This Perspective manuscript by Lenormand, Harmand and Gallet addresses the concept of 'cost of resistance', which is commonly defined as the reduction in fitness in the absence of drug (compared to the wild-type) associated with a drug resistance mutation. They trace how this concept developed historically and then highlight several of its shortcomings. My impression is that the authors have reviewed the literature quite thoroughly (with a few suggested additions noted later) and thus their perspective is well-grounded. They raise valid issues with the definition and usage of the term 'cost of resistance', and I believe this article can provide a worthwhile contribution to the literature by highlighting and bringing together several problems that, while not entirely new, have been underappreciated.

As a reader with a primarily theoretical background, I enjoyed reading this manuscript and was generally convinced by the authors' arguments. However, my main concern is that this message may fail to reach a broader readership, including experimentalists, in its present form. The authors may choose to ignore this critique if this is not their target audience, but I believe their manuscript has the potential to have a much greater impact if they address this point. Specific suggestions are detailed below.

Secondly, while the geometric model is appealingly simple and useful for illustration, it comes across rather dominantly in the current version of the manuscript. Although the ideas that the authors put forward are not specific to this model formulation, the generality of the points they raise could easily be lost due to the emphasis on the geometric model (including all 4 figures of the manuscript). More clearly conveying this generality could be achieved by adding some discussion and illustration using a model that deals directly with fitness, e.g. by linking to dose-response curves (see below). I don't suggest to replace the geometric model, which is elegant and useful, but rather to complement this with some broader discussion.

Finally, I suggest to devote a bit more space on how to move forwards beyond 'cost'. Although the authors do touch on this point (including in the Abstract, where they propose "to study, measure and analyze the fitness effects of mutations across environments and to better distinguish those effects from 'pleiotropic effects' of those mutations"), the manuscript would benefit from a dedicated section providing concrete suggestions, including stronger links to experimental approaches.

As a technical point, it would be great if the authors could add page and line numbering to the next version of their manuscript, in order to facilitate the commenting process.

More specific comments on the content follow.

Model description:

* It would be useful to have a slightly more detailed introduction to the geometric model and its assumptions, as this forms a large part of the exposition but may not be familiar to all readers, even some theoreticians.

* In particular, although it is written that "we can assume that fitness declines with the distance from the peak in any given environment", please give the mapping from phenotype to fitness explicitly, and highlight the key assumption that fitness depends only on (Euclidean?) distance from the optimum in multi-dimensional trait space. This mapping is needed in order to make deductions such as "The difference between these two distances [AR and AO in Fig. 1] scales with the selection coefficient of the resistance mutation in the treatment environment" and that all points P_1 such that $|AR| = |AP_1|$ "confer the same benefit in the treated environment" (i.e. have the same fitness).

* Since the geometric model is rather abstract, it would be helpful to include where possible any intuition or discussion of how it relates to measurable quantities – most importantly in this context, how the definitions of "resistance" and "cost" in this model relate to more commonly used empirical measures.

- In the geometric model, "resistance" is represented by a point in phenotypic space that is closer to the optimum in the treated environment than the wild type is (i.e. |AR| < |OR| in Fig. 1). With a mapping from phenotype to fitness, this could be directly related to the common practical definition of resistance as an increase in minimum inhibitory concentration (MIC), meaning the resistant strain can still grow at higher drug concentration than the wild type.
- Similarly, "The cost of resistance is shown by the distance OR, as it is defined as the fitness effect of the resistance mutation in the non-treated environment" (pp. 6-7). However, there is a subtle distinction to make here: this sentence first suggests that cost is in units of distance in trait space, but then that it is rather a difference in relative fitness. These are two different measures, which will be linked by the phenotype-to-fitness mapping assumed in the model. In practice, "cost" is usually measured directly in the currency of fitness (or rather, some proxy such as relative growth rate).

* 1st paragraph on p. 7: "Should these [pleiotropic] effects be totally compensated, the phenotype would be in P_2 and it would indeed enjoy a greater fitness in both the treated and non-treated environments." How strongly does this conclusion (enhanced fitness in both environments) depend on the assumptions of the geometric model?

Additional conceptual links and references:

In general, these are not mandatory to add, but may be of interest to the authors and in my opinion would strengthen the manuscript!

* *The context dependence of fitness effects* (p. 3) points out that "thinking in terms of averages" is not always valid. This important point could usefully be expanded. Firstly, thinking in terms of averages can be misleading not only in terms of ecological conditions as the authors already mention, but also in terms of genetic background in the opposite case to that they describe, i.e. for the many relevant species that reproduce asexually or with limited recombination or horizontal gene transfer. Furthermore, even if averaging (over genetic backgrounds or environments) might be considered reasonable for predicting long-term dynamics, in the initial establishment of rare resistance alleles when stochastic effects dominate, the context in which the allele first arises can be extremely important. Finally, while the authors mention that the spatial scale of dose variation (relative to dispersal) is relevant, an analogous point could be made for the temporal scale of dose variation (relative to generation time). A useful reference here would be Cvijović et al. (2015), *PNAS* E5021-E5028 (doi:10.1073/pnas.1505406112).

* More generally, the authors refer to spatially heterogeneous models in several places. These points could often be extended to temporal heterogeneity, with links made to the extensive literature on pharmacokinetics and pharmacodynamics (PK/PD) of drug dosing.

* What immediately sprung to my mind when reading this manuscript was the relationship to "dose-response curves", which relate some demographic parameter (e.g. net population growth rate, which can be considered a measure of fitness) to drug dose. That these are never mentioned struck me as a glaring omission, particularly in the discussion of varying drug doses on pp. 11-12, which would provide a natural link. I think there are several reasons why it would be useful to bring these up:

- Using keywords such as “dose-response curve” (and perhaps “reaction norm”, of which dose-response curves are an example) will catch the attention of more readers.
- Dose-response curves are more easily related to empirical measures than the geometric model, thus raising the interest for experimentalists.
- By including a model that deals directly in the currency of fitness (or some measurable proxy), in addition to the geometric model, the authors would have the opportunity to highlight that the conceptual issues they raise are general.
- Many of the authors’ ideas could be very nicely illustrated by plotting dose-response curves, e.g. showing different “costs” of resistance mutations in different genetic backgrounds or environments, and illustrating the issues that arise at varying drug doses. A figure or two like this could help provide some balance by indicating at a glance that the manuscript is not only about the geometric model.
- There is a natural link between the authors’ ideas and existing literature highlighting the limitations of using single-parameter measures of “resistance” or “fitness” (such as the MIC) to predict population dynamics, particularly in PK/PD models. A few relevant references:
    - Regoes et al. (2004), *Antimicrob. Agents Chemother.* 48:3670 (doi: 10.1128/AAC.48.10.3670-3676.2004)
    - Sampah et al. (2011), *PNAS* 108:7613-7618 (doi: 10.1073/pnas.1018360108)
    - Gehring & Riviere (2013), *Vet J* 198:15-18 (doi: 10.1016/j.tvjl.2013.07.034)

* Another potential reference, which echoes the authors’ point about the dependence of fitness on ecological context: Day, Huijben, Read (2015). *Trends Microbiol.* 23:126-133.

* Middle of p. 7, regarding compensation of pleiotropic effects: here one could also cite work by Dan Andersson’s group (e.g. the Andersson & Hughes 2010 review already included in the references, and/or Andersson & Hughes 2012, *Drug Resistance Updates* 15:162-172.)

Discussion of varying drug doses (pp. 11-12):

* The idea that different drug concentrations could represent either different intensities of selection or different phenotypic optima is certainly interesting conceptually, and is clearly explained in the context of the geometric model. However, it could be more clearly explained in practical terms as well. Are there relevant empirical examples, e.g. for the statement that “it is fairly easy to imagine two mutations R_1 and R_2 that would qualify as resistance mutations, in each of the two environments, but not in the other”? My guess (although unfortunately I cannot offer a reference off the top of my head) is that there may well be such cases, e.g. where gene overexpression or amplification, an efflux pump, or enzymatic degradation of an antibiotic confers “resistance” at low doses, but only a target modification confers “resistance” at high doses.

* I think this whole discussion would flow better with a bit of reorganization. The argument (p. 12) that “it is difficult to conceive that adding a vanishingly small quantity of drug suddenly shifts away phenotypic requirements, and that further increases in dose only change the selection intensity” is convincing, and I think this could usefully be moved up front to where the idea of different optima versus different selection intensity is first introduced on p. 11. Likewise, the admittance that this has not been demonstrated empirically could be moved along with it. The discussion on benefits and costs at different drug doses would seem to flow more naturally afterwards. Indeed, the point that fitness is more generally a function of drug dose can be made without relying on the (rather abstract) distinction between distinct optima vs. different selection intensity.

* “This [association of strong resistance with high costs] may well be true, but not necessarily” (p. 12). It would be great to back this up with empirical counterexamples, if available.

* Is the last sentence on p. 12 (“In any case, representing evolution of resistance as convergence to a phenotypic optimum has received some empirical support”) specific to this section’s discussion of varying drug doses, or is it more general? It sounds like a more general point that could provide an important connection to empirical literature, and thus could be made more prominent in the manuscript.

Making the manuscript more accessible and relevant to a broader audience, including experimentalists:

* Several of the above suggestions already go towards increasing accessibility of the paper and relating it to empirical approaches. In addition, the authors could strengthen their case that the concept/terminology of “cost of resistance” is a “hindrance” not only in models, but also in practice. I think they could readily argue that over-simplifying the fitness effects of “resistance” mutations will hinder prediction of the evolution of resistance, given that natural environments are multi-faceted and heterogeneous.

* The authors could also devote more attention to what could be improved in practice (e.g. what should be measured in empirical studies). Here, it may be worth acknowledging that while their suggestions are clear and “simple” conceptually, they are not necessarily simple to implement: e.g. in the concluding section on p. 13, saying that “it may be safer in most cases to simply discuss and measure the fitness effects of mutation in different environments” sounds good in theory, but entails a lot of work (and decisions about which environmental factors to vary) in practice. Can the authors point to empirical studies that have made progress in this direction?

Minor comments on wording:

* In a couple of places, the authors refer to the importance of “ecological conditions” when really environmental conditions, more broadly defined (both abiotic and biotic), are relevant. E.g. beginning of *The context dependence of fitness effects* (“the selective effects of mutations depend on ecological conditions”) and middle paragraph on p. 4 (“it helped introduce some ecology…”).

* p. 3, 1st sentence under *The cost of resistance*: “This is where the concept of ‘cost of resistance’ becomes important” – I find this sentence too vague.

* p. 4 (section *The cost of resistance*): “In particular, all the paper[s] on local adaptation, clines and all the field of ecological genetics developed without the need to refer to this concept [cost].” This statement, without further qualification on “all the paper[s]”, feels a bit too sweeping. Perhaps the authors could rephrase to mention the time frame they are referring to? It might also be more enlightening to summarize these topics as general models of adaptation in heterogeneous environments, if that is an accurate assessment. Again, could models involving temporal heterogeneity, as well as spatial heterogeneity, be included here?

* pp. 4-5: In the paragraph on how cost arose from life history theory: I don’t really see how it became “natural to think that the cost can evolve to be reduced, or even eliminated” only in later interpretations.

* p. 5, 1st paragraph: “the best proof for this reasoning is that cost-free mutations are sometimes found…”. This might be better reworded as “the best support for this reasoning is that apparently

cost-free mutations are sometimes found". Moreover, given the direction of the authors' following arguments, it would be helpful to follow up this statement with caveats or counter-examples, instead of ending this paragraph with the impression that evolution of reduced or eliminated costs is the norm.

* p. 5, 2nd paragraph: I don't understand what is meant by "an essentialization of mutation/genotypes".

* The section title "Costs of resistance are not pleiotropic effects" might be better worded as "Cost of resistance are not equivalent to pleiotropic effects". Similarly, the following section title "Resistance mutations do not have a cost" could be misleading, and would be clearer if expanded to "Resistance mutations do not have a single, well-defined cost", or else modified to something like "'Cost of resistance' is poorly defined" or "'Cost of resistance' is a problematic/misleading term".

* p. 7: In the discussion of compensation of pleiotropic effects ("amelioration") vs. cost evolution, it might be helpful to illustrate these two different processes on the figure, or at least refer back to the figure (e.g. amelioration of pleiotropic effects would correspond to moving from point R to point P_1 in Fig. 1, but this endpoint is still associated with a non-zero cost represented by the distance OP_1).

* p. 8, 1st paragraph: "For instance, habitat quality varies and can even obscure the relationship between 'absolute' measures of fitness and environment variables…" This statement and its connection to the following E. coli example aren't entirely clear. It might help to clarify that the finding that E. coli grows faster at temperatures slightly higher than 37 degrees was presumably obtained in lab conditions, where many other variables may also differ from the human host, which could result in the apparent non-optimality of the evolved wild type.

* p. 8, 2nd paragraph: "Worse, this cost of resistance may not even actually be a 'cost'." This sentence is confusing and I think it would be better rephrased or left out, as the meaning is much clearer in the following sentence.

* p. 9: Is "phenotypic trade-off" a standard term? I am familiar with trade-offs in terms of fitness – while there is an implicit mapping from phenotype to fitness in this model, I'm not sure whether it is usual to refer to trade-offs directly on the phenotype level.

* Despite the authors' emphasis earlier in the paper that mutations are not inherently beneficial or deleterious, but rather that their fitness effects depend on context, later on there are several instances where the authors refer only to a "beneficial mutation" without being clear about the corresponding environment. Specifically: on p. 10 ("the mutation R illustrated on Figure 2 would still be a beneficial mutation"); in the caption of Fig. 3 ("they are therefore both beneficial mutations"); at the end of the 1st paragraph on p. 11 ("resistance mutations versus mere beneficial mutations").

* p. 10: "This would be in general clearer and more insightful." I find this sentence too vague. If it is meant as the introduction of a new, more useful definition of resistance, this should be made clearer and more prominent. This would be helpful later, e.g. on p. 12, when the authors continue to discuss "resistance mutations" and it is not always clear precisely how they are now being defined.

* The authors should be careful to distinguish "wild-type" from "genotype that is optimal in a particular non-treated environment". For example, in the last sentence on p. 10, the authors refer to both "a wild-type in O" and "a wild-type in B". More accurately, there is only a single wild-type (at O, as previously used), whereas point B represents an optimum in a particular non-treated environment (e.g. that being tested in the lab, which may not represent the ancestral environment

in which the wild-type evolved). The same issue comes up in the captions to Figs. 3 & 4, and on p. 13 ("failing to measure costs relative to a well-adapted wild-type to the non-treated environment…").

* Beginning of p. 11: Here the authors seem to suggest that fitness is always measured relative to a wild-type. It is true that competition experiments are one common way of quantifying relative fitness in the lab. However, it is also common to measure absolute fitness of a given strain in isolation, as in dose-response curves where the "response" is e.g. the net rate of population growth or decline when exposed to the drug.

* Having debunked the concept of "cost of resistance", what the authors mean when they nonetheless continue to refer to "cost" towards the end of the paper (pp. 12-13 and Figs. 3-4 captions) becomes blurry. It would be clearer to now avoid using the term "cost" all together, or else be careful to state precisely what is now meant by "cost".

* p. 12 minor wording clarification: "Studying 'cost' and 'benefit' at one particular dose may give the illusion…" (suggest adding the underlined part).

* In Figs. 3 & 4, please illustrate "cost" in the plots if possible. Otherwise, the statements about cost do not belong in the figure captions.

Citations:

* The reference list is actually quite extensive, including both theoretical and empirical references, and the authors' perspective overall seems to be well-founded based on their broad reading. However, there are a several statements in the text that would be better justified by adding specific citations (at least as examples):

- section *Resistance mutations as beneficial mutations*: "classically, the fitness benefit of a resistance mutation…depends on the fraction of the population exposed to the drug…", and later, "This selective advantage…is often thought to represent an inherent property of the mutation itself."
- bottom of p. 2 (near beginning of section *The context dependence of fitness effects*): "Rather, it [the fitness effect of a mutation] depends on the ecological conditions, the genetic background, and on other alleles."
- middle of p. 4: "something well known in ecological genetics"
- top of p. 5: "resistance mutations are simply viewed [in recent interpretations] as pleiotropic".
- 2nd paragraph of p. 7: "pleiotropic effects and the 'cost of resistance' are two different things… contrary to what is usually considered". (It's the part about what is usually considered that calls for citations.)
- end of 1st paragraph on p. 8: The *E. coli* example needs a citation. Subsequently, the claim "There are many other examples like this" should either be backed by citations, or simply cut.