Adaptation to the cost of resistance: a model of compensation, recombination, and selection in a haploid organism

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Populations of pathogenic organisms often evolve resistance in response to the use of pesticides or antibiotics. This rise of resistance may be followed by a fall when chemical control is suspended and resistance alleles carry a fitness cost. Another possibility is that mutations at secondary loci compensate for the cost, usually without loss of resistance. This enables resistant types to withstand invasion by the susceptible wild-type; resistance then persists in the population, which reduces the efficacy of future pesticide or antibiotic use. We examined a two-locus model of a haploid organism that adapts to the cost of resistance by a single compensatory mutation. We addressed the question how different combinations of cost and compensation and different levels of recombination affect the consequences of a single pesticide application. Resistance will become fixed in the population when the fraction of the population exposed to pesticide exceeds the cost of resistance. Compensatory mutations reduce the cost of resistance and therefore this threshold level of pesticide use. In the absence of pesticide, recombination promotes stability of equilibria. In the presence of pesticide, recombination accelerates the fixation of resistance and compensating alleles; recombination may also enable the persistence of compensated resistant types after pesticide use.

Keywords: cost of resistance; compensatory evolution; pesticides; epistasis; resistance

1. INTRODUCTION

The use of chemicals to control populations of pathogenic organisms has often led to the rapid spread of alleles conferring resistance to these chemicals (Palumbi 2001). Resistance frequently comes at a cost, however, so that the susceptible wild-type has a higher fitness than resistant mutants when antibiotics or pesticides are absent. On the positive side, this fitness difference may cause susceptible types to return to their dominant position in pathogen populations when chemical control is suspended (Levin *et al.* 1997; Andersson & Levin 1999; Lipsitch 2001). On the negative side, a cost of resistance selects for mutations that reduce this cost, usually without loss of resistance. Recent experiments on micro-organisms show the ease with which the cost of resistance is reduced by mutations at secondary loci: the cost of resistance was rapidly compensated for in streptomycin-resistant *Escherichia coli* (Schrag & Perrot 1996; Schrag *et al.* 1997), rifampin-resistant *E. coli* (Reynolds 2000) and *Helicobacter pylori* (Björkholm *et al.* 2001), fusidic acid-resistant *Staphylococcus aureus* (Nagaev *et al.* 2001), and fluconazole-resistant *Candida albicans* (Cowen *et al.* 2001).

Compensatory mutations may also have occurred in organisms where cells are attacked from within, e.g. by restriction enzymes or toxins in bacteria (Hayes 2003). In these systems, cells without protection destroy themselves while protection without toxicity is probably costly.

Mutations that modify the cost can facilitate the evolution of both attack and defence mechanisms. Compensatory adaptation not only applies to resistance mutations, however, but also to deleterious mutations in general. Moore *et al.* (2000) showed that deleterious effects of random insertion mutations in evolving lines of *E. coli* were compensated for while retaining these mutations (see also Poon & Otto (2000) for a short review of the experimental literature).

Compensatory mutations are not only of practical importance but also of theoretical interest: they provide an opportunity to study the origin and maintenance of gene interactions (epistasis) in adaptive evolution. Compensating second site mutations make a return to the susceptible state unlikely, as back mutations at either the resistance locus or the compensatory locus result in genotypes with a lower fitness than both the uncompensated susceptible and the compensated resistant genotypes. Hence, it shows how two loci can become parts of a group of coadapted genes that effectively prevent reverse evolution (Teotónio & Rose 2001).

Pesticide and antibiotic resistance have been modelled extensively (reviewed by Peck 2001) and a cost of resistance is often taken into account. Subsequent modification of the cost is rarely dealt with, however. As compensatory mutations may reduce the efficacy of chemical control by slowing the decline of resistance, understanding their dynamics is critical. Here we present a simple model of a haploid organism, e.g. a pathogenic fungus, that adapts to the cost of resistance by a single compensatory mutation. We use both analytical solutions (for continuous pesticide use) and

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Table 1. Fitness values of the susceptible-uncompensated (*SU*), susceptible-compensated (*SC*), resistant-uncompensated (*RU*) and resistant-compensated (*RC*) genotypes in unexposed (W_u) and exposed (W_e) environments. (These environments occur with frequencies $1-f$ and f , respectively. W_i is the mean fitness across environments of genotype i . The selection coefficients s_r , s_{rc} and s_c represent the cost of resistance without compensation, the cost of resistance with (or after) compensation, and the cost of compensation, respectively.)

genotype	frequency	W_u	W_e	W_i
<i>SU</i>	x_1	1	0	$1-f$
<i>SC</i>	x_2	$1-s_c$	0	$(1-f)(1-s_c)$
<i>RU</i>	x_3	$1-s_r$	$1-s_r$	$1-s_r$
<i>RC</i>	x_4	$1-s_{rc}$	$1-s_{rc}$	$1-s_{rc}$

simulations (for temporary pesticide use) to examine how selection and recombination affect populations dominated by the wild-type (susceptible and uncompensated) or the double mutant (resistant and compensated) genotypes.

2. MODEL

Consider a large, random-mating population of a haploid organism with discrete generations. A fraction, f , of this population is exposed to a pesticide, leaving a fraction, $1-f$, unexposed. At the first (or resistance) locus the wild-type *S* allele renders its bearer susceptible to this pesticide; the mutant *R* allele confers resistance but this resistance comes at a cost ($s_r > 0$). At the second (or compensatory) locus the mutant *C* allele compensates for (part of) the cost of resistance (reducing s_r to s_{rc} , which may result in $s_{rc} < 0$) whereas the wild-type *U* allele has no effect on the cost. The *C* allele may be costly, however, when associated with an *S* allele. Table 1 lists the genotypes and their fitnesses. The expected frequency of genotype i after selection becomes $x'_i = W_i(x_i - \delta r D) / \bar{W}$, where $\delta = 1$ (for $i = SU$ and *RC*) or -1 (for $i = SC$ and *RU*), r is the recombination rate, $D = x_1 x_4 - x_2 x_3$, and $\bar{W} = \sum (x_i - \delta r D) W_i$. We ignore stochastic effects, migration and mutation so that we can focus exclusively on the effects of selection and recombination.

The different outcomes of our model can be explored by considering the behaviour around the boundary equilibria of the four genotypes. The matrix of partial derivatives (the Jacobian matrix) describes the linearized dynamics of the system around these equilibria. The local stability of equilibrium points can be determined by inspection of the eigenvalues of the Jacobian matrix evaluated at equilibrium; stability is indicated when the absolute values of all four eigenvalues are smaller than unity.

- (i) Equilibrium 1: $x_1 = 1$, $x_2 = 0$, $x_3 = 0$ and $x_4 = 0$. The eigenvalues of the Jacobian matrix are 0, $1-s_c$, $\frac{1-s_r}{1-f}$ and $\frac{(1-r)(1-s_{rc})}{1-f}$.
- (ii) Equilibrium 2: $x_1 = 0$, $x_2 = 1$, $x_3 = 0$ and $x_4 = 0$. The eigenvalues of the Jacobian matrix are $\frac{1}{1-s_c}$, 0, $\frac{(1-r)(1-s_r)}{(1-f)(1-s_c)}$ and $\frac{1-s_{rc}}{(1-f)(1-s_c)}$.
- (iii) Equilibrium 3: $x_1 = 0$, $x_2 = 0$, $x_3 = 1$ and $x_4 = 0$. The eigenvalues of the Jacobian matrix are $\frac{1-f}{1-s_r}$, $\frac{(1-f)(1-r)(1-s_c)}{1-s_r}$, 0 and $\frac{1-s_{rc}}{1-s_r}$.
- (iv) Equilibrium 4: $x_1 = 0$, $x_2 = 0$, $x_3 = 0$ and $x_4 = 1$. The eigenvalues of the Jacobian matrix are $\frac{(1-f)(1-r)}{1-s_{rc}}$, $\frac{(1-f)(1-s_c)}{1-s_{rc}}$, $\frac{1-s_r}{1-s_{rc}}$ and 0.

Figure 1. The effect of different initial conditions after 25 000 generations. The grey area corresponds to initial allele frequencies that result in fixation of the *SU* genotype; initial allele frequencies in the white area result in fixation of the *RC* genotype. Parameter values: $f = 0$, $s_r = 0.25$, $s_{rc} = 0.05$, $s_c = 0.1$ and $r = 0.25$.

We will now consider how the conditions for stability are affected by different values of s_r , s_c , s_{rc} and r in both the absence and presence of pesticide use.

3. RESULTS

(a) No pesticide use ($f = 0$)

The first thing to note is that equilibria 2 and 3 will never be stable when, as we assume, $s_c > 0$ and $s_{rc} < s_r$. Without recombination ($r = 0$), the first equilibrium is stable when $s_r > 0$, $s_{rc} > 0$ and $s_c > 0$; equilibrium 4 is unstable in this case. With $r > 0$, equilibrium 1 is stable when $s_r > 0$, $s_c > 0$ and $s_{rc} > 1 - \frac{1}{1-r}$ (note that because $0 \leq r \leq 0.5$, non-zero values for r allow $s_{rc} < 0$); equilibrium 4 is stable when $s_{rc} < s_c$ and $s_{rc} < r$. The conditions for stability of these two equilibria are not mutually exclusive and which equilibrium will be attained depends on the initial allele frequencies (figure 1). With recombination the numerators of eigenvalues containing r (these eigenvalues are associated with genotypes containing alleles not present in the fixed genotype) become smaller and the conditions for stability will therefore be more readily fulfilled than without recombination.

(b) Continuous pesticide use ($f > 0$)

Applying pesticide does not inevitably lead to a stable equilibrium of one of the resistant genotypes. With $r = 0$, equilibrium 1 remains stable as long as $f < s_r$, $f < s_{rc}$, and

Figure 2. The effect of recombination (r) and the level of spraying (f) on the outcome of pesticide use after 25 000 generations. Grey area: fixation of SU genotype; white area: fixation of RC genotype. Parameter values: $s_r = 0.25$, $s_{rc} = 0.05$ and $s_c = 0.1$. The initial frequency of both the S and the U allele is 0.9999.

$s_c > 0$; when $f > s_r$ and $f > s_{rc}$, equilibrium 4 is stable. We therefore have a threshold level of spraying $f > s_r$ that marks the transition from equilibrium 1 to equilibrium 4 (we assume $s_{rc} < s_r$ so the condition $f > s_{rc}$ is always met when $f > s_r$). With $r > 0$ and $s_{rc} < f < s_r$ the outcome is determined by the amount of recombination (figure 2): recombination rates lower than $\frac{1}{1-s_{rc}}f - s_{rc}$ result in equilibrium 4 whereas higher values for r yield equilibrium 1. When $f \gg s_r$ and $f \gg s_{rc}$ the time to fixation of the RC genotype depends on the reduction of the cost of resistance (figure 3).

(c) Temporary pesticide use

So far we have assumed the continuous presence of pesticide. With increasing values of f , however, the number of generations of pesticide use (τ) that suffices to cause fixation of both R and C alleles decreases; this number can be found by simulation. Obviously, τ decreases rapidly when s_{rc} approaches zero (figure 4a), but recombination also reduces τ (figure 4b). This effect of recombination only occurs for low levels of pesticide use; for high values of f there is even a slight increase (not visible in the figure) in τ with increasing amounts of recombination.

When pesticides are used for only a short period of time the transition from equilibrium 1 ($x_1 = 1$) to equilibrium 4 ($x_4 = 1$) may occur via equilibria with $x_1 = 0$, $x_2 > 0$, $x_3 = 0$ and $x_4 > 0$. This is illustrated in figure 5a: starting with a mainly susceptible and uncompensated population less than 39 generations spraying yields equilibrium 1 while more than 49 generations spraying results in equilibrium 4; 39–49 generations of pesticide use, however, causes fixation of the C allele but not of the R allele. Figure 5b shows how this result is arrived at for 40 generations of pesticide use: the R allele goes to near-fixation quickly, but its frequency then declines and remains ‘frozen’ at a frequency of 0.92. The C allele, however, rises only slowly in frequency. The timing and duration (in terms of number of generations of pesticide use) of the internal equilibria depend on f : the higher the value of f , the earlier and shorter this transitional spraying period.

Figure 3. Changes in allele frequency under continuous pesticide use of the R allele (solid line) and the C allele (dashed lines). Each dashed line shows a different amount of compensation ($s_{rc} = 0, 0.025, 0.05$ or 0.075) with compensation decreasing from left to right. Parameter values: $f = 0.9$, $r = 0$, $s_r = 0.1$, $s_c = 0.025$. The initial frequencies of both the R and the C alleles are 0.0001.

4. DISCUSSION

The evolution of sets of interacting genes (coadapted gene complexes) is a long-standing problem in evolutionary biology (Whitlock *et al.* 1995; Michalakis & Slatkin 1996; Fenster *et al.* 1997). Gene interactions are central to Wright’s shifting balance theory of evolution where they are necessary (but not sufficient) for the occurrence of fitness peaks and valleys. A difficulty is how to cross fitness valleys: a high-fitness double mutant is unlikely to arise when intermediate genotypes are rare because of their lower fitness. It is therefore not surprising that solutions to this problem (e.g. Phillips 1996; Christiansen *et al.* 1998) usually involve an important role for genetic drift so that intermediate types can rise in frequency by chance. The evolution of the cost of resistance points to a way of circumventing this problem. When selection temporarily favours an intermediate genotype it allows this intermediate to reach a high frequency; the emergence of the double mutant is then much more likely. If such an episode of selection leaves no traces (e.g. it did not affect other loci) then, in hindsight, the widespread occurrence of the double mutant is puzzling.

Adaptation to the cost of resistance makes it easier for resistance to invade a predominantly susceptible population during pesticide use and to persist in that population once the pesticide has disappeared from the environment. The conditions for the RC genotype to persist in the absence of chemical control are still stringent, however, and require recombination when compensation is not complete. Fungi are predominantly haploid; however, recombination is important in outcrossing and the parasexual cycle. In strictly asexual organisms, however, other mechanisms are needed to maintain RC types in a population. Levin *et al.* (2000) suggest that bottleneck effects can account for the long-term persistence of RC genotypes. When compensatory mutations occur more often than reversals they are more likely to pass through bottlenecks; they can therefore remain in the population despite their lower fitness. This mechanism will especially work for mutations

Figure 4. The minimum number of generations spraying (τ) that results in fixation of the RC genotype for different levels of pesticide use (f); and (a) different reduced costs of resistance (s_{rc}) and (b) different recombination rates (r). Parameter values: (a) $s_r = 0.2$, $s_c = 0.1$ and $r = 0$; (b) $s_r = 0.3$, $s_{rc} = 0.2$ and $s_c = 0.1$. The initial frequency of both the S and the U allele is 0.9999.

with small effect as these are expected to arise more often than mutations with large effect (cf. Burch & Chao 1999).

Some of the results we presented apply to evolutionary rather than ecological time-scales. When only a small part of the pathogen population is exposed to pesticide it may take thousands of generations until the RC genotype becomes fixed. However, when most of the population is exposed, fixation occurs within tens of generations, especially when there is recombination. Important here is our assumption that compensating alleles are already present in the population. An additional 'waiting time' would have to be taken into account when compensating alleles have to be introduced into the population by mutation or migration. Phillips (1996) looked at the time to fixation of compensatory mutations in a situation comparable to our unsprayed environment (i.e. both resistance and compensatory alleles are deleterious individually). He showed that the average waiting time can be extremely long in both small populations (where mutations are rare) and large populations (where selection is very effective). In our case, however, resistance and compensatory alleles may already be at a high frequency as a result of recent pesticide use.

In the absence of pesticide use two boundary equilibria are possible, depending on the initial allele frequencies and recombination rate. The initial allele frequencies will mainly depend on the history of selection (i.e. pesticide use). The effect of recombination is a consequence of

Figure 5. The result of temporary pesticide use. (a) The outcome after 25 000 generations with pesticide use in the first 30–50 generations. Open circles, SU genotype; filled circles, RC genotype; squares, SC genotype. Parameter values: $r = 0.125$, $f = 0.5$, $s_r = 0.15$, $s_{rc} = 0.05$, $s_c = 0.05$. The initial frequency of both the S and the U allele is 0.9999. (b) Changes in allele frequencies after 40 generations of pesticide use. Solid line, R allele; dashed line, C allele. Parameter values: $r = 0.125$, $f = 0.5$, $s_r = 0.15$, $s_{rc} = 0.05$, $s_c = 0.05$. The initial frequencies of both the R and the C allele are 0.0001.

breaking up the combinations RC (at the SU boundary) or SU (at the RC boundary). At the SU boundary, recombination even allows s_{rc} to take negative values so that RC types have a higher fitness than the wild-type SU. With constant pesticide levels recombination affects the equilibria when $s_{rc} < f < s_r$: in this range little or no recombination (and therefore little or no decoupling of R and C alleles) benefits the RC genotype whereas high recombination rates cancel the RC genotype's fitness advantage in the sprayed part of the population. A similar effect is seen with temporary pesticide use where recombination has the effect of considerably shortening the time to fixation.

So far we have not dealt with the nature of the cost of resistance nor have we been explicit about the mechanism(s) by which compensation is achieved. However, by assigning fitness values $1 - s_r$ and $1 - s_{rc}$, respectively, to the RU and RC genotypes in both the presence and absence of pesticide we implicitly assumed that the cost is not conditional on the presence or absence of pesticide. When resistance is costly only in the absence of pesticide (and the

RU and RC genotypes have fitness 1 instead of $1-s_r$ and $1-s_{rc}$ in the sprayed part of the environment) both resistance and compensation will spread even faster than our results indicate.

More realism can be added to our model in several ways. We considered a single compensatory mutation, but as long as compensation is not complete there will be selection for any additional mutation that reduces the remaining fitness cost. These second (third, etc.) compensatory mutations may be conditional on the first (second, etc.) mutation and thereby add to the complexity of the model. Furthermore, we assumed that $s_c > 0$, i.e. the compensatory mutation is slightly deleterious on a susceptible background, to account for its low frequency in the population before spraying. However, the C allele will still be compensatory (and epistatic) as long as its fitness enhancing effect is stronger when associated with an R allele than with an S allele. When $s_c < 0$, equilibrium 1 becomes unstable and the conditions for stability of equilibria 3 and 4 become more stringent. A stable equilibrium 2 becomes a possibility when $s_c M < 0$, however, resulting in the continued presence of compensatory alleles in a susceptible population.

Extension of the model to a diploid pest organism requires that we consider the degree of dominance of both resistance and compensatory mutations. The degree of dominance of the resistance mutation is an important issue in the 'high-dose/refuge' (HRD) strategy. This strategy aims at delaying the evolution of resistance by creating a refuge (i.e. an untreated area) for susceptible genotypes that can keep most of the resistance alleles in heterozygous condition. With a high dose heterozygotes are killed; resistance is then effectively recessive which slows its spread through the population. The concepts of effective dominance or recessivity do not apply to haploid organisms, however, and the HRD strategy is therefore unlikely to work against haploid pests (Rausher 2001). Moreover, our model shows that leaving part of the environment ($1-f$) untreated is an important factor in determining the RC genotype's success. Clearly then, there is the need to know if compensatory evolution can interfere with the HRD strategy in diploid organisms such as insects.

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