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EPISTATIC INTERACTIONS CAN LOWER THE COST OF RESISTANCE TO MULTIPLE CONSUMERS

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Abstract.—It is widely assumed that resistance to consumers (e.g., predators or pathogens) comes at a "cost," that is, when the consumer is absent the resistant organisms are less fit than their susceptible counterparts. It is unclear what factors determine this cost. We demonstrate that epistasis between genes that confer resistance to two different consumers can alter the cost of resistance. We used as a model system the bacterium *Escherichia coli* and two different viruses (bacteriophages), T4 and λ, that prey upon *E. coli*. Epistasis tended to reduce the costs of multiple resistance in this system. However, the extent of cost savings and its statistical significance depended on the environment in which fitness was measured, whether the null hypothesis for gene interaction was additive or multiplicative, and subtle differences among mutations that conferred the same resistance phenotype.

Key words.—Bacteria, cost of resistance, epistasis, resistance, viruses.

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Organisms have evolved numerous mechanisms for resisting pathogens, predators, and herbivores, and such resistance can have profound effects on the structure and function of ecological communities. The evolution of resistance can, for example, increase diversity by allowing the coexistence of competitors (Leibold 1996) and alter the regulation of food webs (Polis and Strong 1996; Bohannan and Lenski 1997). Understanding the evolution of resistance is therefore essential for understanding many aspects of ecology.

It is widely assumed that resistance comes at a "cost," that is, when the consumer (e.g., pathogen) is absent the resistant organisms are less fit than their susceptible counterparts (Simms 1992). Theoretically, the cost of resistance is an important determinant of the equilibrium level of resistance. Researchers have attempted to measure the cost of resistance empirically; in some cases costs of resistance have been detected, whereas in others they have not (Simms and Rausher 1987; Lenski 1988a; Simms 1992). It is unclear what factors determine whether resistance incurs a cost. Here, we demonstrate that epistasis between genes that confer resistance to two different consumers can alter the cost of resistance. We used as a model system the bacterium *Escherichia coli* and two different viruses (bacteriophages), T4 and λ, that prey upon *E. coli*. Epistasis tended to reduce the costs of multiple resistance in this system. However, the extent of cost savings and its statistical significance depended on the environment in which fitness was measured, whether the null hypothesis for gene interaction was additive or multiplicative, and even subtle differences among mutations that conferred the same resistance phenotype.

MATERIALS AND METHODS

We isolated mutants of *E. coli* B (Lenski 1988a; Lenski et al. 1991) that were resistant to either T4 or λ-*vir* (a lytic variant of λ), and we also isolated double mutants resistant to both T4 and λ. Cultures started from single cells of the sensitive progenitor *E. coli* genotype were grown overnight and then exposed to high concentrations of either T4 or λ-*vir* on agar plates, such that only resistant mutants could survive to form colonies. For each virus, we isolated 10 independent resistant mutants. We chose only T4-resistant mutants that were not cross-resistant to another virus, T7. (Two classes of T4-resistant mutants exist, those that are cross-resistant to T7 and those that are not. Those that are cross-resistant incur a much higher cost of resistance [Lenski 1988a]. We sought to obtain a relatively homogeneous set of mutations; hence we excluded T4-resistant mutants that were cross-resistant to T7.) From each λ-resistant genotype, we obtained a double mutant that was resistant to both viruses by exposure to T4. The double mutants were also screened for cross-resistance to T7, and cross-resistant mutants were excluded. All 30 resistant genotypes and their sensitive progenitor were stored as clones at −80°C to prevent any subsequent evolution.

We estimated the cost of resistance by determining the fitness of each resistant genotype relative to its sensitive progenitor, using methods described previously (Lenski 1988a; Lenski et al. 1991). Briefly, the resistant and sensitive strains were coinoculated into minimal medium free of any virus, allowed to grow in direct competition for 24 h, and enumerated via plate counts. We calculated relative fitness as the ratio of the Malthusian parameters of the two competing genotypes (Lenski et al. 1991). The sensitive competitor used in these assays was genetically identical to the sensitive progenitor, with the exception that the sensitive competitor has a single mutation that allows it to grow on the sugar arabinose. Arabinose utilization is selectively neutral in the environments we used to measure fitness (Travisano and Lenski 1996), and this trait was used as a genetic marker to distinguish the competitors during the assays. We used three different competitive environments: glucose-limited medium, maltose-limited medium, and trehalose-limited medium. We used Davis minimal medium (Carlton and Brown 1981) supplemented with 2×10^{-3} μg thiamine hydrochloride per ml

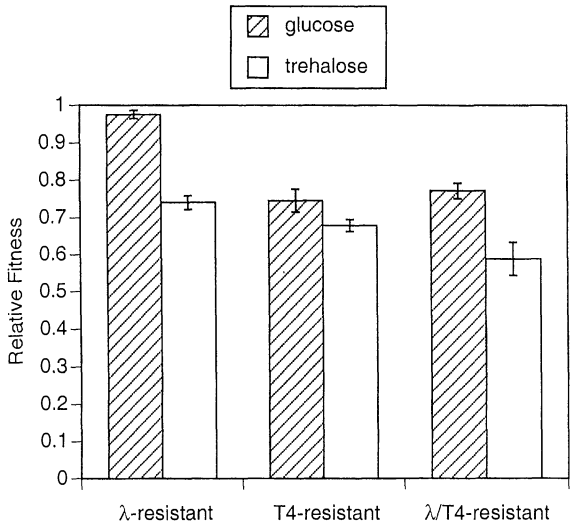

FIG. 1. Effect of environment on the fitness of *Escherichia coli* resistant to virus λ, to virus T4, or to both λ and T4. Fitnesses were measured in glucose- and trehalose-limited environments, and all values are expressed relative to the progenitor strain that is sensitive to both viruses. Each value is the mean fitness for 10 independent mutant genotypes, with two replicate fitness assays run for each genotype. Error bars indicate standard errors of the mean, using degrees of freedom based on the number of independent genotypes.

and 25 μg per ml of the limiting sugar. Two replicates of each competition were performed in each environment.

We tested for epistasis between T4-resistance and λ-resistance genes using two different null models, multiplicative and additive. Let W_L and W_T be the fitnesses of the λ-resistant and T4-resistant single mutants, respectively, and let W_{LT} be the fitness of the double mutant; all fitnesses are expressed relative to the sensitive competitor. According to the multiplicative model, $W_{LT} = W_L W_T$, whereas the additive model gives $W_{LT} = 1 - (1 - W_L) - (1 - W_T) = W_L + W_T - 1$. Both null models were used because each one has certain advantages and disadvantages (Elena and Lenski 1997).

We calculated the *expected* fitness for each double mutant using the estimates of fitnesses obtained from the single mutants. For each double mutant, we had the exact λ-resistant single mutant that was used to construct the double mutant. We then combined (either multiplicatively or additively) the fitness of that particular λ-resistant single mutant with each of 10 fitness values from the 10 independent T4-resistant single mutants. The average of these 10 values (sums or products) is the expected fitness of the double mutant, whereas the range of these values gives an indication of the spread that can be expected given sampling variation and heterogeneity among T4-resistant mutants. We then compared the *observed* fitness of each double mutant with its corresponding expected value. Finally, we used a paired *t*-test to determine whether the mean fitness of the 10 double mutants was significantly different from the mean expected value (Sokal and Rohlf 1995). Any significant difference between observed and expected fitnesses indicates directional epistasis between the two classes of resistance mutation.

TABLE 1. Comparison between observed and expected fitnesses of double mutants in glucose medium, under both multiplicative and additive null models. Expected fitness values are the average of 10 estimates calculated using the exact λ-resistant progenitor and 10 independent T4-resistant mutants. + denotes that the observed fitness of a λ-resistant/T4-resistant double mutant is higher than any of the 10 values that were averaged to produce the corresponding expected fitness. SED, standard error of mean difference.

Double mutant ID	Observed fitness	Expected multiplicative	Observed extreme?	Expected additive	Observed extreme?
6591	0.7704	0.7599		0.7645	
6594	0.6784	0.6980		0.6815	
6596	0.8097	0.7329		0.7283	
6598	0.6957	0.6981		0.6817	
6599	0.6576	0.6953		0.6780	
6602	0.8351	0.7351		0.7312	
6603	0.9070	0.7005	+	0.6849	+
6605	0.7814	0.7732		0.7823	
6619	0.8118	0.7260		0.7191	
6649	0.7740	0.7667		0.7736	
Mean difference		0.0435		0.0496	
SED		0.0233		0.0239	
Paired *t*		1.8663		2.0755	
P		0.0949		0.0678	

RESULTS

The cost of resistance was highly conditional on the assay environment (Fig. 1). In maltose, most λ-resistant mutants and most double mutants were unable to grow, even in the absence of the sensitive competitor (data not shown). Hence, this environment was not suitable for quantifying epistasis. In contrast, there was little or no cost due to λ-resistance in glucose. In trehalose, λ-resistance imposed an intermediate cost. The pattern was quite different for T4-resistance, which imposed a significant cost in all three environments, but did not preclude growth.

The extent of epistasis between the two classes of resistance mutations also depended on the competitive environment. In the glucose environment, the double mutants tended to have slightly higher fitness than expected based on the separate effects of the T4- and λ-resistant mutations (Table 1). However, this effect was not significant for either the multiplicative ($P = 0.0949$) or additive ($P = 0.0678$) null model, based on two-tailed tests. Moreover, in only one case was the observed fitness of a double mutant more extreme than any of the 10 component values that went into calculating each expected value, which is no greater than expected by chance alone.

In the trehalose environment, the differences between the observed and expected fitness values were more pronounced, again in the direction indicating that the two mutations together were less costly than expected from their separate effects (Table 2). On average, this effect was nonsignificant relative to the multiplicative null model ($P = 0.1851$). By contrast, the effect was significant using the additive model ($P = 0.0221$), which had a lower expectation and hence gave a larger discrepancy between observed and expected values. These results become much clearer when one compares each observed fitness value with the range of values used to calculate the expected fitness. Given variation in the measure-

TABLE 2. Comparison between observed and expected fitnesses of double mutants in trehalose medium, under both multiplicative and additive null models. Expected fitness values are the average of 10 estimates calculated using the exact λ-resistant progenitor and 10 independent T4-resistant mutants. + denotes that the observed fitness of a λ-resistant/T4-resistant double mutant is higher than any of the 10 values that were averaged to produce the corresponding expected fitness, whereas − denotes that the observed fitness of a double mutant is lower than any of these 10 values. SED, standard error of mean difference.

Double mutant ID	Observed fitness	Expected multiplicative	Observed extreme?	Expected additive	Observed extreme?
6591	0.9020	0.5353	+	0.4672	+
6594	0.5556	0.3933	+	0.2583	+
6596	0.6240	0.5072	+	0.4259	+
6598	0.6449	0.4721	+	0.3743	+
6599	0.8591	0.5379	+	0.4712	+
6602	0.4453	0.5242	−	0.4509	
6603	0.4542	0.5222		0.4480	
6605	0.3341	0.5630	−	0.5080	−
6619	0.6300	0.5120	+	0.4330	+
6649	0.4341	0.4685		0.3689	+
Mean difference		0.0848		0.1677	
SED		0.0591		0.0608	
Paired *t*		1.4350		2.7608	
P		0.1851		0.0221	

ment of fitness as well as possible heterogeneity among T4-resistant mutants, the probability that the observed fitness for a particular double mutant will, by chance, be more extreme—either higher or lower—than any of the 10 values used to calculate the expected fitness is 0.18 (two of 11). Given 10 double mutants, one would expect perhaps two or three such extreme cases by chance alone. In fact, however, the observed fitnesses in trehalose for eight of the 10 double mutants were more extreme than any of the 10 values used to compute their corresponding expectations (Table 2). Based on the binomial distribution, the exact likelihood that this many, or more, extreme values would be seen by chance alone is very small, only 0.000038. Therefore, this result indicates that there is frequent epistasis among the λ-resistance and T4-resistance mutations with respect to fitness costs in the trehalose environment. However, the epistatic effects are evidently variable, sometimes going in one direction and sometimes in the other, thus obscuring their overall effect when the analysis is based only on the mean effect across many mutations. Nonetheless, in most cases where the double mutants have extreme fitness—six of eight cases under the multiplicative model and seven of eight for the additive model—the direction of the epistatic effect is such that the two resistance mutations cost less together than expected on the basis of their separate fitness effects.

DISCUSSION

Our results indicate that the cost of resistance depends on the specific phenotype as well as the environment in which the cost is measured. Whether there are significant epistatic interactions among resistance mutations with respect to fitness depends on the environment and the precise form of the null hypothesis. The sign and strength of the epistasis also depends on subtle differences among mutations within a given resistance class. Overall, there is a tendency for the cost of bacterial resistance to these two viruses to be somewhat less than expected from their separate costs of resistance, especially in the trehalose environment.

Other researchers have also reported effects of the environment on the cost of resistance. For example, the costs of resistance to downy mildew and to leaf-root aphid in lettuce increase when plants are grown in nutrient-poor soil (Bergelson 1994). Previous studies have also seen epistatic effects that depend on the environment. For example, in tobacco the epistatic variance component for several traits is more pronounced in "extreme" environments than in "normal" environment (Jinks et al. 1973). Still other studies have detected epistatic effects of unknown genetic factors on the cost of resistance. The costs of resistance to downy mildew and leaf-root aphid in lettuce (Bergelson 1994), to crown rust in oats (Frey and Browning 1971), to mosaic virus in cotton (Legg et al. 1979), and to mildew in barley (Bjornstad and Aastveit 1990) are all influenced by genetic background. Modifier genes in *E. coli* lower the cost of resistance to virus T4 (Lenski 1988b) and to the antibiotic streptomycin (Schrag et al. 1997), and modifiers in the sheep blowfly reduce the cost of resistance to the insecticide diazinon (McKenzie et al. 1982).

However, to the best of our knowledge, no previous study has examined the separate and combined costs of resistance to two or more consumers. Additional studies are thus needed to determine whether there is a general tendency for the combined cost of resistance to multiple consumers to be less than expected from the separate costs to each consumer, as we have observed. Such an effect has important implications for the evolution of resistance to consumers. For example, by lowering the cost of resistance to multiple consumers, such epistasis would presumably broaden the conditions for invasion and persistence of organisms that are resistant to multiple consumers. Also, to the extent that the environment influences the nature of epistatic interactions between resistant alleles, as we observed in this study, different coevolutionary trajectories may arise between organisms and their consumers in different environments.

Of course, epistatic interactions are not unique to mutations that confer resistance. Two recent studies using *E. coli* (Elena and Lenski 1997) and *Aspergillus niger* (De Visser et al. 1997) examined epistatic interactions among large numbers of genes in terms of their effects on fitness. Both studies observed that epistatic interactions were quite common, but that antagonistic and synergistic interactions—in which the combined effects are, respectively, less than or greater than expected from the separate effects—were approximately equally common. Thus, there is no strong net effect on fitness when summing over all interactions, and detailed pairwise analyses were necessary to discern the true extent of epistasis. Our results, in particular the fitness values measured in trehalose, are consistent with this interpretation, and they show that heterogeneity in the epistatic effects can occur even among mutations that appear superficially identical based on their resistance phenotypes. Therefore, the coevolutionary trajectory between an organism and its consumers will depend not only on the environment, but also on subtle differ-

ences among resistance mutations in fitness costs (Lenski 1988a) and their epistatic interactions with other mutations that confer resistance to different consumers (this study).

Finally, physiological explanations for epistatic interactions among mutations are generally not well understood and may often be quite complex (Travisano 1997). The following hypothesis might explain why the combined cost of resistance in *E. coli* to viruses T4 and λ is less than expected from the separate costs for each virus alone, especially in trehalose. Mutations that confer resistance to λ usually cause loss or structural modification of the LamB protein, the outer membrane receptor to which λ initially binds to the bacterial cell (Szmelcman and Hofnung 1975; Arber 1983). LamB is involved in the uptake of maltose and trehalose, but not glucose (Klein and Boos 1993; Travisano and Lenski 1996). Mutations that confer resistance to T4 cause the truncation of outer membrane lipopolysaccharide, the receptor to which T4 initially binds (Prehm et al. 1976; Wright et al. 1980; Lenski 1988a). Such truncation inhibits the trimerization of several outer membrane proteins, including LamB, which is necessary for their activity (Laird et al. 1994). Thus, in trehalose, the costs of resistance to both T4 and λ are probably due, at least in part, to the loss of activity of the LamB protein. It is reasonable that once the cost of resistance has been "paid" to one virus, the cost of resistance to the other one comes at a "discount" because it involves the same physiological deficiency. We recognize that this explanation is speculative, but we believe that mechanistic hypotheses such as this are useful in suggesting why the combined costs of resistance to multiple selective agents may depend on subtle details of genetics and physiology (Raymond et al. 1989).

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