J. 1971 Chromosomes de Glossina fusca congelensis. *C. r. Acad. Sci.*, Paris **272**, 2561–2564.
Kidwell, M.G., Kimura, K. & Black, D.M. 1988 Evolution of hybrid dysgenesis potential following P element contamination in *Drosophila melanogaster*. *Genetics* **119**, 815–828.
Kidwell, M.G & Ribeiro, J.M.C. 1992 Can transposable elements be used to drive disease refractoriness genes into vector populations? *Parasitol. Today* **8**, 325–329.
James, A.C. & Jaenike, J. 1990 'Sex ratio' meiotic drive in *Drosophila testacea*. *Genetics* **126**, 651–656.
Jones, R.N. 1991 B chromosome drive. *Am. Nat.* **137**, 430–440.
Jones, R.N. & Rees, H. 1982 *Chromosomes*. Academic Press.
Langley, C.H., Montgomery, E., Hudson, R., Kaplan, N. & Charlesworth, B. 1988 On the role of unequal exchange in the containment of transposable element copy number. *Genet. Res.* **52**, 223–235.
Lamb, B.C. & Helmi, E. 1989 The effects of gene conversion control factors on conversion-induced changes in allele frequency in populations and on linkage disequilibrium. *Genetica* **79**, 167–181.
Law, R. & Hutson, V. 1992 Intracellular symbionts and the evolution of uniparental cytoplasmic inheritance. *Proc. R. Soc. Lond.* B **248**, 69–77.
Laven, H. 1959 Speciation by cytoplasmic isolation in the *Culex pipiens* complex. *Cold Spring Harb. Symp. quant. Biol.* **24**, 166–173.
Luck, R.F. 1990 Evaluation of natural enemies for biological control: a behavioural approach. *Trends Ecol. Evol.* **5**, 196–199.
Lyon, M.F. 1991 The genetic basis of transmission-ratio distortion and male sterility due to the *t* complex. *Am. Nat.* **137**, 349–358.
Lyttle, T.W. 1977 Experimental population genetics of meiotic drive systems. I. Pseudo-Y chromosomal drive as a means of eliminating cage populations of *Drosophila melanogaster*. *Genetics* **86**, 413–445.
Lyttle, T.W. 1979 Experimental population genetics of meiotic drive systems. II. Accumulation of genetic modifiers of Segregation Disorder (SD) in laboratory populations. *Genetics* **91**, 339–357.
Matthews, R.B. & Jones, R.N. 1982 Dynamics of the B-chromosome polymorphism in rye. 1. Simulated populations. *Heredity* **48**, 345–369.
Miao, V.P., Covert, S.F. & Vanetten, H.D. 1991 A fungal gene for antibiotic resistance on a dispensable ('B') chromosome. *Science, Wash.* **254**, 1773–1776.
Newsome, A. 1990 The control of vertebrate pests by vertebrate predators. *Trends Ecol. Evol.* **5**, 187–191.
Nur, U. & Brett, B.L.H. 1985 Genotypes suppressing meiotic drive of a B chromosome in the mealy bug *Pseudococcus obscuris*. *Genetics* **62**, 543–554.
Ohta, T. & Dover, G.A. 1984 The cohesive population genetics of molecular drive. *Genetics* **108**, 501–521.
O'Neill, S.L., Giordano, R., Colbert, A.M.E., Karr, T.L. & Robertson, H.M. 1992 16S rRNA phylogenetic analysis of the bacterial endosymbionts associated with cytoplasmic incompatibility in insects. *Proc. natn. Acad. Sci. U.S.A.* **89**, 2699–2702.
Rand, D.M. & Harrison, R.G. 1986 Mitochondrial DNA transmission in crickets. *Genetics* **114**, 955–970.
Rigaud, T., Mocquard, J.P. & Juchault, P. 1992 The spread of parasitic sex factors in populations of *Armadillidium vulgare* Latr (Crustacea, Oniscidea): effects on sex ratio. *Genet. Sel. Evol.* **24**, 3–18.
Rousset, F. & Raymond, M. 1991 Cytoplasmic incompatibility in insects: why sterilize females? *Trends Ecol. Evol.* **6**, 54–57.
Rousset, F., Bouchon, D., Pintureau, B., Juchault, P. & Solignac, M. 1992 *Wolbachia* endosymbionts responsible

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

for various alterations of sexuality in arthropods. *Proc. R. Soc. Lond.* B **250**, 91–98.

Rubin, G.M. & Spradling, A.C. 1982 Genetic transformation of *Drosophila* with transposable element vectors. *Science, Wash.* **218**, 348–353.

Spradling, A.C. 1986 P element-mediated transformation. In *Drosophila, a practical approach* (ed. D. B. Roberts), pp. 175–197. Oxford: IRL press.

Shaw, M.W. & Hewitt, G.M. 1990 B chromosomes, selfish DNA and theoretical models: where next? In *Oxford surveys in evolutionary biology*, vol. 7 (ed. D. Futuyama & J. Antonovics), pp. 197–223. Oxford University Press.

Silver, L.M. 1993 The peculiar journey of a selfish chromosome: mouse *t* haplotypes and meiotic drive. *Trends Genet.* **9**, 250–254.

Slatkin, M. 1986 Interchromosomal biased gene conversion, mutation and selection in a multigene family. *Genetics* **112**, 681–698.

Southern, D.I. & Pell, P.E. 1973 Chromosome relationships and meiotic mechanisms of certain *morsitans* group tsetse flies and their hybrids. *Chromosoma* **44**, 319–334.

Taylor, D.R. 1990 Evolutionary consequences of cytoplasmic sex ratio distorters. *Evol. Ecol.* **4**, 235–248.

Temin, R.G., Ganetzky, B., Powers, P.A., Lyttle, T.W., Pimpinelli, S., Dimitri, P., Wu, C. & Hiraizumi, Y. 1991 Segregation distorter in *Drosophila melanogaster*: genetic and molecular analyses. *Am. Nat.* **137**, 287–331.

Turelli, M. & Hoffmann, A.A. 1991 Rapid spread of an inherited incompatibility factor in Californian *Drosophila*. *Nature, Lond.* **353**, 440–442.

Turelli, M., Hoffmann, A.A. & McKechnie, S.W. 1992 Dynamics of cytoplasmic incompatibility and mtDNA variation in *Drosophila simulans* populations. *Genetics* **132**, 713–723.

Wallace, B. 1968 Polymorphism, population size, and genetic load. In *Population biology and evolution* (ed. R. C. Lewontin), pp. 87–108. New York: Syracuse University Press.

Walsh, J.B. 1985 Interaction of selection and biased gene conversion in a multigene family. *Proc. natn. Acad. Sci. U.S.A.* **82**, 152–157.

Walsh, J.B. 1986 Selection and biased gene conversion in a multigene family: consequences of interallelic bias and threshold selection. *Genetics* **112**, 699–716.

Wood, R.T., Cook, L.M., Hamilton, A. & Whitelaw, A. 1977 Transporting the marker gene *re* (red eye) into a laboratory cage population of *Aedes aegypti* using meiotic drive at the M^p locus. *J. med. Entomol.* **14**, 461.

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