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Engineered Underdominance Allows Efficient and Economical Introgression of Traits into Pest Populations

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A novel form of underdominance is suggested as a mechanism that is able to drive desired genes into pest populations through the release of transgenic individuals over one or more generations. Such a mechanism is urgently needed by those working to reduce the impact of malaria by releasing strains of *Anopheles*, the vector of the disease, that are not susceptible to malaria parasites. We use simple population genetics models to quantify the benefits conferred when heterozygous genotypes, arising from matings between introduced and wild individuals, are not viable. In a randomly mating population, underdominant systems accelerate introgression of desired alleles and allow the release of individuals to be discontinued once the frequency of transgenic alleles attains a threshold. A set of two constructs, which together are selectively neutral but lethal when one is carried without the other, are found to produce dynamics that are characteristic of underdominant systems. When these constructs are carried on non-homologous chromosomes, then the ratio of released to natural born individuals need only be greater than 3 : 100 for introgression to occur. Furthermore, the threshold for the gene frequencies over which the introduced genes are expected to become fixed upon discontinuing the release of transgenic individuals is surprisingly low. The location of the threshold suggests that the introduced genes are expected to spread in space, at least locally. For the first time, the prospect of a practical drive mechanism for the genetic manipulation of pest populations is raised.

1. Introduction

The potential of genetic manipulation for the control of diseases and pests critically depends on finding a mechanism which can drive genes into wild populations. The search for such a mechanism has ranged far and wide, fuelled by the prospect of reducing the impact of malaria by genetic manipulation of its vector (Curtis, 1994). The recent and rapid spread of *P* elements in natural populations of *Drosophila melanogaster* (Engels, 1997) motivated suggestions that transposable elements might provide a viable means of driving transgenes into arthropod populations. A second suggestion due to Curtis & Sinkins (1998) has been cytoplasmic incompatibility (CI). Turelli & Hoffmann (1999) modelled a number of approaches to using CI as a drive mechanism for transgenes but did not express confidence that any of the approaches were practical. Underdominance has a long history as a candidate for introducing desired genes into insect populations. Curtis (1968) was the first to propose the use of translocations to fix desirable genes and

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went on to test his ideas in the laboratory (Curtis & Adak, 1974; Curtis, 1976) and the field (Curtis *et al*., 1982). He was preceded by Serebrovsky (1940) who suggested that since translocation heterozygotes are usually semi-sterile, the release of translocation homozygotes might lead to the eradication of a pest due to a population-level reduction in fertility. In this manuscript, we propose an engineered form of underdominance as a general mechanism able to fix desirable genes into free-ranging pest populations.

Selection is the natural mechanism for the spread of a new allele, usually a mutation, where individuals which carry the allele are conferred an advantage. However, it is important for applications to pest populations that the fitness of the population not be increased. Underdominance is a form of selection but only reduces the fitness of heterozygous genotypes relative to homozygous genotypes. In its simplest form, underdominance acts at a single locus where there are two alleles, say A and a. It occurs when the heterozygote, genotype Aa, is less fit than either the AA homozygote or the aa homozygote. Such systems are unusual because they are unstable. For example, when the two homozygotes are of equal fitness, then, whichever allele is initially predominant becomes rapidly fixed in the population while the alternative vanishes. This propensity to fix alleles or alternatively, to eliminate alleles, is characteristic of underdominant systems (Crow, 1986) and is the reason as to why they are relevant to genetic manipulation of pest populations.

We present the theoretical behaviour of two constructs consisting of two copies of the desired gene, two pairs of suppressors and promoters which regulate the expression of two different lethal genes and the lethal genes themselves. These constructs, denoted by α and β, are conceptually portrayed in Fig. 1. We also note that α and β do not necessarily have to carry the same desired gene. If a desired *trait* was required, necessitating the introduction of two new genes, then this is also possible.

The theoretical behaviour of underdominant systems is well established. General models of selection operating at a single locus, where underdominance is discussed, are given by Spiess (1977), Crow (1986) and Crow & Kimura (1970).

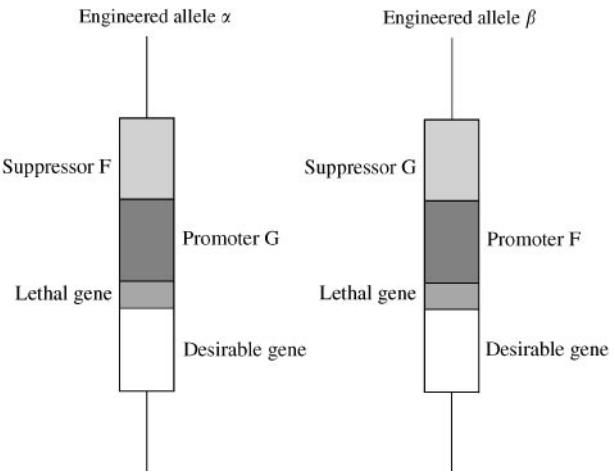

FIG. 1. A set of two constructs that produce an underdominant system. Individuals carrying both α and β alleles will survive to breed and are assumed to have equal fitness relative to individuals that carry neither, i.e. wild type. Individuals that carry one or more copies of one of the constructs but no copies of the other are not viable. For these genotypes, one of the lethal genes will be promoted. Expression of the lethal genes may be delayed such that death would occur at the onset of sexual maturity but this need not be the case.

The constructs in Fig. 1, however, are novel and are not covered by these models. In this paper, we establish that the constructs represent a drive mechanism which is well suited to ensuring the persistence and spread of desirable genes in pest populations. We apply a long-established mathematical approach—difference equations for a large, randomly breeding population having non-overlapping generations—to evaluate the potential of the constructs to improve genetic manipulation of pest species.

2. Extreme Underdominance

Consider an extreme example of underdominance where the difference in fitness between the heterozygote and the two homozygotes is absolute—the former genotype is not viable while the latter two genotypes are. This system represents the simplest scenario with which we may contrast the behaviour of the constructs in Fig. 1.

We first model the tendency of extreme underdominance to rapidly fix one allele and eliminate the alternative allele. Suppose allele a was introduced into a wild population (all of genotype AA)

by releasing individuals of genotype *aa*. Genotype *Aa* is not viable, but genotypes *aa* and *AA* are of equal fitness. Suppose further that *a* is introduced into a species having a lifespan and breeding behaviour such that the population consists of discrete non-overlapping generations. Let u_k, v_k and w_k denote the proportions of the k-th generation that are of genotypes *AA*, *Aa* and *aa*, respectively. Individuals of genotype *Aa* do not make a genetic contribution to subsequent generations. We denote the proportion of gametes produced by the $(k-1)$-th generation which carry the *A* allele by A_k. With this notation, the genotype proportions u_k, v_k and w_k are given by A_k^2, $2A_k(1-A_k)$ and $(1-A_k)^2$ but if only viable individuals are included, then, only those of homozygous genotype are to be counted. In this case, in order to calculate the gene frequencies which will determine the genotype frequencies in the next generation, the proportions A_k^2 and $(1-A_k)^2$ must be rescaled by the proportion which is viable. For example, if the heterozygote dies upon attaining sexual maturity then, these frequencies do not apply to the generation once it has become sexually mature. If the population is allowed to move through generations without further interference then the governing equation for the wild-type allele is

$$A_{k+1} = \frac{u_k}{u_k + w_k} = \frac{A_k^2}{A_k^2 + (1-A_k)^2} \tag{1}$$

$$= \frac{A_k^2}{2A_k^2 - 2A_{k+1}} \tag{2}$$

which has fixed points $A^* = 0$, 1/2 and 1.

To analyse the stability of these fixed points, we calculate

$$\frac{\mathrm{d}}{\mathrm{d}A_k}\left(\frac{A_k^2}{2A_k^2 - 2A_k + 1}\right)\bigg|_{A_k = A^*} = \frac{2A^*(1-A^*)}{(2A^{*2} - 2A^* + 1)^2}. \tag{3}$$

From eqn (3) we see immediately that both 0 and 1 are stable fixed points. Substituting $A^* = 1/2$ gives

$$\frac{\mathrm{d}}{\mathrm{d}A_k}\left(\frac{A_k^2}{2A_k^2 - 2A_k + 1}\right)\bigg|_{A_k = 1/2} = \frac{1/2}{1/4} = 2 > 1 \tag{4}$$

and so is unstable [see Kaplan & Glass (1995)]. The location and stability of the three fixed points suggest that unless A_0 is exactly equal to 1/2 then the allele that is predominant, whether it be *A* or *a*, will become fixed.

Now consider the deliberate introgression of *a* into a wild population (all of genotype *AA*), achieved by the sustained release of genotype *aa*. Unless the number of individuals released is large enough for the new allele to instantly become the predominant allele, introgression will be hampered during the initial release because of the inherent tendency to fix the predominant allele, i.e. *A*. However, assuming that the frequency of *a* can be increased over a number of generations such that it becomes the predominant allele, introgression will be accelerated.

The "strength" of the tendency of an underdominant system to fix alleles can be quantified by the effort required to reverse the allele which is predominant in the system. With the deliberate introduction of a new allele into a wild population, the wild-type allele will be initially fixed in the population, so, we are interested in measuring the effort required to eliminate the wild-type allele. In the simple example we have developed so far, the effects of underdominance are symmetric so that the strength of the tendency to fix either allele is the same. We quantify the tendency to fix alleles by modifying the governing equations, which was previously simplified to eqn (2), so as to include the deliberate and sustained release of individuals carrying *a*. The expressions for the genotype frequencies must now include the effect of releasing genotype *aa* in every generation. We find,

$$u_k = q_k A_k^2, \tag{5}$$

$$v_k = q_k 2A_k(1-A_k), \tag{6}$$

$$w_k = q_k(1-A_k)^2 + (1-q_k), \tag{7}$$

and

$$A_{k+1} = \frac{u_k}{u_k + w_k} = \frac{A_k^2}{2A_k^2 - 2A_k + (1/q_k)}, \tag{8}$$

where q_k represents the proportion of the k-th generation which arises from natural breeding.

The remaining proportion of the k-th generation, $1 - q_k$, are released individuals of genotype *aa*. If we assume that $q_k = q$ then, eqn (8) is amenable to analytic methods of analysis. The alternative to making this assumption is to model the effect of releasing transgenic individuals on the density of the population. This in turn requires a further set of equations for the population dynamics of the target population. The numbers may be inflated by the addition of homozygous individuals, or alternatively, the loss of heterozygotes may decrease the size of the population. Our immediate interest is the dynamics of the gene frequencies in the population, so we assume that the population size remains constant and the number of transgenics released is also constant. These two assumptions imply that $q_k = q$.

Setting $q_k = q$ in eqn (8), and solving the resulting equation for fixed points we find that $A_{k+1} = A_k = A^*$ must satisfy

$$A^* = \frac{(A^*)^2}{2(A^*)^2 - 2A^* + (1/q)}. \tag{9}$$

This implies $A^* = 0$, or,

$$A^* = \frac{1}{4}\left(3 \pm \sqrt{9 - \frac{8}{q}}\right). \tag{10}$$

If $q < 8/9$, then the expression under the square root of eqn (10) is negative so that $A^* = 0$ is the only real fixed point. If $q > 8/9$ then there are three real fixed points. We conclude that for values of q larger than 8/9, the tendency to fix the predominant allele will keep the frequency of allele A at approximately 3/4. If the number of *aa* individuals released is high enough, such that $q < 8/9$, then the introduced allele a will become fixed. This is borne out in Fig. 2. Whether or not it is feasible in practice to require that q be less than 8/9—meaning that for every eight individuals produced by natural breeding, one or more transgenic individuals must be released—is both species-specific and application-specific. Nevertheless, we have found that the initial pressure to fix the A allele can be overcome by releasing sufficiently high numbers of genotype *aa*.

The tendency to fix the wild-type allele during the initial stages of introgression will also decrease the rate of introgression while the wild-type allele is predominant. We compare in Fig. 3, the rate of introgression when heterozygotes survive and breed [taken from Davis & Fulford (1999)] with the rate obtained when underdominance is operating such that heterozygotes are not viable. The latter is given by iterating eqn (8). Fig. 3 illustrates that introgression is indeed

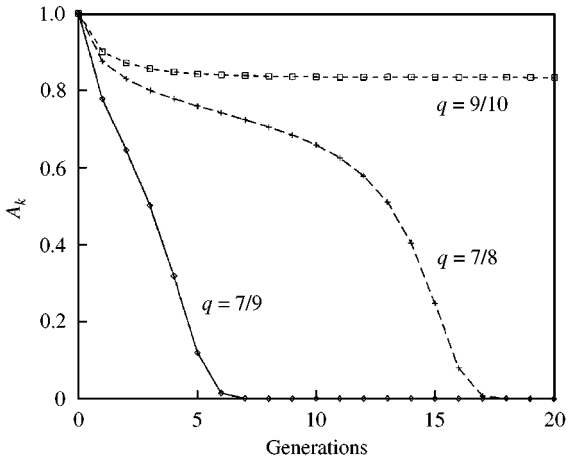

FIG. 2. Introgression of a new allele, a, where genotype *Aa* is not viable. For $q = 9/10$ ($> 8/9$) the wild-type allele (A) remains predominant. For $q = 7/8$ and 7/9 (both smaller than 8/9) A vanishes and the new allele quickly becomes predominant.

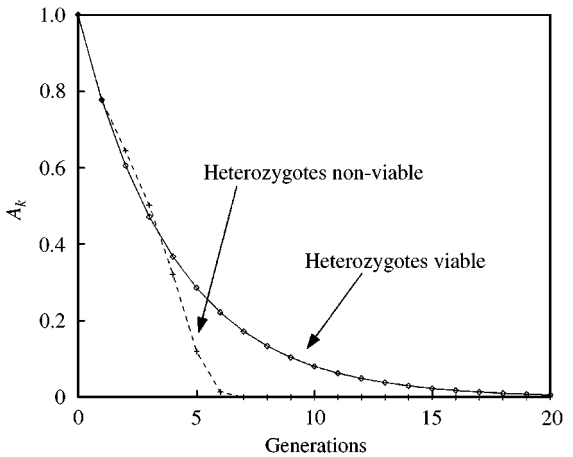

FIG. 3. A comparison of rates of introgression with $q = 7/9$, between when heterozygotes are viable (the solid line) and when they are not (the broken line). For a short time, the frequency of the wild-type allele decreases at a faster rate for the former than the latter. However, once $A_k < 1/2$, the rate of introgression for the case where heterozygotes are not viable increases appreciably as the instability in the system works to fix the new allele rather than hamper introgression.

hampered by underdominance while $A_k > 1/2$, but is accelerated once $A_k < 1/2$. The benefit of the latter clearly outweighs the delay of the former, at least when $q = 7/9$. What is even more remarkable is a comparison of the tails of the two curves. After 20 generations, when heterozygotes are viable, the frequency of the transgenic allele is still visibly tending to zero rather than being indistinguishable from zero. In contrast, when heterozygotes are not viable, the frequency of the wild-type allele is indistinguishable from zero after the seventh generation.

3. Engineering Underdominance

The model in the previous section represents extreme underdominance operating at a single locus. Success in engineering a single allele which generates extreme underdominance—the heterozygote not viable while the two homozygotes have equal fitness—appears to be unlikely (Peter Grewe, pers. comm.). A less daunting alternative is the creation of the two constructs portrayed in Fig. 1. The salient feature of α and β is that the promoters and suppressors for the two toxin genes are cross linked. In the absence of the matching suppressor, promoter G and promoter F will cause an expression of the toxin gene below them, resulting in the death of the individual. This means that individuals who carry one or more copies of α but no copies of β, or alternatively, one or more copies of β and no copies of α, are not viable. Individuals who carry one or more copies of both constructs are viable because both suppressors are present in the genome and neither toxin gene is expressed.

With two constructs to be inserted into the genome, there are three distinct configurations which are possible. If the insertion of new DNA can be directed at a specific site, then it is theoretically possible for engineered individuals to carry α and β at one locus. Otherwise, if the constructs insert randomly across the genome, then it is more than likely that they will appear at two different loci on non-homologous chromosomes (the second configuration). Finally, there is a probability that they will insert on the same pair of homologous chromosomes but at different loci. The three possibilities are illustrated in Fig. 4. The third case is a theoretical possibility but will be a rare event if integration sites are random, and if integration sites are not random, then this configuration would be avoided. We therefore do not consider the third case.

If α and β appear at the same locus (the first case) then the released genotype would be $\alpha\beta$. If α and β appear on unlinked loci (the second case) then the released genotype would be $\alpha\alpha\beta\beta$. The F_0 and F_1 generations resulting from inter-breeding between released and wild-type individuals for the first configuration are illustrated in Fig. 5a. The F_0, F_1 and F_2 generations for the second configuration are illustrated in Fig. 5b.

In Fig. 5b, the genotypes $A\alpha\beta\beta$ and $\alpha\alpha B\beta$ are shown as viable. This assumes that the presence of a single copy of suppressor F (or G) is enough to prevent the two copies of the relevant promoter from expressing the toxin gene. This is inherently likely since the suppressor codes for a protein then binds to the promoter. The binding action of the protein may be imperfect, but this will be the case regardless of whether there are one or two copies of the suppressor.

3.1. HOMOLOGOUS

We first consider releasing individuals of genotype $\alpha\beta$. These individuals carry the two engineered constructs at a single locus. As depicted in Fig. 5a, when there are 3 alleles—α, β and A (which denotes the wild-type allele)—there are six possible genotypes. Only two of those genotypes survive to produce offspring. The remaining four are not viable due to the action of α in the absence of β or β in the absence of α.

Suppose that due to the once-off release of individuals of genotype $\alpha\beta$, both alleles are present in a wild population. We will once again assume that this wild population has a lifespan and breeding behaviour such that the population consists of discrete non-overlapping generations. We let α_k represent the frequency of gametes carrying the first engineered allele, β_k the second allele and A_k the wild-type allele. These three frequencies apply to the gametes produced by the $(k-1)$-th generation. Now let G_k denote the frequency of genotype G in the k-th generation (before the actions of α and β take affect) where G is one of AA, $A\alpha$, $A\beta$, $\alpha\alpha$, $\beta\beta$ or $\alpha\beta$.

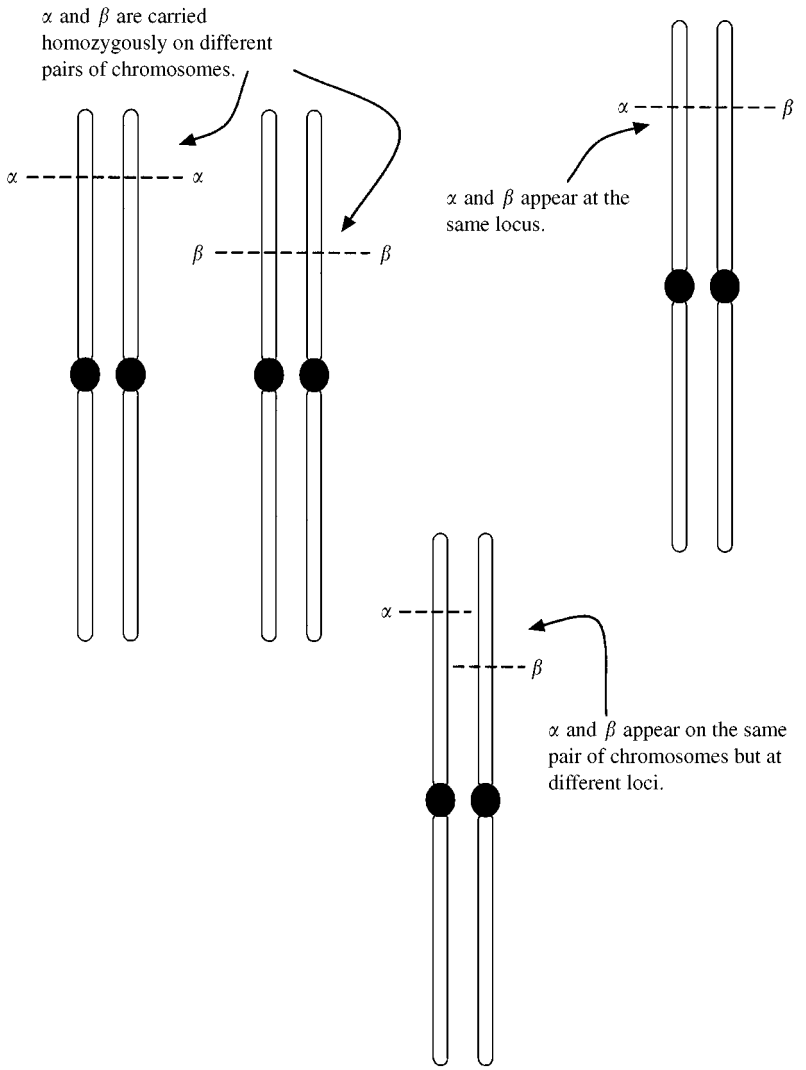

FIG. 4. If there are two engineered alleles, α and β, then there are three possible arrangements for how they might be carried on a set of chromosomes. Chromosomes are depicted as homologous pairs.

Assuming that the population mates at random, the G_k will fall into the usual Mendelian ratios,

$$AA_k = A_k^2, \tag{11}$$

$$A\alpha_k = 2A_k\alpha_k, \tag{12}$$

$$A\beta_k = 2A_k\beta_k, \tag{13}$$

$$\alpha\alpha_k = \alpha_k^2, \tag{14}$$

$$\beta\beta_k = \beta_k^2, \tag{15}$$

$$\alpha\beta_k = 2\alpha_k\beta_k. \tag{16}$$

However, four of these genotypes ($A\alpha$, $A\beta$, $\alpha\alpha$ and $\beta\beta$) are not viable and we account for the loss of non-viable genotypes by rescaling the gametic contributions of viable genotypes by the proportion of the population which actually contributes

$$A_{k+1} = \frac{AA_k}{AA_k + \alpha\beta_k}, \tag{17}$$

$$\alpha_{k+1} = \beta_{k+1} = \frac{\frac{1}{2}\alpha\beta_k}{AA_k + \alpha\beta_k}. \tag{18}$$

The three allelic proportions—α_k, β_k and A_k—must sum to one, implying $\alpha_k = \beta_k = \frac{1}{2}(1 - A_k)$.

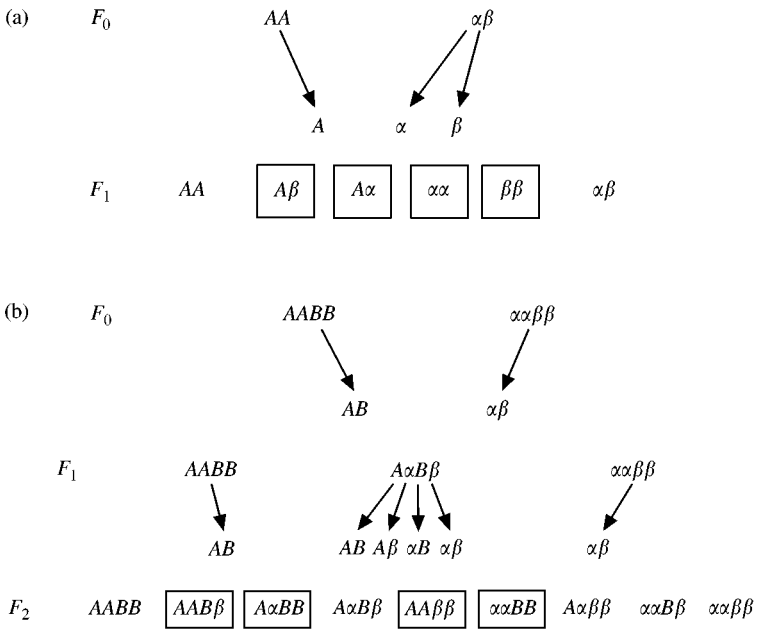

FIG. 5. The F_0, F_1 and F_2 generations when the two engineered alleles depicted in Fig. 1 appear at (a) a single locus, and (b) two unlinked loci. Arrows indicate haplotypes produced by genotypes. Boxed genotypes are not viable and there is no difference in fitness between the remaining genotypes.

Using this equation and substituting eqn (11) into eqn (17), the nine difference equations reduce to a single difference equation which is the equation parallel to eqn (2),

$$A_{k+1} = \frac{2A_k^2}{3A_k^2 - 2A_k + 1}. \tag{19}$$

Equation (19) has fixed points $A^* = 0$, 1/3 and 1 and as before, we differentiate with respect to A_k to obtain

$$\left.\frac{\mathrm{d}}{\mathrm{d}A_k}\left(\frac{2A_k^2}{3A_k^2 - 2A_k + 1}\right)\right|_{A_k = A^*}$$

$$= \frac{4A^*(1 - A^*)}{(3(A^*)^2 - 2A^* + 1)^2} \tag{20}$$

so that, as one might expect, $A^* = 0$ and 1 are stable while

$$\left.\frac{\mathrm{d}A_{k+1}}{\mathrm{d}A_k}\right|_{A^* = 1/3} = 2 \tag{21}$$

so that $A^* = 1/3$ is unstable. This analysis reveals a system that behaves much like the extreme example of underdominance. There is the same tendency to fix alleles, either the wild-type allele becomes fixed ($A^* = 1$) or the wild-type allele vanishes ($A^* = 0$), but the system is not symmetric and favours the former outcome—the wild-type allele will become fixed if the initial frequency of the wild-type allele is above 1/3.

If we now modify eqn (19) further so as to model the ongoing and regular release of individuals of genotype $\alpha\beta$, we obtain the equivalent of eqn (8). As before, the modification to the governing difference equation is slight but significant. Recalling that q is the proportion of each generation that arises from natural breeding then

$$AA_k = qA_k^2, \tag{22}$$

$$A\alpha_k = 2qA_k\alpha_k, \tag{23}$$

$$A\beta_k = 2qA_k\beta_k, \tag{24}$$

$$\alpha\alpha_k = q\alpha_k^2, \tag{25}$$

$$\beta\beta_k = q\beta_k^2, \tag{26}$$

$$\alpha\beta_k = 2q\alpha_k\beta_k + (1 - q) \tag{27}$$

and

$$A_{k+1} = \frac{AA_k}{AA_k + \alpha\beta_k} \tag{28}$$

become, upon simplification,

$$A_{k+1} = \frac{2A_k^2}{3A_k^2 - 2A_k - 1 + (2/q)}. \tag{29}$$

This first-order difference equation has fixed points

$$A^* = 0 \text{ and } \frac{1}{3}\left(2 \pm \sqrt{7 - \frac{6}{q}}\right). \tag{30}$$

We conclude that the critical value of q, for this underdominant system, is 6/7. This means that the introgression of genotype $\alpha\beta$ will occur if for every six individuals recruited naturally, one or more $\alpha\beta$ individuals are released.

The two critical values of the system—the unstable fixed point for A_k and the value of q below which the wild-type allele is expected to vanish—both indicate that more effort is required to fix the α and β alleles than to fix the hypothetical a in the first example of underdominance that we analysed. The release of transgenics must not stop until A_k is less than 1/3 and this value will not be reached unless $q < 6/7$. If either of these conditions is not satisfied then, the original allele will persist.

3.2. NON-HOMOLOGOUS

Now consider releasing individuals of genotype $\alpha\alpha\beta\beta$. The two engineered alleles, α and β, are carried on separate homologous pairs of chromosomes. A denotes the wild-type alternative at the locus at which α appears, and B denotes the wild-type alternative at the locus at which β appears. Since the constructs appear at two loci, there are a total of nine possible genotypes (only five of which are viable) and four haplotypes. Fig. 5(b) illustrates how inter breeding of the two genotypes $AABB$ (the only genotype present prior to the release of transgenic individuals) and $\alpha\alpha\beta\beta$ (the genotype released) gives rise to the $A\alpha B\beta$ genotype in the first generation. The full suite of genotypes appears in the second generation. We are concerned with whether the system displays the same tendency to fix alleles as the previous systems did. To verify this, we assume an initial presence of haplotypes carrying α and β and model the frequencies of all haplotypes in subsequent generations.

We represent the proportion of AB, $A\beta$, αB and $\alpha\beta$ haplotypes produced at breeding (which determine the genotype frequencies of the k-th generation) by AB_k, $A\beta_k$, αB_k and $\alpha\beta_k$, respectively. Similarly, G_k represents the proportion of the k-th generation, prior to sexual maturity, that is of genotype G where G is one of $AABB$, $AABb$, $A\alpha BB$, $A\alpha B\beta$, $AA\beta\beta$, $\alpha\alpha BB$, $A\alpha\beta\beta$, $\alpha\alpha B\beta$ or $\alpha\alpha\beta\beta$. We write down the governing equations by rescaling the gametic contributions from viable genotypes by the proportion of the generation that is viable,

$$AB_{k+1} = \frac{AABB_k + \frac{1}{4}A\alpha B\beta_k}{AABB_k + A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k}, \tag{31}$$

$$A\beta_{k+1} = \frac{\frac{1}{2}A\alpha\beta\beta_k + \frac{1}{4}A\alpha B\beta_k}{AABB_k + A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k}, \tag{32}$$

$$A\beta_{k+1} = \frac{\frac{1}{2}\alpha\alpha B\beta_k + \frac{1}{4}A\alpha B\beta_k}{AABB_k + A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k}, \tag{33}$$

$$\alpha\beta_{k+1} = \frac{\alpha\alpha\beta\beta_k + \frac{1}{2}A\alpha\beta\beta_k + \frac{1}{2}\alpha\alpha B\beta_k + \frac{1}{4}A\alpha B\beta_k}{AABB_k + A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k}, \tag{34}$$

where

$$AABB_k = AB_k^2, \tag{35}$$

$$A\alpha B\beta_k = 2AB_k\alpha\beta_k + 2A\beta_k\alpha B_k, \tag{36}$$

$$A\alpha\beta\beta = 2A\beta_k\alpha\beta_k, \tag{37}$$

$$\alpha\alpha B\beta = 2\alpha B_k\alpha\beta_k, \tag{38}$$

$$\alpha\alpha\beta\beta_k = \alpha\beta_k^2. \tag{39}$$

The substitution of eqns (35)–(39) into eqns (31)–(34) eliminates the genotype frequencies and leaves a system of four equations for the haplotype frequencies. This set of four reduces to just two by noting that (a) if $A\beta_0 = \alpha B_0$ then $A\beta_k = \alpha B_k$ for all k, and (b) the gamete proportions sum to 1. Substituting

$$A\beta_k = \alpha B_k = \tfrac{1}{2}(1 - AB_k - \alpha\beta_k) \qquad (40)$$

finally yields

$$AB_{k+1} = f(AB_k, \alpha\beta_k) = \frac{AB_k^2 + \frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1 - AB_k - \alpha\beta_k)^2}{AB_k^2 + \alpha\beta_k(2 - \alpha\beta_k) + \frac{1}{2}(1 - AB_k - \alpha\beta_k)^2}, \quad (41)$$

$$\alpha\beta_{k+1} = g(AB_k, \alpha\beta_k) = \frac{\alpha\beta_k - \frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1 - AB_k - \alpha\beta_k)^2}{AB_k^2 + \alpha\beta_k(2 - \alpha\beta_k) + \frac{1}{2}(1 - AB_k - \alpha\beta_k)^2}. \quad (42)$$

By inspection, (1, 0) and (0, 1) are fixed point solutions of eqns (41) and (42) and, respectively, represent the wild-type (AB) and transgenic ($\alpha\beta$) haplotypes becoming fixed in the population. A third fixed point, $(\alpha\beta^*, AB^*) \approx (0.17, 0.63)$, represents the co-existence of alleles α and A, and β and B.

We take advantage of the fact that when $A\beta_k = \alpha B_k$ then $AB_k + 2A\beta_k + \alpha\beta_k = 1$ and map a number of trajectories onto a triangular grid to create a DeFinetti diagram [Hoppensteadt & Peskin (1992)]. To do this, we iterate eqns (41) and (42) from four sets of initial conditions for AB_0 and $\alpha\beta_0$. For each trajectory, the resulting set of points $(AB_k, \alpha\beta_k)$ are mapped to $AB_k\mathbf{p} + \alpha\beta_k\mathbf{q} + (1 - AB_k - \alpha\beta_k)\,\mathbf{r}$ where $\mathbf{p}$, $\mathbf{q}$ and $\mathbf{r}$ were chosen to be $(-1, 0)$, $(1, 0)$ and $(0, \sqrt{3})$. The results are shown in Fig. 6. Associated with fixed points $(AB^*, \alpha\beta^*) = (1, 0)$ and $(0, 1)$ (the bottom left and right-hand vertices of the triangle) are basins of attraction. The trajectories which begin in these regions will converge to the associated fixed point. The curve which separates the two basins (denoted by the broken line in Fig. 6) includes the third (unstable) fixed point. It is referred to as the separatrix. Of the four trajectories in Fig. 6, two have initial values $(AB_0, \alpha\beta_0)$ which lie to the left of the separatrix and so converge to the fixed point (1, 0) while the remaining two have initial values which lie to the right of the separatrix and so converge to (0, 1). Finally, the trajectories shown in Fig. 6 promptly converge to a set of points referred to as the centre manifold [see for example Kaplan & Glass (1995)]. The trajectories which begin away from the centre manifold are strongly attracted towards it.

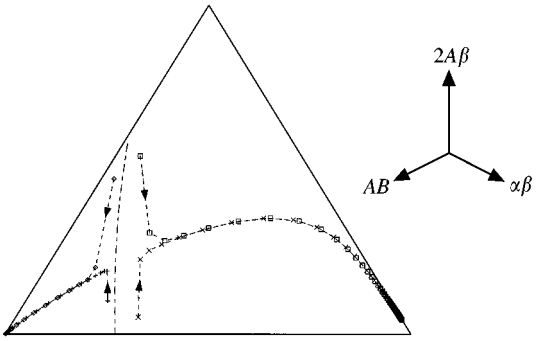

FIG. 6. DeFinetti diagram showing trajectories for the two loci non-homologous system. There is a clear tendency for the system to eliminate alleles from both loci so that either the wildtype alleles remain or the transgenic alleles remain. The four trajectories converge to a stable manifold which leads either to the bottom right-hand vertex, representing the point at which the transgenic alleles become fixed, or, the bottom left-hand vertex, representing the point at which the wild-type alleles become fixed. The two bottom vertices correspond to the two stable fixed points of the system with which is associated a basin of attraction. The dashed curve running vertically through the DeFinetti diagram separates the two basins of attraction and is known as the separatrix of the system. The separatrix was found numerically by iterating from a fine grid of initial conditions and recording the end-point of each trajectory.

With respect to the fixed points of the system, the non-homologous case has demonstrated the characteristic behaviour of underdominant systems—that there are three fixed points, one unstable and two stable, where the two stable fixed points represent alternative sets of alleles becoming fixed. However, as a means of comparison, Fig. 6 is inadequate because the homologous case is one dimensional, whereas, the non-homologous case is two dimensional. Despite this, the separatrix in Fig. 6 (which determines which haplotype becomes fixed) appears promisingly close to the fixed point (1, 0), which would be the

starting point for the deliberate introgression of α and β. A fairer means of comparison between the three scenarios is the critical value of q which determines whether the sustained release of transgenics leads to α and β becoming fixed or whether it merely means persistence of the constructs at low frequencies. In the very first example of underdominance, the critical value of q was 8/9, for the homologous case with two alleles it was 6/7. To determine the critical value of q for the non-homologous case, we modify eqns (41) and (42) (as we have done previously) so as to include the release of individuals of genotype $\alpha\alpha\beta\beta$ in each generation. Equations (35)–(39) become

$$AABB_k = qAB_k^2, \tag{43}$$

$$A\alpha B\beta_k = 2qAB_k\alpha\beta_k + 2A\beta_k\alpha B_k, \tag{44}$$

$$A\alpha\beta\beta = 2qA\beta_k\alpha\beta_k, \tag{45}$$

$$\alpha\alpha B\beta = 2q\alpha B_k\alpha\beta_k, \tag{46}$$

$$\alpha\alpha\beta\beta_k = q\alpha\beta_k^2 + (1-q) \tag{47}$$

and now eqns (31)–(34) simplify to

$$AB_{k+1} = \frac{q(AB_k^2 + \frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1-AB_k-\alpha\beta_k)^2)}{q(AB_k^2+\alpha\beta_k(2-\alpha\beta_k)+\frac{1}{2}(1-AB_k-\alpha\beta_k)^2)+1-q}, \tag{48}$$

$$\alpha\beta_{k+1} = \frac{q(\alpha\beta_k - \frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1-AB_k-\alpha\beta_k)^2) + 1 - q}{q(AB_k^2+\alpha\beta_k(2-\alpha\beta_k)+\frac{1}{2}(1-AB_k-\alpha\beta_k)^2)+1-q}. \tag{49}$$

The critical value of q below which the only physically possible fixed point solution to eqns (48) and (49) is (0, 1), was determined numerically. It lies within the interval (0.97, 0.975). Fig. 7 illustrates the behaviour of the system for three values of q—0.98, 0.97 and 0.9. For $q \leqslant 0.97$ the α and β constructs become fixed. This does not represent a criterion which is difficult to satisfy. For example, releasing three individuals of genotype $\alpha\alpha\beta\beta$ for every 100 individuals recruited naturally will achieve the introgression of α and β and therefore an introgression of the desired genes.

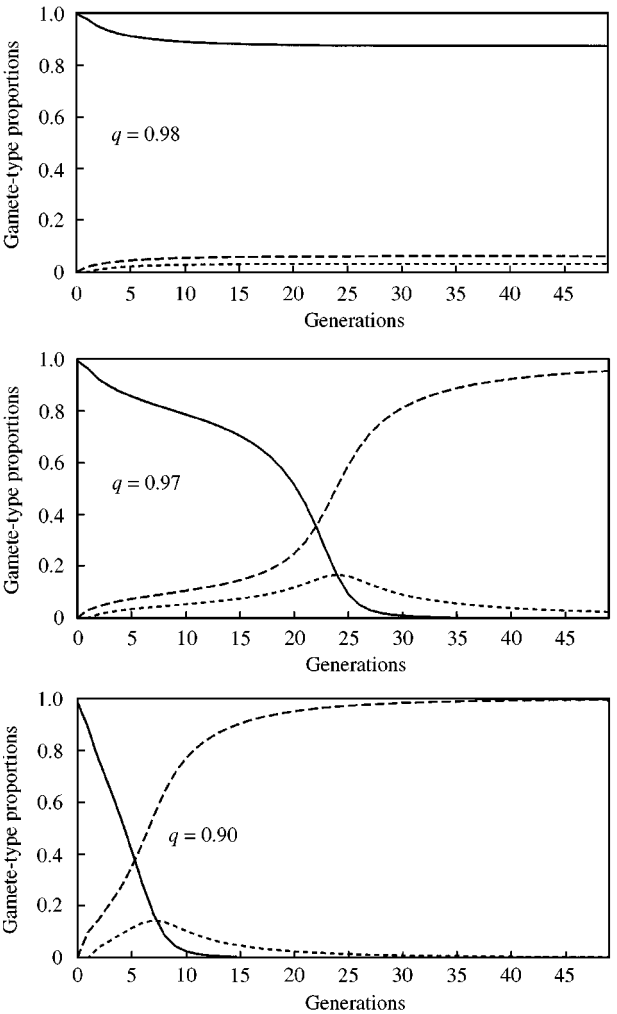

FIG. 7. The haplotype frequencies as a function of the number of generations since the release of genotype $\alpha\alpha\beta\beta$ into a randomly breeding population. The results illustrate the sensitivity of the dynamics to q. For $q = 0.98$, the new haplotype fails to become predominant while for $q = 0.97$ all other haplotypes decline to 0. The results for $q = 0.9$ demonstrate how quickly the haplotype AB can vanish from the population 33. (——) AB_k; (– – – –) $\alpha\beta_k$; (- - - - -) αB_k.

4. Control

The introgression of inducible genes through the sustained release of transgenic animals was proposed as a form of biological control for common carp, *Cyprinus carpio* in Australia (Grewe, 1996). Accelerating the introgression of inducible

genes into free-ranging pest populations is a suitable application of the α and β constructs.

The expression of inducible genes is controlled by a trigger. Exposure to the trigger causes death or sterility in individuals that carry the transgene, but the trigger is otherwise benign. Since very low concentrations of the trigger may be enough to cause expression, control using inducible genes may have advantages over traditional methods of control. If death results from exposure to the trigger then the gene is known as an inducible fatality gene (IFG) and if sterility results then it is an inducible sterility gene (ISG). Genes for insecticide susceptibility may be viewed as inducible genes, where application is specific to the management of insect populations and the trigger is of course the appropriate insecticide. At present, inducible gene technology for vertebrates is being developed at the individual species level but is a costly and time-consuming process.

The original strategy, as proposed by Grewe (1996), was to release transgenic young to mature with each generation of naturally born individuals, until the proportion of the population carrying the transgene was close to 1 [see also Grewe (1997)]. Exposing the population to the trigger would then (ideally) cause the population to collapse, either through a large number of immediate deaths for an IFG or a large reduction in the number of births in the case of an ISG. If predominance of the inducible gene can be recovered while population density is low, then the inducible gene may be triggered periodically to achieve control (Davis *et al*., 2000). However, the introgression of a selectively neutral gene into a wild population (an inducible gene is assumed to be neutral while the trigger is absent) is slow and requires that a regular release of transgenic individuals be sustained indefinitely (Davis & Fulford, 1999; Davis *et al*., 1999). A second problem is that the use of the trigger to reduce density invariably creates selection against the inducible gene. Even if release of transgenics is sustained indefinitely the presence of the inducible gene in the population can be reduced to ineffectual levels by too frequent use of the trigger [see Davis *et al*. (2000)].

For the underdominant systems we have modelled, once introgression has progressed beyond a critical stage, it will continue even if managers choose to stop releasing transgenics. This constitutes a limited form of self-propagation that significantly improves the practicality of using an IFG or ISG because it means that the ongoing release of transgenics is no longer a condition for long-term control.

Apart from increasing the rate at which introgression occurs and reducing the effort required, underdominant systems may reduce density during introgression. This was in fact pointed out by Serebrovsky (1940) who long ago proposed the release of translocation homozygotes as a form of pest control. The proportion of a new generation which is not viable depends directly on the allele frequencies in the previous adult generation. For example, in the first and simplest of the underdominant systems we have considered, the proportion of a generation that is heterozygous (and so not viable) is given by

$$2A_k(1 - A_k). \tag{50}$$

This expression attains a maximum of a half when $A_k = 1/2$. Therefore, while A_k is close to 1/2, a fraction of each new generation is not viable. However, if A_k is close to either 0 or 1 then the fraction of each new generation that is not viable is low.

For the engineered form of underdominance we have modelled, when α and β appear at the same locus, only two of the possible six genotypes are viable, $\alpha\beta$ and AA. This means that none of the offspring resulting from a cross between the two genotypes are viable and only 1/2 of the offspring from a self cross of genotype $\alpha\beta$ are viable. An interesting consequence is that if the whole population were all of genotype $\alpha\beta$, then only half of their offspring would be viable.

Finally, when α and β appear on unlinked loci, then, the behaviour is more like simple extreme underdominance—the proportion of the population not viable reaches a maximum during introgression of the two constructs but then decreases to zero when the constructs become fixed.

For all of these cases, the effect of the loss of individuals on population density depends on the strength and timing of any compensatory density-dependent effects. In any case, such reductions to density are not intended to be the

principal means of control though it is a useful side-effect. To achieve substantial reductions to abundance, the original strategy of exposing the population to the trigger—which will induce death or sterility in those carrying transgenic alleles α or β—is to be used.

Davis *et al.* (2000) have shown that using a trigger to bring about large reductions in abundance also creates high selection pressure against IFG or ISG alleles. This is true regardless of whether underdominance is operating or not. The difference that underdominance creates is the presence of an opposing selection force that counters the selection for wild-type alleles due to an exposure of a pest population to the trigger. Where underdominance is operating, managers can play off the tendency of an unstable system to fix alleles against the selection pressure introduced by control measures. The intensity of selection pressure is partly determined by the effectiveness of whatever delivery mechanism is used to expose the population to the trigger. If every individual in the population is reached by the delivery mechanism, then the only surviving members of the population would be wild type. While this may have killed or sterilized almost all of the population, the distribution of genotypes will be as it was before the introduction of transgenic individuals. This means that control over the population is lost, introgression must begin again, and the long-term advantages of using an underdominant system are irrelevant. It is in fact difficult to imagine a delivery mechanism which is capable of reaching every single individual in a wild population and it is obviously not advantageous to do so anyway. Rather, if the effectiveness of the delivery mechanism was manipulated such that the proportion of wild-type alleles never became so large as to reverse the introgression achieved, then, the means of control would not be lost.

So we have arrived at the key question for the use of inducible genes in pest control: what is a "safe" proportion of the population for the delivery mechanism to reach? If it is too high then, even if underdominance is at work, the trend towards transgenic alleles becoming fixed may be reversed. In the long term, this would constitute a significant waste of time and effort. If it is too low then reductions to abundance may not be of satisfactory significance. In response to this dilemma we return, at least initially, to the first and simplest underdominant system we considered. This system involved the two alleles a and A. We now assume that not only does the hypothetical allele a cause underdominance but that, it also acts as an inducible fatality gene. This means that genotype aa is vulnerable to exposure to the trigger. We assume that genotype Aa is absent from the breeding population due to the effect of underdominance. Modifying eqn (2) so as to include the impact of exposure of the population to the trigger on the frequency of genotype aa gives

$$A_{k+1} = \frac{u_k}{u_k + (1-x)w_k} = \frac{A_k^2}{A_k^2 + (1-x)(1-A_k)^2}, \tag{51}$$

where x is the proportion of the population exposed to the trigger and exposure occurs in every generation. The higher the value of x, the more efficient is the delivery mechanism and the higher the selection pressure created by using the trigger. If we require that $A_{k+1} \leqslant A_k$, then the resulting inequality specifies a bound for x such that upon satisfying this bound, exposure of the population may occur "safely" every year.

$$x < \frac{1-2A_k}{1-A_k}. \tag{52}$$

Alternatively, if it is x that is fixed, we can rewrite the condition as a requirement on A_k, a level of introgression required before the trigger may be safely used

$$A_k < 1 - \frac{1}{2-x}. \tag{53}$$

For example, if $x = 0.9$ then $A_k < \frac{1}{11}$, or, if $x = 0.5$ then $A_k < \frac{1}{3}$.

The analogous problem for the two allele non-homologous case is less simple. At what stage of introgression, in terms of the haplotype proportions $\alpha\beta_k$ and AB_k, is it safe to begin triggering with efficiency x each generation. Modifying the governing system of equations to include the impact of exposing the population to the trigger

gives the following system

$$AB_{k+1} = \frac{AABB_k + \frac{1}{4}(1-x)A\alpha B\beta_k}{AABB_k + (1-x)(A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k)}, \tag{54}$$

$$A\beta_{k+1} = \frac{\frac{1}{2}(1-x)A\alpha\beta\beta_k + \frac{1}{4}(1-x)A\alpha B\beta_k}{AABB_k + (1-x)(A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k)}, \tag{55}$$

$$A\beta_{k+1} = \frac{\frac{1}{2}(1-x)\alpha\alpha B\beta_k + \frac{1}{4}(1-x)A\alpha B\beta_k}{AABB_k + (1-x)(A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k)}, \tag{56}$$

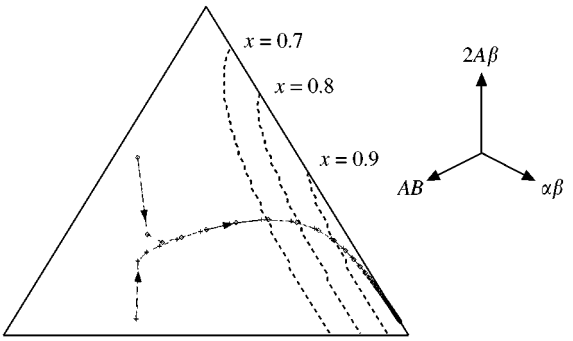

FIG. 8. Separatrixes for the system given by eqns (58) and (59) and two trajectories taken from Fig. 6. The trajectories show where the centre manifold of the undisturbed system intersects the separatrixes for the triggering systems when $x = 0.7$, 0.8 and 0.9. The separatrixes were found numerically by iterating from fine grids of initial conditions and recording the end-point of each trajectory.

which again reduces to a much simpler system of two linked difference equations

$$AB_{k+1} = \frac{AB_k^2 + (1-x)(\frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1 - AB_k - \alpha\beta_k)^2)}{AB_k^2 + (1-x)(\alpha\beta_k(2-\alpha\beta_k) + \frac{1}{2}(1 - AB_k - \alpha\beta_k)^2)}, \tag{58}$$

$$\alpha\beta_{k+1} = \frac{(1-x)(\alpha\beta_k - \frac{1}{2}AB_k\alpha\beta_k + \frac{1}{8}(1 - AB_k - \alpha\beta_k)^2)}{AB_k^2 + (1-x)(\alpha\beta_k(2-\alpha\beta_k) + \frac{1}{2}(1 - AB_k - \alpha\beta_k)^2)}. \tag{59}$$

Figure 8 shows three separatrixes for this system on a DeFinetti diagram where x has been fixed to 0.7, 0.8 and 0.9. We assume that prior to exposure, the population is free from outside interference so that the haplotype proportions will be following the centre manifold depicted in Fig. 6. This means that the intersection of the centre manifold and the separatrixes arising from

$$\alpha\beta_{k+1} = \frac{(1-x)\alpha\alpha\beta\beta_k + \frac{1}{2}(1-x)A\alpha\beta\beta_k + \frac{1}{2}(1-x)\alpha\alpha B\beta_k + \frac{1}{4}(1-x)A\alpha B\beta_k}{AABB_k + (1-x)(A\alpha B\beta_k + A\alpha\beta\beta_k + \alpha\alpha B\beta_k + \alpha\alpha\beta\beta_k)} \tag{57}$$

eqns (58) and (59) are critical points. Triggering with efficiency x prior to reaching this point will lead to the loss of the α and β constructs from the pest population and therefore, triggering will have little or no impact on pest density.

5. Overlapping Generations

The inclusion of overlapping generations complicates equations such as eqn (8), but if a stable age structure is assumed, then the underlying dynamics are exactly the same and the critical value for q remains unchanged. If m is the age at sexual maturity, M is the maximum lifespan, and a_i is the proportion of the breeding population of age i then, for example

$$A_k = \sum_{i=m}^{M} a_i \frac{A_{k-i}^2}{2A_{k-i}^2 - 2A_{k-i} + 1/q}, \tag{60}$$

where

$$\sum_{i=m}^{M} a_i = 1, \tag{61}$$

is the equation parallel to eqn (8) where there are $M - m$ overlapping generations. Looking for fixed points by setting $A_k = A_{k-1} = \cdots A_{k-M} = A^*$ gives the same fixed points as for the non-overlapping case.

The rate of introgression is a central concern for the genetic manipulation of pests as the practicality of the technique requires that the time periods involved be short. While the inclusion of overlapping generations will not change the underlying dynamics of the underdominant systems we have presented, it is certain to slow introgression. Davis *et al.* (1999) have studied the effect of lifespan on the rate of introgression of a neutral gene. As a consequence, it was suggested that species with long lifespans are not good candidates for genetic control. The length of time that the original wild population still contributes genetically to new generations is critical in determining rates of introgression. If this length of time is a long period, then there is little hope for fast results even with the advantages of underdominance. Genetic manipulation, not used in conjunction with any other control method, remains effectively restricted to species having short lifespans.

6. Leakiness

An assumption behind the results we have presented is that the expression of all genetic constructs is stable. That is, transgenic individuals never revert to wild-type individuals. There are thought to be a number of epigenetic mechanisms through which expression is lost, all of which frustrate attempts to engineer plants and animals. For the systems we have presented, there are a number of places where failure of the introduced DNA to express will change the behaviour of the system. For example, the combination of alleles illustrated in Fig. 1 may be "leaky"—that is, the binding action of the protein to either promoter F or G due to the presence of suppressor F or G may be imperfect such that individuals carrying alpha and beta may not be viable. Alternatively, those genotypes which are boxed in Fig. 5 may survive. Depending on the frequency with which this occurs, the instability of the system may be compromised to the extent that the benefits we have discussed vanish. In the case of control using inducible genes, if the IFG or ISG fails to be expressed when the population is exposed to the trigger, then control over the pest will be limited. The future of genetic manipulation of pests may depend on the success of geneticists in creating transgenic lines selected for an ability to remain stable after crossing with wild-type individuals.

7. Spatial Dynamics

The implications of spatial dynamics for achieving introgression of the α and β constructs are unclear. The dynamics of hybrid zones, assumed to be maintained by dispersal and selection against hybrids, have been analysed by Barton (1979) and various co-workers (Pialek & Barton, 1997; Barton & Hewitt, 1989). We follow the example of Turelli & Hoffmann (1999) and identify this body of theory as most relevant to the spatial behaviour of α and β.

For a single locus underdominant system, the results of Barton (1979) suggest that the critical value for the unstable equilibrium is 0.5. If the unstable equilibrium is below this value, then the introduced allele is expected to spread through a homogeneously distributed population, assuming that it has been introduced at a sufficiently high frequency to become locally predominant. If on the other hand, the unstable equilibrium is above 0.5, then dispersal and selection will combine to eliminate the introduced allele, even if it was once locally fixed. The implication of these results when α and β are carried at the same locus is immediately apparent: the relevant unstable equilibrium is 2/3 and so the constructs are not expected to spread. When the constructs are carried on non-homologous chromosomes, then, the theoretical results are not directly applicable since the unstable equilibrium is no longer represented by a single point. However, if the value of 0.5 simply represents equal frequencies of the alternative alleles, then the same state can be represented for the two-locus system of concern

by a vertical line that exactly bisects the triangle in Fig. 6. We now see that the separatrix of the two-loci system shown in Fig. 6 sits well to the left of such a vertical line, so, we would conclude that the α and β constructs would spread in space. Having reached this conclusion, we note, as did Turelli & Hoffmann (1999), that the work of Pialek & Barton (1997) suggests that even though the conditions for local spread are met, spatial spread on a larger scale can be easily prevented by natural barriers to gene flow.

8. Conclusions

We began by developing a model for a hypothetical system where extreme underdominance operated at a single locus and was determined by just two alleles. The tendency to fix alleles was quantified by the effort required to replace the predominant allele with the alternative allele by regularly releasing genotypes carrying the alternative allele. Effort was measured by the value of q below which introgression was successful. Recall that q represents the proportion of each generation born in the wild so that the lower the value of the threshold for q, the more the effort required. The value of q was found to be 8/9, and since the system is symmetric, this value applied to both alleles.

We then considered the behaviour of the constructs α and β when engineered individuals carried them at the same locus. In this case, there are three alleles, the third being the wild-type allele. The resulting system was unstable, either the wild-type allele became fixed or it vanished from the population. The critical initial value for the frequency of wild-type alleles was 1/3. If the frequency of the wild-type allele was less than 1/3, then the wild-type allele was expected to vanish, resulting in a population entirely of genotype $\alpha\beta$. If the frequency of the wild-type allele was greater than 1/3 then α and β were expected to vanish from the population, resulting in a population entirely of genotype AA. It was noted that if the entire population was of genotype $\alpha\beta$ then only 1/2 of the offspring of such a population would be viable since a self-cross of genotype $\alpha\beta$ produces genotypes $\alpha\alpha$ (not viable), $\alpha\beta$ (viable) and $\beta\beta$ (not viable) in the ratio of 1 : 2 : 1. However, the critical value of q for this system was 6/7, indicating that a high degree of effort would be required to achieve a successful introgression of α and β if they were carried in this configuration.

A second configuration for α and β is for the constructs to appear at two unlinked loci. This system was unstable as well and we found that the initial frequencies of two of the haplotypes determined which sets of alleles became fixed in the population. In the previous two systems, the initial value of a single allele frequency determined the outcome. In this case, the outcome is determined by which side of a curve in two-dimensional space the initial point (determined by two initial haplotype frequencies) lies. This meant that while the qualitative behaviour was the same, the results cannot be compared directly with the single values found for the previous two systems. The position of the critical curve did, however show that the system is asymmetric. Furthermore, the asymmetry favours the α and β alleles becoming fixed rather than the equivalent wild-type alleles becoming fixed. For example, if all the allele frequencies were initially equal, then the engineered constructs would be fixed while the wild-type alleles vanished. The second critical value—that for q—was found to be 0.97. This is significantly closer to 1 than either 8/9 or 6/7. It indicates that less effort is required to ensure a successful introgression of α and β when they are carried at unlinked loci. Finally, for this configuration for α and β, the theoretical results of Barton (1979) suggest that α and β will propagate locally in space.

For any particular application, the models are too simple and care must be taken to improve the models to incorporate the population biology of a target population. However, our results suggest that the pair of constructs, α and β, represent a practical and general drive mechanism for the genetic manipulation of wild pest populations.

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