# Resistance to xenobiotics and parasites: can we count the cost?

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**The nature and cost of single genes of major effect is one of the longest running controversies in biology. Resistance, whether to xenobiotics or to parasites, is often paraded as an obvious example of a single gene effect that must carry an associated fitness 'cost'. However, a review of the xenobiotic resistance literature shows that empirical evidence for this hypothesis is, in fact, scarce. We postulate that such fitness costs can only be fully interpreted in the light of the molecular mutations that might underlie them. We also derive a theoretical framework both to encompass our current understanding of xenobiotic resistance and to begin to dissect the probable cost of parasite resistance.**

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Models of the evolution of resistance to both xenobiotics (Box 1) (e.g. pesticides and drugs) and pathogens (Box 1) often share the central assumption that resistance is associated with a fitness cost. This assumption is based on three premises broadly derived from the fields of evolutionary biology, population genetics and physiology. First, the acquisition of any adaptation to a new environment, such as resistance, involves a large modification of the previous phenotype(s). Because these phenotypes have been shaped by various selection pressures to fit the different environments faced by one species, any large phenotypic modifications are therefore argued to be deleterious within the context of the ancestral environment. Second, although genetically determined resistance confers a selective advantage in the presence of either xenobiotics or pathogens, resistance genes are rarely fixed in natural populations. Assuming that populations have reached an evolutionary equilibrium, this maintenance of polymorphism is thought to result from the occurrence of a counterbalanced selection pressure, which decreases the frequency of the resistance gene in the absence of the corresponding pesticide or pathogen (or fluctuating selection that favours alternative alleles at the same locus). Third, knowledge of the physiological modifications involved in xenobiotic resistance has promoted hypotheses based on altered function(s) of the associated proteins.

In all fields of resistance (herbicides, fungicides, insecticides, antibiotics and plant pathogens), several functional explanations of cost have been proposed and tested[1–3]. Typically, changes in receptors or enzymes are seen as simple disruptions to their normal roles, although overexpression of an enzyme or receptor might divert energy from other fitness-enhancing functions. Curiously, in the case of animal resistance to parasites, although the precise physiological mechanisms involved in resistance are poorly documented, most of the evolutionary literature is based on the central assumption of a costly investment in defence functions, leading to a tradeoff between resistance and other fitness-related traits. More specifically, the cost of resistance is often presented as the cost to maintain the immune and/or defence machinery, which is required to mount a successful immune response[4]. Because the cost of resistance is central to our ability to predict the behaviour of resistance genes, many studies have focused on this perceived tradeoff between resistance and other fitness-related traits. However, although many have confirmed the expected cost of resistance, others have failed to detect it altogether[5]. Several reasons have been proposed for failure to detect a cost. First, resistance costs might only be apparent under a specific set of environmental conditions, which were not encountered in the experimental set-up. Second, the associated antagonistic pleiotropy (Box 1) might not be statistically detectable[6]. Third, and most provocatively, there might be no real cost of resistance[7] – this latter possibility is important because it would make most of the current models inappropriate.

Another complicating factor is the potential confusion in the literature between related concepts of resistance cost. Thus, resistance cost can be defined either as a resistance-associated change in physiology and/or in a life history trait, or as the selection pressure acting against the involved mutation in natural populations. These concepts are obviously related but not equivalent. Given the need for a clear distinction between these two aspects, our choice is to refer to the 'physiological cost of resistance' (Box 1) when addressing the functional basis of the effects (often seen as potentially deleterious) of resistance-associated mutations, and to counter-selection (Box 1) when addressing the selection pressure acting against these mutations. In the context of alleles (*A* or *a*) of a single gene, we therefore define the cost of resistance as *s* and the relative gain *g* in the following equation: where *AA* is 1, *Aa* is $(1-h_1s)(1+h_2g)$ and *aa* is $(1-s)(1+g)$, and where $h_1$ and $h_2$ are the relative penetrance of each allele in heterozygous form. It is noteworthy that, in this context and in terms of evolutionary time, a chance linkage between a resistance gene and a deleterious mutation in another gene reflects no physiological cost of resistance except in the case of strictly clonal organisms, where the whole genome is in complete linkage.

The aim of this perspective is to ask if it is possible to use our knowledge of the molecular mechanisms of resistance to make assumptions about the strength of predicted counterselection. We examine this hypothesis by phrasing several related consecutive questions. (1) When does theory predict a cost of resistance? (2) What are the molecular mechanisms of resistance to xenobiotics and do these help us to develop a framework for the prediction of cost-associated mechanisms? (3) What is the molecular basis of parasite resistance and can we use our framework to predict probable costs for a different type of resistance?

## When does theory predict a cost of resistance?

### *Predictions from xenobiotic resistance*

For resistance to xenobiotics, expectations relative to costs derive from a model of adaptation developed by Fisher[8]. In this model, independent selection pressures shape the present (almost) optimal phenotypes through complex gene coevolution. This inferred gene interdependence makes any mutation with a large phenotypic effect likely to induce severe deleterious effects. Therefore, the key point determining the likelihood of a counterselection of resistance gene(s) is the shift in distribution of viable phenotypes from the ancestral to the new environment. If

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their distributions largely overlap, then adaptation to the new environment can be achieved by the spread of many mutations of small effect [presumably associated with little or no cost, i.e. $(1-s)^n$ or $(1-s)$], rather than by one mutation of major effect (associated with significant cost)[9]. By contrast, if the environmental gap is such that the two distributions are distinct, then adaptation can be achieved by only a single mutation of large effect, even in the presence of a severe cost[10]. In this latter case, subsequent evolution is likely to involve a few additional mutations, which would decrease this original counterselection. From Fisher's model, Orr[11] described the bout of adaptive evolution experienced by a population colonizing a totally new environment. He showed three important properties: first, a clear and robust exponential trend is observed for the phenotypic effect among subsequent mutations; second, the leading mutation fixed during adaptation can have a large phenotypic effect; and third, mutations with the largest effect tend to count among the first to be fixed.

Orr[11] argued that his model, through a new and fixed optimum, tends to lead to the evolution of many (but not all) adaptations. We feel that his model is also appropriate for the evolution of resistance to xenobiotics. Xenobiotics tend to define a 'new environment' where the treated populations (i.e. the current phenotypes of weeds, bacteria or insects) are not viable. In most cases, resistance to xenobiotics effectively defines a sudden change towards a new phenotypic optimum that remains fixed during the period of use of a particular xenobiotic. Therefore, this theoretical framework predicts three main trends. First, that a single major mutation should be involved in resistance to xenobiotics; second, that such a mutation should be associated with a significant physiological cost, thus leading to net counterselection in the absence of xenobiotics; and third, that this cost can potentially be corrected by epistasis with subsequent mutations (i.e. modifiers).

**Box 1. Glossary**

**Counterselection:** selection against an allele (in this case, usually against the resistant allele).

**Fluctuating asymmetry:** population wide deviation from an expected perfect symmetry. Usually inferred to reflect developmental problems of either genetic and/or environmental origin. In the case of *Lucilia cuprina*, the morphological structure measured (bristle number) was symmetrical in resistant or susceptible flies carrying the modifier, but asymmetrical in resistant flies lacking the modifier.

**Pathogen:** used here to encompass pathogenic organisms, including prokaryotes (e.g. bacteria) and eukaryotes (e.g. parasitoids).

**Physiological cost (of resistance):** the functional basis of the effects of resistance-associated mutations (often seen as potentially deleterious).

**Pleiotropy:** used here in terms of multiple phenotypes (often cost-related) associated with a single mutation.

**Temperature-sensitive paralysis:** used to define the transient immobilization of insects observed when the temperature is raised to 38°C. Full mobility is restored after the insects are returned to room temperature.

**Trans-acting:** a term used to describe a regulatory effect observed between loci not in direct linkage.

**Xenobiotics:** literally, biological compounds 'foreign' (xeno-) to the organism and, here, including mainly pesticides (herbicides, insecticides, fungicides and drugs).

## What are the molecular mechanisms of xenobiotic resistance?

### *A diversity of molecular mechanisms*

Recently, the molecular basis of xenobiotic resistance has been extensively reviewed elsewhere[2,12,13]. Four classes of mechanisms are observed: (1) constitutive overproduction; (2) constitutive underproduction of one gene product; (3) alteration of a target or receptor; and (4) an inducible change in gene regulation. Therefore, the expected 'cost' varies for each mechanism. The first class includes mechanisms that lead to a constitutive overproduction of drug-inactivating enzymes, transporters involved in cell influx and/or efflux processes, or drug-target proteins; for example, the overproduction of esterases sequestering and hydrolysing organophosphorus and carbamate insecticides[14,15]. These phenomena can be spectacular: one specific esterase (E4) can constitute up to 3% of the total protein content in resistant aphids[14]. Similarly, overexpression of the highly conserved P-glycoprotein transporters is associated with multidrug resistance in a broad range of taxa, including bacteria, protozoa, nematodes, insects and even human cancer cells[16]. Whether such resistance mechanisms result from the transcription of amplified genes, from a mutation leading to a constitutive overtranscription or from a combination of both, they intuitively fit the concept of the internal trade-off in allocation of resources because they lead to a significant overproduction of gene product(s).

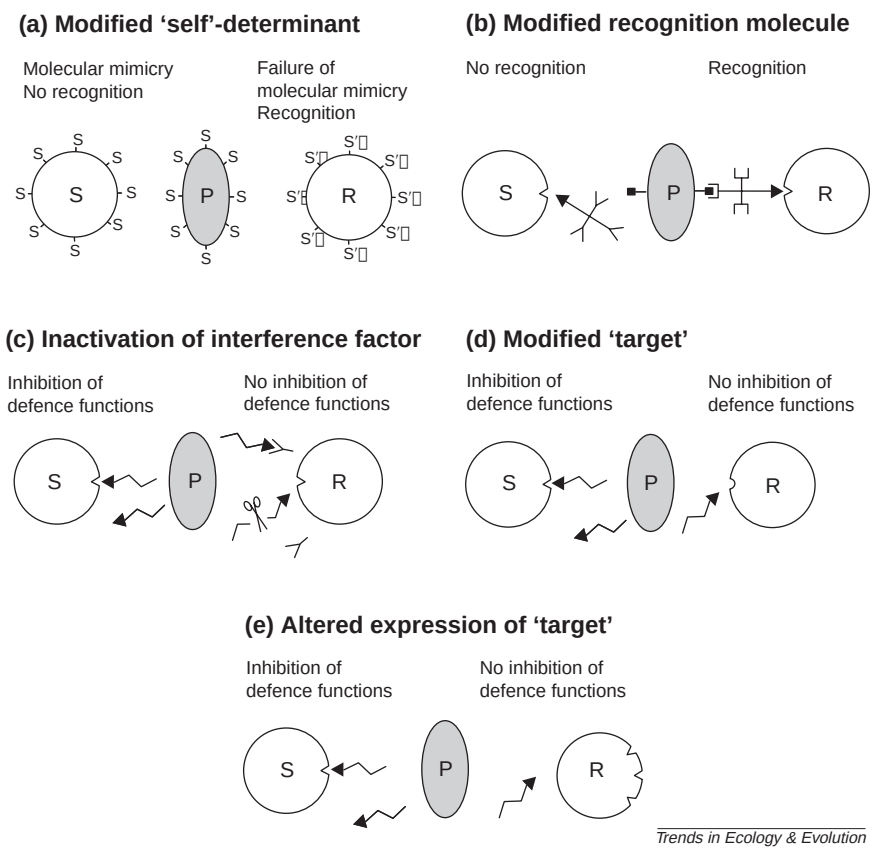
Constitutive downregulation of either drug targets or drug-activating enzymes constitute the second class. Here, resistance is expected to result mainly from a reduction in, or loss of, function[2]. The third class involves qualitative modifications of a single gene product – usually a drug receptor or a drug target[2]. For instance, several mutations in β-tubulin genes confer benzimidazole resistance in various helminths, yeasts and fungi[17,18] by affecting the binding affinity of benzimidazole (a tubulin disruptor) to tubulin[17]. These modifications are expected to induce deleterious effects by disrupting the pathways within which targets or receptors are usually involved[19]. By contrast to the former classes, no general expectation on physiological resistance cost can be proposed when resistance is achieved by the fourth class, namely gene regulation induced by environmental signals.

### *A diversity of physiological costs?*

A growing body of data supports the simple expectation that the physiological cost of resistance is a direct extension of the molecular basis of resistance. For example, cyclodiene insecticide resistance is associated with a point mutation in the *Resistance to dieldrin* gene, which encodes a γ-aminobutyric acid (GABA) receptor subunit[20]. Here, the resistant receptor spends less time in its 'drug-preferred' desensitized (not responsive to agonist) state, a change correlated with temperature-sensitive paralysis (Box 1) in the whole fly, and, notably, a phenotype also seen in DDT and pyrethroid resistant sodium channels[21]. P450 enzymes, which are involved in a diverse array of physiological activities, also illustrate how a precise understanding of the changes involved is crucial to our knowledge of the origin of physiological cost[22]. Thus, trans-acting regulatory loci, which upregulate P450s capable of insecticide metabolism, might also coregulate genes involved in other physiological functions (e.g. hormone metabolism)[22]. This absence of specificity directly raises the opportunity for a physiological cost via negative pleiotropy[22].

Genetic 'modifiers' (defined here as genes not involved in resistance, which modulate the physiological resistance cost) are also a useful way of trying to understand the physiological basis of cost. In *Aspergillus nidulans*[17], the *benA33* mutation in the β-tubulin gene induces benzimidazole resistance and an absence of mitosis at a restrictive temperature. This temperature sensitivity is associated with hyperstability of microtubules, as a result of structural alteration of β-tubulin. However, normal microtubule stability can be restored by a mutation altering the structure of a protein partner, the α-tubulin[17]. In a second example, diazinon resistance in the Australian sheep blowfly *Lucilia cuprina* is due to

**Box 2. Possible parasite resistance mechanisms**




Possible mechanisms of resistance to parasites (Fig. I). (Fig. Ia) The parasite (P) expresses and displays host-like determinants that mimic self (molecular mimicry). The resistant host (R) displays modified 'self' determinants (S′) and, therefore, recognizes the parasite determinant as nonself. (Fig. Ib) The parasite takes advantage of a lack of the proper recognition molecules in the susceptible host (S). Modification of a recognition molecule permits the resistant host (R) to recognize the parasite. (Fig. Ic) Products from the parasite actively interfere with one or more functions of the effector cells from the susceptible host (S). Modification or overproduction of humoral factors in the resistant host (R) inactivates the parasite-interfering factor by degradation or binding. (Fig. Id) The resistant host (R) presents a modified 'target' receptor that prevents the interfering factor from binding and/or from inducing a proper transduction signal. (Fig. Ie) Effector cells from the resistant host (R) present an altered expression of target receptor (overproduction or underproduction), thus leading to the failure of the parasite to interfere with defence functions[44].

allelic substitution at the *Rop-1* gene, which encodes a carboxylesterase whose normal function is not known. Ten years after resistance appeared, field-collected resistant flies showed similar fitness to susceptible ones in a diazinon-free environment[23]. However, when their genetic background was disrupted by repeated backcrossing to a laboratory susceptible strain, the relative fitness of the resistant phenotype declined[23]. The modifier, a single locus of major and dominant effect[24], corrects several deleterious effects, including increased mortality during overwintering, lowered fitness in laboratory conditions and increased fluctuating asymmetry (Box 1), thus suggesting that both genes could be involved in early developmental processes[25]. Finally, insecticide resistance mediated by the overproduced esterase E4 in the aphid *Myzus persicae* might provide a third unusual example of a potential modifier. In this case, E4 resistant aphids can display various levels of gene amplification[26], but they can also actually 'switch off' esterase production in their clonal progeny, thus leading to apparent reversion to susceptibility[27] by differential methylation of the DNA in the 5′ end of the E4 genes themselves[28]. However, in the context of fitness traits studied to date, the contribution of this 'reversion' to the net fitness of these aphid clones is far from clear. Thus, for example, revertant aphids still suffer from the same overwintering mortality as their counterparts[29].

Population-level studies can also elucidate the link between the nature of physiological cost and resistance mutations, as seen in the allele replacement observed in the *Culex pipiens* acetylcholinesterase (*Ace*) locus in the South of France[30]. *Ace.1*$^{S}$, an allele which encodes enzymes sensitive to insecticide inhibition, is presently found alongside two other alleles conferring resistance: *Ace.1*$^{R}$, which encodes a variant insensitive to insecticide inhibition, and the duplicated allele *Ace.1*$^{RS}$, which encodes both sensitive and insensitive variants. *Ace.1*$^{RS}$ is postulated to have appeared 14 years after *Ace.1*$^{R}$, but appears to be quickly replacing it. However, the wild-type function of this particular insecticide target is well understood because it is involved in the turnover of the neurotransmitter acetylcholine (ACh). The amount of ACh in synapses results from a balance of acetylcholinesterase (AChE, which degrades ACh) and choline acetyltransferase or CAT (which synthesizes ACh) activities. Thus, this amount might be crucial in determining fitness because mortality can be caused either by an excess or a deficit of ACh. Because the enzyme encoded by *Ace.1*$^{R}$ is four times less active than that encoded by *Ace.1*$^{S}$, the replacement of *Ace.1*$^{R}$ by *Ace.1*$^{RS}$ might be because the more successful allele codes for both a highly active susceptible AChE and a resistant one of lower activity. Consequently, *Ace.1*$^{RS}$ should increase overall AChE activity relative to *Ace.1*$^{R}$ and, thus, should partially restore the equilibrium.

### *How well does the xenobiotic data fit the theory?*

Empirical data on resistance to xenobiotics generally agrees with the theoretical framework already described. Thus, resistance is usually achieved by mutations of major effect, some of which have documented physiological costs. Further, even a few examples of modifiers are reported. However, experimental studies of bacterial evolution provide counterexamples, where resistant mutations associated with no physiological cost of resistance can occur. Thus, resistant mutations can be fixed in antibiotic-treated, as well as in antibiotic-free, populations without any evidence of epistatic action[31,32]. Similarly, the long-term survey of *C. pipiens* insecticide resistance illustrates the replacement of costly resistance alleles by less costly ones at two loci. In conclusion, there are still too few well documented studies to settle whether counterexamples are exceptions or the rule.

## What is the molecular basis of parasite resistance?

### *What do we mean by parasite resistance?*

In reference to parasites, the term 'resistance' is often used in a broad context, referring to all mechanisms contributing to a decrease in the detrimental effect of the parasite[33–35]. These mechanisms

**Table 1. Potential mechanisms of resistance to parasites and predicted costs**

| Molecular modifications | Possible resistance mechanisms[a] | Expected physiological costs | Counterselection in parasite absence |
|---|---|---|---|
| Constitutive overproduction of a gene product:<br>Gene amplification<br>Gene overtranslation | Enhanced defence function(s) (case e) | Reallocation of resources | Probable |
| Constitutive underproduction of a gene product | Underrepresentation of the parasite target (case e) | Loss of function | Possible |
| Mutations leading to structural changes in: | | | |
| Self-determinant or recognition molecules | 'Abnormal' recognition of compatible parasite (cases a and b) | Altered recognition of other parasites? | None |
| Cellular machinery or excreted molecules | Failure of parasite to exploit host internal milieu (i.e. malaria) or to interfere with defence molecule (case c) | Altered function | Possible |
| Membrane receptor | Modified parasite target (case d) | Altered function | Possible |
| Inducible regulation of gene expression | Inducible defence | Probably none | Probably none |

[a]Statements in parentheses refer to the cases discussed in Box 2.

include acquisition of avoidance behaviour, expression of inducible defences and modification of life history traits. A more specific meaning of resistance refers to the biochemical and physiological changes preventing proper parasite establishment, survival and/or development – here, we will focus only on this aspect.

Most studies of resistance to parasites have been interpreted as a differential investment in defence and/or immune functions and, therefore, the physiological cost of resistance is inferred to be a tradeoff in allocation of the associated resources[36]. Generally, the distinction between the effector mechanisms of antiparasite defence and the underlying resistance mechanisms themselves is not well made. For example, although an insect selected for resistance to a given parasitoid species will mount an energetically expensive melanotic encapsulation response to eliminate parasitoid eggs or larvae[37], the melanotic encapsulation only reflects the mode of elimination of the parasite. Without evidence that resistance results from an enhanced defence system, the 'resistance gene(s)' could be any gene that causes the effector system to be abnormally activated (i.e. melanotic encapsulation). We feel that this distinction between carrying the gene associated with resistance and expressing a downstream immune response is fundamental, because both the nature and the evolutionary consequences of potential costs associated with these mechanisms will be different. On the one hand, the cost of mounting a defence response will only be expressed after infection (such as a computer virus detection program that only scans for and deletes viruses every time you put a disk in) and this reaction confers a selective advantage relative to the susceptible host. On the other hand, the potential cost of the resistance-associated mutations might be expressed in the absence of the parasite itself, thus leading to counterselection within some environments.

### *Parasite resistance mechanisms: what do we really know?*

Unfortunately, there are few examples where the precise mutations involved in parasite resistance have been identified. In human resistance to *Plasmodium*, heterozygous carriers of the sickle-cell haemoglobin gene have some resistance to severe malaria compared with carriers of the normal globin gene. A single point mutation of the β-globin gene is involved in partial resistance to malaria[38]. Here, the cost associated with resistance is obviously not related to any investment in defence function but is related to the sickle-cell anaemia resulting from the disrupting mutation in the β-globin gene. Although significant progress has been made in characterizing genes associated with resistance to various pathogens, including viruses[35], data on the precise mutations and physiological modifications are still lacking. However, our current knowledge on host–parasite immunobiology does allow us to speculate on the probable mechanisms involved.

### *Potential resistance mechanisms*

Most organisms have evolved immune systems that are capable of recognizing self and nonself, and subsequently of eliminating invaders[39,40]. Because organisms showing susceptibility to a specific parasite are nevertheless capable of eliminating other parasite species[41,42], susceptibility often relies more on the parasite's ability to evade the defence response rather than on host deficiency. Evidence for immune evasion has been extensively documented in a wide variety of organisms, including protozoa, parasitoid insects and helminths[43–45]. This immune evasion might be accomplished by various mechanisms, such as preventing recognition as nonself (molecular masking, molecular mimicry and location in 'nonimmunocompetent sites'), or by modifying or suppressing defence functions (immunomodulation or immunosuppression). Although host–parasite compatibility results from potentially complex interactions, we predict that single major resistance mechanisms will be associated with disruption, modification and/or abnormal expression of specific gene products, thus making parasites unable to evade host defences (Box 2) or unable to exploit the host environment (e.g. human resistance to malaria). Among observations supporting the hypothesis of modified recognition processes (Box 2, Fig. Ia,b) is evidence for a role of the major histocompatibility complex in resistance of mice to gastrointestinal nematodes[46]. However, observations supporting the hypothesis that the parasite fails to interfere with host defences (Box 2, Fig. Ic–e) are poorly documented. Thus, although it is possible that some cases of parasite resistance rely on enhanced defence functions, it seems more probable that major resistance mechanisms will be associated with mutations altering, or adding, functions to existing gene products, in a fashion reminiscent of xenobiotic resistance.

### Can we predict the probable cost of parasite resistance?

Theory on the evolution of host resistance to parasites and its potential associated cost has been derived from models based on host–parasite genetic interactions, rather than on models of adaptation to new environments. Two types of interaction have been investigated in particular (for details and comments see Refs 47,48). Matching-allele models correspond to symmetric frequency-dependence models, where the outcome of parasite infection is determined by the correspondence of one allele at a host locus with one allele at a parasite locus. Such models do not require counterselection in parasite-free environments to explain the maintenance of polymorphism. Indeed, individuals carrying an allele for resistance to a specific parasite genotype would, however, be susceptible to the other parasite genotypes. Therefore, counterselection of each resistance allele would be driven by the presence of the parasites that do not carry the proper allele at the matching locus, thus leading to frequency-dependent dynamics. The dynamics of such systems have been explored at both population and metapopulation levels and, interestingly, they define some properties compatible with the phenotypic distribution of host resistance and parasite virulence[49,50]. Alternatively, gene-for-gene models involve asymmetrical frequency dependence[50], where mutations for resistance could be at an advantage when interacting with any genotype of a given parasite species. To explain the maintenance of resistance polymorphisms, such models require a physiological cost of resistance, thus leading to counterselection in parasite-free environments[47,48].

A clear distinction between these basic models, although crucial for drawing precise expectations on resistance costs, appears difficult to establish even in the well studied cases of plant–pathogen interactions[47,48]. In addition, because these models have been developed to investigate host–parasite coevolution, they are based on the assumption that selection pressures are driven mostly by host–parasite interactions. Alternatively, the putative pleiotropy of resistance genes facilitates the evolution of resistance in natural populations driven by forces other than parasitism. In other words, mutated genes might be involved in one or several complex metabolic pathways, leading to several important phenotypic changes including resistance to a given parasite. This possibility, also referred to as resistance being a 'chance product'[51], would be compatible with the observation that resistance is sometimes detected in populations that have never been exposed to the corresponding parasite. However, this possibility has been the object of little investigation at either the theoretical or empirical level.

In conclusion, the environmental conditions defining counterselection of resistant mutants are apparently much more diverse among cases of resistance to parasites than of resistance to xenobiotics, and do not strictly correspond to the absence of a particular parasite species.

### Prospects

Although there has been considerable recent progress in our understanding of both the theoretical, ecological and molecular basis of parasite resistance, our level of understanding still lags behind that of xenobiotic resistance. However, based on our knowledge of xenobiotic resistance, we can begin to make some predictions as to when fitness costs are likely to occur (Table 1). In this framework, costs associated with changes in resource allocation are restricted to one specific set of mutations, namely constitutive overproduction of defence molecules. For all other classes of mutations, we predict that experiments trying to quantify perceived fitness in parasite-free environments might be ineffective. Thus, mutations affecting the structure of self-determinant (or parasite-recognition) molecules are good candidates for evolving, as described by the matching-allele model (i.e. for inducing, as a unique side effect, a susceptibility to alternative parasite genotypes)[47]. Guidelines for future studies of resistance costs in parasite resistance are therefore beginning to emerge (Table 1), but identification of the molecular and/or physiological changes involved in resistance should be viewed as a crucial step enabling us to take this further. The importance of a molecular understanding is further stressed when costs are associated with pleiotropy. Indeed, negative pleiotropic effects of resistance genes cannot be guessed *a priori* and are difficult (if not impossible) to detect using conventional methods[6]. However, as discussed above, testable hypotheses can emerge when molecular changes are identified. For example, in the case of insecticide resistance in *L. cuprina*, where molecular characterization has helped in detecting modifier genes, and in identifying candidate functions of both the modifiers and the resistance genes themselves. Finally, and perhaps most importantly, the identification of parasite resistance-associated mutations will help us to assess levels of molecular polymorphism at these loci and thus, in turn, to address the question of the forces driving the evolution of resistance. In this respect, it is noteworthy that the few studies of xenobiotic resistance performed at both the molecular and population level have revealed unexpected differences in the evolution of resistance costs[15,23,27,31]. Given the expected complexity of host–parasite systems, similar efforts to couple gene and population studies should provide new insights on the probable diversity of resistance costs and their evolution.

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# A fossil valentine

Fossil Crinoids
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Crinoids, better known as sea lilies (Echinodermata: Crinoidea), have left among the most extensive, diverse and abundant of macroinvertebrate fossil records. Typically, the endoskeleton of a single crinoid accounts for at least 85% of body mass, and can contribute up to several hundred thousand individual skeletal grains to the sediment when the organism dies and disarticulates[1]. Crinoid skeletal material is the primary component of vast limestone deposits, called regional encrinites, known from the Ordovician to the Jurassic. The Lower Mississippian Burlington and Keokuk Limestones alone are 53-m thick, and span more than 74 000 km$^2$ of Missouri, Iowa and Illinois[2].

Despite this largesse, the great bulk of the crinoidal fossil record consists of disarticulated skeletal pieces that tend to be ignored chiefly because they are normally impossible to identify. Nevertheless, research on complete and partially complete fossil animals has generated an extensive, if chiefly taxonomic and biostratigraphic, literature. However, although bizarre new species and whole new faunas continue to emerge[3,4], the past three decades have seen a tremendous expansion in the kinds of research tools brought to bear on these animals, much of it depending directly on the enormous morphological and/or stratigraphic database. As examples, Baumiller[5] used dynamic survivorship analysis on the stratigraphic ranges of 838 Paleozoic genera to determine that crinoids with finely meshed filtration crowns (restricted to higher energy environments) had higher extinction and origination rates than those with coarsely meshed crowns (found across all environments). Foote[6] included 1195 species in tracing patterns of morphological diversification associated with Paleozoic and Mesozoic crinoid radiations. Furthermore, the use of SCUBA and manned submersibles has generated a growing body of actualistic studies on ecology, biomechanics, growth and taphonomy[7,8]. Although extant crinoids are still sometimes viewed as 'living fossil' relicts holding on to a precarious existence in the deep sea, the modern fauna is actually diverse, abundant and, especially on Indo–West Pacific reefs, common in shallow water. More than half of the 540 species of living unstalked feather stars (the largest extant group of crinoids) occurs at shelf depths. A single square meter of New Guinean reef might support up to 115 specimens of a dozen different species[9]. Stalked