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Coupling between tolerance and resistance differs between related (*Eimeria*) parasite species: implications for coevolution with their mouse hosts

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Dear Prof. Dr. Wolf Blanckenhorn,

We wish to resubmit an original research article (newly) entitled “Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for co-evolution with their mouse hosts” for consideration by Journal of Evolutionary Biology. We build on previous research showing that resistance and tolerance should be studied jointly, and show that coupling of the two can differ between closely related parasite taxa.

Testing whether closely related parasite species could show differences in coupling between tolerance and resistance, we found a trade-off between resistance and tolerance to one, *E. falciformis*, but not to its close relative *E. ferrisi*. Our work has direct implications for the evolutionary question of effects of parasites in hybrid zones. Moreover, we argue that the framework of resistance-tolerance coupling allows to prioritize research questions to be addressed with different parasites: broad questions of relevance for the host species as a whole with parasites showing no coupling, questions of local adaptation and host-parasite co-evolution with parasites showing coupling.

We think that this work will be of both general interest for evolutionary biologists working on parasites, and for specialised research on the house mouse hybrid zone.
This work is original and has not been published elsewhere, nor is it currently under consideration for publication elsewhere, we have no conflicts of interest to disclose, its submission for publication has been approved by all relevant authors and institutions, all persons entitled to authorship have been so named, all authors have seen and agreed to the submitted version of the manuscript. The full text (excluding abstract, references, tables and figure legends) contains 4242 words.

Thank you for your consideration of this manuscript.

Sincerely,

The authors

JEB MS JEB-2020-00032

Dear Prof. Dr. Blanckenhorn,

with this letter we provide a revised manuscript. Below we respond to your and the reviewers' comments in detail.

Re: Reject with resubmission allowed

Dear Author,

Thank you for submitting your manuscript "Decoupling of resistance and tolerance against one of two related parasites (*Eimeria*) in mice" (JEB ms JEB-2020-00032) to the Journal of Evolutionary Biology. Your work has now been considered by two reviewers, whose comments are enclosed. Overall, and this includes my assessment, there seems to be some potential in your work being interesting for evolutionary biologists. However, as currently presented we must reject your manuscript for multiple reasons outlined by the reviewers below.

We want to thank both reviewers for the thorough reviews. We are confident we were able to substantial improve our manuscript taking into account all comments and issues raised. We changed the manuscript substantially, including the title, which now reads: "Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for co-evolution with their mouse hosts"

C1. First and foremost, as mentioned by both reviewers, the writing, particularly the main set-up of your manuscript, leaves much to be desired and seems to be generally geared more to parasitologists than to evolutionary biologists. In particular, your work is taxonomically too focused on your system, and you fail to cite work on other species that is relevant in this context.

We now focus the manuscript on the evolutionary implications of coupling (or the absence thereof) between resistance and tolerance. We argue, these are, that host-parasite co-evolution can be expected

in the presence of coupling, much less in the absence. We then present our test for local (host subspecies) adaptation for *E. ferrisi* in this framework and conclude that our negative result for this might be explained with an absence of resistance-tolerance coupling.

C2. Second, reviewer 1 asks what the relevance or specificity of your work is in relation to the mouse hybrid zone, which of course would be very interesting to know from an evolutionary point of view but currently remains highly unclear and unargued.

We now highlight implications of our main result (resistance-tolerance coupling for one but not the other parasite) for the house mouse hybrid zone in the discussion (lines 354-366). We hope this shows the relevance of our findings within our system. This has direct implications for the evolutionary question of effects of parasites in hybrid zones. We also show that this serves an example beyond our systems (as we try to balance taxonomically focused with broad evolutionary implications as suggested by the reviewers). We argue that the framework of resistance-tolerance coupling allows to prioritize research questions to be addressed with different parasites: broad questions of relevance for the host species as a whole with parasites showing no coupling, questions of local adaptation and host-parasite co-evolution with parasites showing coupling.

C3. Third, both reviewers question your measure of tolerance. Reviewer 1 asks why you did not use the measured suggested in this context by another author that you cite, and reviewer 2 outright dismisses your index as statistically shaky if not flawed. I definitely agree with the latter assessment, which apparently is still a recurring problem in ecological and evolutionary studies.

We agree with the reviewers than our index calculated was based on measurements for host individuals. Following the advice of the reviewers, we changed it for a genotype based measurement, within each group. We described our approach in material and method as follows (lines 211-216):

> "Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness (or health condition if that is the parameter of interest) on infection intensity per genotype (Simms, 2000; Råberg et al., 2009). Thus tolerance was assessed as the slope of maximum relative weight loss compared to day 0 on number of OPG at the day of maximal shedding, within each

mouse strain and for each parasite isolate. A steep slope indicates a low tolerance (high weight lost for a given parasite burden)."

C4. Fourth, a control group is missing.

Each animal present individual variation in weight at the time of infection. We are convinced that each individual at starting day is a better control for it's own weight loss. The assessment of harm caused in animal infection experiments (for potentially necessary humane endpoints) does e.g. not allow comparison to a control group, but only to the starting weight of the same individual. We agree with this regulations and consider the "internal control" more relevant than uninfected control groups (which we are frankly not allowed to use in this experiment). We implement this within our analysis using a fixed a null intercept, as detailed in the previous comment.

These are but the most important criticisms of your work, and both reviewers made even more useful suggestions for change. In light of these comments and my own reading of your manuscript, I therefore cannot recommend your paper for publication and thus reject it.

We hope that you consider our revision for another round of review and that we were able to convincingly answer the reviewer's concerns as detailed below.

However, I leave the option open for you to resubmit a substantially revised version of your article at some point in the future, if you think you can fix the main criticisms and make this work more interesting for evolutionary biologists. Any resubmitted manuscript will be treated as a new submission, and there can of course be no guarantee that the paper will ultimately be published by JEB. It is very likely that the new MS would be sent back to at least one of the original reviewers, so please think carefully about the value of resubmission (i.e. can you present new data/analyses or clarify issues in a way that is likely to satisfy the reviewer).

We added four more mouse strains and changed our analytical framework for tolerance, we hope that the revised conceptional framework satisfy the reviewers.

If you resubmit a revised version, please include a letter in which you describe how you have

responded to each of the referees' comments. Please number the comments and refer to line numbers in the original and revised paper for easy reference. A marked-up revision is also helpful. Please upload this letter with your other files so it forms part of the PDF.

We did not provide a marked-up version, as the our changes are so substantial that such document would not be useful. The text is ~70% rewritten and the remaining ~30% are reorganized.

Please submit the paper on the JEB website (http://mc.manuscriptcentral.c) as a RESUBMISSION, providing the original ms number. In your Author Center in ScholarOne Manuscripts you will find on the left a list entitled My Manuscripts. Click the Manuscripts with Decisions in this list; in the resulting list you will find this manuscript with on the right under Actions the option Create a Resubmission. By clicking this link you will be guided through the resubmission process.

Sincerely,

Wolf Blanckenhorn
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****
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Reviewer: 1

Comments to the Author

In this manuscript about host-parasite interactions, resistance (parasite load) and tolerance (the maintenance of health despite infection) against Eimeria protozoan parasites of different species and strains are experimentally studied in house mice. Both resistance and tolerance should entail fitness costs, and high resistance will reduce selection on evolution of tolerance, and vice versa, so that one might expect species to exhibit either high resistance or high tolerance, but not both, predicting a negative correlation between the two. The house mice hosts were inbred strains derived from two Mus musculus musculus and two Mus musculus domesticus populations. The main result is that with infection with one Eimeria species, the two subspecies appear to show a negative correlation between resistance and tolerance, while none is evident upon infection by the second Eimeria species, which is quite interesting.

C5. I didn't understand the rationale for carrying out this experiment on Eimeria strains derived from the house mice hybrid zone – what do we learn from this that we wouldn't learn from Eimeria strains safely within the domesticus side or the musculus side of the hybrid zone? Are potential adaptations of Eimeria to mice of the hybrid zone important? Do the two Eimeria species not coincide elsewhere?

We thank the reviewer for this comment that will allow improvement of our manuscript. We now focus our conceptual framework and highlight two aims of our study: to test (1) presence/absence of resistance-tolerance trade-offs for each parasite and (2) local adaptation of *E. ferrisi*. We argue that presence or absence of coupling of resistance and tolerance allows expectation on the presence of local adaptation.

We are confident that this is now clearly pronounced in the introduction, lines 101-109:

> "We tested if coupling between resistance and tolerance differs between both parasite species and discussed the implication for parasite-host coevolution. As coevolving hosts and parasites can adapt to their local antagonist, we tested local adaptation of *E. ferrisi* to *Mus musculus*, using a parasite isolated in a *M. m. domesticus* host and one in a *M. m. musculus* host. Parasite local adaptation corresponds to a higher parasite fitness in sympatric than in allopatric host, and host local adaptation corresponds to a higher host fitness when infected with sympatric than

> allopatric parasite (Schulte et al., 2011). If found, local adaptation would be indirect evidence for 109 coevolution of this parasite with *Mus musculus*."

and in the discussion, lines 385-386:

> "Moreover, our results did not support local adaptation of *E. ferrisi*, which might be explained by the absence of host-parasite coevolution caused by uncoupling of parasite and host fitness"

C6. I also am not sure why it is advantageous to carry out this experiment on highly inbred mice, capturing little genotypic variation, rather than on mice with natural within- population variation. I found the answer in Raberg et al. 2009, who advocate such an approach to allow an estimate of the slope of the relationship between fitness and infection intensity, which they define as tolerance, per genotype. The authors of this manuscript refer to this as the usual way to measure tolerance, but this slope is not estimated here, instead a tolerance index is used – an individual based measure, rather than a genotype based measure. There is however no explanation given as to what governed this choice, why the tolerance index is preferable to the standard way, especially given that they use "fully inbred" mice (what does fully-inbred mean?).

We revised our analysis to a "slope per genotype" based measure, as described earlier in this document (**C3**):

> "Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness (or health condition if that is the parameter of interest) on infection intensity per genotype (Simms, 2000; Råberg et al., 2009). Thus tolerance was assessed as the slope of maximum relative weight loss compared to day 0 on number of OPG at the day of maximal shedding, within each mouse strain and for each parasite isolate. A steep slope indicates a low tolerance (high weight lost for a given parasite burden)." (lines 211-216)

C7. The introduction mentions that "understanding how resistance and tolerance are coupled is necessary to conclude on health effects of parasitism". I'm not sure I understand how data on the relationship between resistance and tolerance help to understand fitness costs. The introduction implies a prediction that resistance and tolerance are negatively correlated, but reports that one study found them to be uncoupled. A quick look at the literature led me to several more examples that are relevant,

although on different host-parasite systems. The discussion is very focused on house mice and Eimeria. There needs to be broadening out of the discussion and comparison with other systems.

We now focus much less on fitness effects on the host *per se* but make (potentially more bold, we admit) conclusion on the implications of resistance-tolerance coupling. In the discussion, we developed the relevance of our results for our system. We frame this as an example to better understand the appearance of the co-evolution in host–parasite systems. We argue that co-evolution in more likely in systems presenting tolerance-resistance coupling.

C8. Does sex play a role in either resistance or tolerance? Males and females were included in this study but pooled for data analysis.

We are not aware of reasons to hypothesize higher or lower resistance or tolerance in *Eimeria* infections in one sex. For this reason, we chose to not use sex as factor in the model, but rather used a sex-balanced design within each isolate infection group to add more (meaningful) variance and obtain more conservative results.
Lines 163-164:

> "Mice were randomly allocated to experimental groups ensuring homogeneous distribution of ages and sexes between groups."

C9. The experimenters ran into some trouble during the experiment, as some mice that tested negative to Eimeria infection prior to entering the experiment, turned positive by the day the experiment started. It would be helpful to indicate what the time frame was here – how much earlier was the negative test compared to the start of the experiment? This was unfortunate, and the authors laudably make it clear that this occurred, but argue that they found no sign that it affected the outcome of the experiment, by examining the data with and without the affected trials.

We thank the reviewer to appreciate our efforts on honesty. We now make the timeframe for infections of the different batches (including the problematic batches) more clear. See lines 161 to 186:

> "All individuals were negative for *Eimeria* at the beginning of our experiment (before infection of first batch, as described in the next paragraph). In total, 168 mice were infected. Mice were randomly allocated to experimental groups ensuring homogeneous distribution of ages and

sexes between groups. Our experiments were conducted in four (partially overlapping) consecutive batches for logistical reasons. The first two batches were infected with the two *E. ferrisi* isolates (Brandenburg64 and Brandenburg139), the third and fourth by one *E. ferrisi* isolate (Brandenburg64) and one *E. falciformis* isolate (Brandenburg88). Our experimental design is summarized in **Table 1** (chronology of experimental batches can be scrutinized in **Supplementary Table 1**).

Nematode infection is common in breeding facilities (Baker, 1998) and could interact with *Eimeria* (Clerc et al., 2019). Nematode eggs were observed in flotated feces of mice belonging to all genotypes before the experiment. Despite treatment of the first infection batch of mice (B1, 22 mice) with anthelminthics (Profender®, Bayer AG, Levekusen, Germany) following the protocole of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the protocole of Floyd, Rogers, Lambshead, & Smith, 2005) in randomly sampled fecal samples a week later. We therefore decided not to treat mice of the following infection batches. Moreover, we observed *Eimeria* oocysts in the feces of 28 mice belonging to the last experimental batch (batch B4) at the day of infection, likely due to cross-contamination between batches. For following statistical analyses, we considered along with the full data set (N=168) a conservative data set in which cross-contaminated animals and animals treated by anthelminthic were removed (N=118). Results obtained on the conservative data set can be found in **Supplementary Material S2**. Despite differences in significance due to a lower statistical power, the main conclusions of our analyses were consistent with those obtained on the main data set."

C10. I think this is an error on page 10 – "SHUNT (Mmd subspecies) shed more OPG at the peak of shedding than both Mmm subspecies, PWD and BUSNA". It looks like it should be "less" not "more".

Thank you for spotting this mistake! We reshaped the document (following other comments), and therefore removed this sentence completely.

C11. Tables 2 until 6 duplicate some information from the figures – the upper diagonal of the tables shows estimate contrasts and SE, while estimates and CI are plotted in the figures. The lower diagonal shows statistics and p values. It would help the reader to indicate the difference between the upper and lower diagonals in the table headings. I would also suggest moving these tables to the Supplement, but keeping the figures in the main document.

We thank the reviewer for this comment. As we reshaped the document (following other comments), we do not discuss in details the differences between each strains any longer.

C12. It seems clear that the controversy about degree of parasite resistance in house mice in the hybrid zones interests the authors – but the links to this study seem fuzzy, as the house mice were not from the house mice hybrid zone, only the parasites were.

As developed earlier (**C2.**), we revised our focus and we now highlight implications of our main result (resistance-tolerance coupling for one but not the other parasite) for the house mouse hybrid zone in the discussion (lines 358-366). We hope this shows the relevance of our findings within our system:

> "In our host system, the house mice, for example, it has been shown that hybrids between *M. m. domesticus* and *M. m. musculus* are more resistant to parasites (Baird et al., 2012), including *Eimeria*, but tolerance could not be measured under natural conditions (Balard et al., 2020). The effect of parasites on host fitness in the evolution of the house mouse hybrid zone is thus still rather ambiguous (Baird & Goüy de Bellocq, 2019). We show that careful distinction between parasite species is necessary when analysing parasite host interaction (see also Jarquin et al 2019) and that it is indispensable to measure both resistance and tolerance in *Eimeria* infections of house mice. "

C13. Were the data analysed twice (once looking at subspecies/species differences and once looking at strain differences) because there wasn't enough data to do one model with strain nested within species, fitting all the variables?

> We previously tried to summarize results at the parasite species and mouse subspecies level. This comment made clear that this was confusing. We thus only report results at the detailed mouse strain level and parasite isolate level. The other "summary analyses" were redundant.

Reviewer: 2

Comments to the Author

C14. In this paper, the authors explore the effect of experimental parasitism by two closely related Eimeria parasites on four widl-derived strains of inbred mice to infections in terms of body mass loss and parasitism load (density of oocysts in feces). They claim to have estimated the level of host defences against experimental infection in terms of resistance and tolerance response as the inverse of maximum density of oocysts and the ratio between body mass loss and maximum density of oocysts,

respectively. I do not think the approach used to estimate tolerance is suitable, for two reasons: first, by using ratios (Instead of reaction norms) tolerance might by confounded with vigour; and second, resistance values are used in the denominator of the equation to estimate tolerance, which by itself could explain the claimed negative association found for one of the parasites.

We revised our analysis to a "slope per genotype" based measure, as described earlier in this document (**C3**):

> "Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness (or health condition if that is the parameter of interest) on infection intensity per genotype (Simms, 2000; Råberg et al., 2009). Thus tolerance was assessed as the slope of maximum relative weight loss compared to day 0 on number of OPG at the day of maximal shedding, within each mouse strain and for each parasite isolate. A steep slope indicates a low tolerance (high weight lost for a given parasite burden)." (lines 211-216)

C15. The paper is quite difficult to follow, and the importance of the study and results in evolutionary scenarios is vaguely introduced. In fact, most results merely describe the effects of experimental infection. The part of the manuscript that in my opinion is more interesting for evolutionary biologist is the study of associations between defensive tolerance and defensive resistance, and the manuscript should therefore be focused in this matter. However, as mentioned before, I think the estimate of tolerance is not appropriate and thus I do not think the conclusions on this experiment are strong enough.

We rewrote it almost entirely, and changed our estimate of tolerance. We are curious how the reviewers perceive this new focus.

C16. There are no line numbers, which make difficult pointing at the sentences to comment. In any case, with the hope of explaining further my point of view, I offer some comments below.

We apologize for this mistake and added line numbers in the current (we previously had the experience that the JEB submission system adds a – then confusing – own set of line numbers).

C17. Abstract has to be completely rewritten with direct and clear messages introducing the importance of the study, methodology employed, results and inferences of result. By now, from the abstract, it is difficult understand the performed work, whereas readers should be able to figure out what has been done. Some examples:
Abstract, 4th line: what do you mean with "hybrid hosts"?
Abstract, 5th line: what kind of fitness effects? What do you mean with modulatory effect?
Abstract: brief explanation of how resistance and tolerance responses were estimated is necessary here, before exposing the results.
Abstract, the two last lines: why findings of resistance in …. have to be interpreted carefully?

We re-wrote the abstract following this advice but also to reflect the revised conceptual focus of our manuscript.

C18. Introduction:
"In natural populations, costs of the two lines of defences against parasites predict that resistance and tolerance are negatively correlated". I do not agree! Tolerance and resistance can be positively, negatively or nor related to each other. It is just that two different lines of resistance defences, which both are costly, can be positively, negatively or nor related to each other.

We agree and modified the introduction highlighting the different possible scenarios of a) negative, b) positive or c) absence of correlation (see lines 55 to 83).

C19. The Introduction is devoted to (i) explain that both resistance and tolerance defensive responses have associated costs, (ii) introduce mouse subspecies immunological characteristics, the two protozoa Eimeria parasites, their prevalence, and their effects in their mouse hots. The authors finish the introduction mentioning that because of differences in prevalence and pathogenicity, associations between resistance and tolerance against the two parasites might differ. I think authors should pay more effort to explain the importance of the study in evolutionary scenarios by, for instance, make clearer the

importance of detecting different defensive strategies of hosts against different parasites in scenarios of host-parasites interactions and evolution.

We entirely rewrote our introduction following this advice, please see in revised manuscript. We focus on the implication of coupling between resistance and tolerance.

C20. Material and methods

Please, try not to use acronyms, or reduce them to a minimum. It makes it very difficult to follow the text.

We replaced all Mmm and Mmd acronyms by species names for clarity.

I am not an expert on the described lab methodologies, and thus I cannot comment on them. Experimental approach:

C21. Mice were orally infected with 150 sporulated oocysts, weight recorded and faeces collected daily.

All 108 used mice we infected and no control group was followed. In my opinion, control groups are necessary given that cross-contamination between batches can occur, and because experimental mice might be infected by nematodes or others kinds of parasites (last paragraph of “experimental infection).

As discussed earlier (**C4.**) each animal present individual variation in weight at the time of infection. We are convinced that each individual at starting day is a better control for it’s own weight loss. The assessment of harm caused in animal infection experiments (for potentially necessary humane endpoints) does e.g. not allow comparison to a control group, but only to the starting weight of the same individual. We agree with this regulations and consider the “internal control” more relevant than uninfected control groups (which we are frankly not allowed to use in this experiment). We implement this within our analysis using a fixed a null intercept, as detailed in earlier comments.

Measures of resistance and tolerance: I am not sure the approach is appropriate or correct to test the idea.

C22. - Resistance: “number of oocysts per gram of feces (OPG) at the day of maximal shedding”. Do you mean the inverse of number of oocysts …. ?

We corrected this point, see lines 192-193:

> "Therefore, as a proxy of (inverse of) resistance we used the number of oocysts per gram of feces (OPG) at the day of maximal shedding. "

C23. - Tolerance: "We defined a tolerance index for each individual, describing how its health varied with infection intensity, between day 0 of infection (weight = 100%, parasite intensity = 0 oocyst per mouse gram) and highest impact (weight = maximum weight loss relative to day 0, parasite intensity = maximum parasite number per gram of feces)."

Two points: (i) But you first mentioned that tolerance is defined as the slope (reaction norm) of the relationship between parasite burden and fitness related variable (… ??). Why didn't you estimate the reaction norms, then?

(ii) Ratios between fitness related variables (or phenotypic quality) and intensity of parasitism are not a good approach to characterise tolerance because it might be confound with individual vigour (i.e., better quality individuals will experience smaller negative effect of a target parasitism burden, but it does not mean they are more tolerant than individuals of lower quality). See discussion on Raberg et al. 2009 and Fry 1993 (Fry JD. 1993. The "general vigor" problem: can antagonistic pleiotropy be detected when genetic covariances are positive? Evolution. 47:327–333.) for the exposition of the namely vigor problem.

(iii) In any case, tolerance is estimated by using the value of resistance in the denominator of the ratio, implying additional problems of interpretation of results, mainly when the aim of the manuscript is to explore association between estimates of tolerance and of resistance tolerance = (maximum relative weight loss … / maximum number of oocysts …), As the maximum number of oocysts is used as a measure of resistance, tolerance is defined on the base of the level of resistance. Thus, it is not unexpected that estimates of resistance and tolerance were negatively related, it is just the mathematical consequence of the methods used to estimate them.

We agree with the reviewers than our index calculated was based on measurements for host individuals. Following the advice of the reviewers, we changed it for a genotype based measurement, within each group. We described our approach in material and method as follows (lines 211-216):

> "Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness (or health condition if that is the parameter of interest) on infection intensity per genotype (Simms,

> 2000; Råberg et al., 2009). Thus tolerance was assessed as the slope of maximum relative weight loss compared to day 0 on number of OPG at the day of maximal shedding, within each mouse strain and for each parasite isolate. A steep slope indicates a low tolerance (high weight lost for a given parasite burden)."

We also discuss possible statistical artifacts that could emerge as well from a linear regression between tolerance (measured now as reaction norm) and resistance (lines 254-265):

> "Of note, tolerance (in absolute value) is measured as the slope α of the linear regression of parasite load (x) on maximum relative weight loss (y) of equation $y = \alpha x + \beta$ (α being the slope and β the intercept, 0 in our case). Therefore, tolerance is expressed as $\alpha = y/x - \beta$. As x and y/x are by definition not independent, testing the correlation between resistance and tolerance can lead to spurious correlation (Brett, 2004). To alleviate the dangers of this statistical artifact, we additionally tested differences in resistance, impact on health and tolerance between mouse strains separately and also the underlying correlation between mean parasite load (x) and mean relative weight loss (y). We use the terminology "coupling" (between resistance and tolerance) to describe genotype-level correlation between tolerance and resistance additionally supported by the absence of positive correlation between health-effect and resistance."

We thank the reviewers for these comments that without doubt have greatly improved our manuscript. We hope that these improvements will make our manuscript acceptable for publication in JEB.

Yours sincerely,
The authors

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Title: Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for co-evolution with their mouse hosts

Running title: Different resistance-tolerance coupling

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Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for coevolution with their mouse hosts

Abstract

Resistance (host capacity to reduce parasite burden) and tolerance (host capacity to reduce impact on its health for a given parasite burden) manifest two different lines of defence. Tolerance can be independent from resistance, traded-off against it, or the two can be positively correlated because of redundancy in underlying (immune) processes. We here tested whether closely related parasite species could show differences in this coupling between tolerance and resistance. We tested this in experimental infections with two parasite species of genus *Eimeria*. We measured proxies for resistance (the (inverse of) number of parasite transmission stages (oocysts) per gram of feces at the day of maximal shedding) and tolerance (the slope of maximum relative weight loss compared to day of infection on number of oocysts per gram of feces at the day of maximal shedding for each host strain) in four inbred mouse strains and four groups of F1 hybrids belonging to two mouse subspecies, *Mus musculus domesticus* and *M. m. musculus*. We found a negative correlation between resistance and tolerance against *E. falciformis*, while the two are uncoupled against *E. ferrisi.* We conclude that resistance and tolerance against the first parasite species might be traded off, but evolve more independently in different mouse genotypes against the latter. We argue that host evolution can be studied largely irrespective of parasite isolates if coupling is absent or weak (*E. ferrisi*) but

host-parasite coevolution is more likely observable and best studied in a system with coupled tolerance and resistance (*E. falciformis*).

Keywords: Resistance, Tolerance, *Eimeria*, Coevolution

Introduction

Host defence mechanisms evolve to alleviate the detrimental effect of parasites. They can be categorised into two components: resistance and tolerance (Råberg et al., 2009). Resistance is the ability of a host to reduce parasite burden, resulting from defence against parasite infection or proliferation early after infection (Schmid-Hempel, 2013). The negative effect of resistance on parasite fitness can lead to antagonistic coevolution. According to theoretical models, fluctuating host and parasite genotypes arise, and balancing selection maintains resistance alleles polymorphic (Boots et al., 2008; Roy & Kirchner, 2000). Resistance has been the classical ”catch all” measure for host-parasite systems, but recently it has been shown to be incomplete, especially with respect to potential fitness effects on the host (Kutzer & Armitage, 2016; Råberg et al., 2009).

Disease tolerance (not to be confused from ”immunological tolerance”, unresponsiveness to self antigens; Medzhitov et al., 2012) is the ability of the host to limit the impact of parasite on its fitness (Kutzer & Armitage, 2016; Råberg et al., 2009; Vale & Little, 2012). By potentially providing a longer-living niche, this defence mechanism improves, or at least does not deteriorate, the fitness of the parasite. Tolerance alleles are thus predicted by theoretical models to evolve to fixation due to positive feedback loops (Boots et al., 2008; Restif & Koella, 2004; Roy & Kirchner,

2000). From a mechanistic perspective tolerance alleviates direct or indirect damage (e.g. excessive immune response underlying resistance against parasites, called immunopathology; Graham et al., 2005) caused by parasites (Råberg et al., 2009). Tolerance mechanisms include modulation of inflammatory response (Ayres & Schneider, 2012), tissue repair (stress response, damage repair and cellular regeneration mechanisms; Soares et al., 2017), and compensation of parasite-induced damage by increase of reproductive effort (Baucom & de Roode, 2011). The resulting metabolic costs of resistance and tolerance, with and without parasite infection, determine the optimal (steady state and infection inducible) extent and of both immune defences (Sheldon & Verhulst, 1996).

Resistance and tolerance can be positively associated if they involve the same metabolic pathway, as was shown in the plant model *Arabidopsis thaliana* in response against herbivory (Mesa et al., 2017). In animals, genetic association studies of resistance and tolerance of *Drosophila melanogaster* against the bacterium *Providencia rettgeri* have shown positively correlated genetic effects, as the same loci were associated with changes of both traits in the same direction (Howick & Lazzaro, 2017).

Nevertheless, resistance and tolerance can also be genetically and physiologically independent, involving different proximate mechanisms. Lack of correlation between both defences was shown for example in monarch butterflies (*Danaus plexippus*) infected by the protozoan parasite *Ophryocystis elektroscirrha*. This study found genetic variation in resistance between butterflies families, but a fixed tolerance (Lefèvre et al., 2010). Similarly, no correlation could be found between resistance and

tolerance for the fish *Leuciscus burdigalensis* in response to infection with its parasite *Tracheliastes polycolpus*. The authors explain the decoupling of both defences by the fact that, in this system, tolerance likely involves wound repair rather than immune regulation, making resistance and tolerance mechanisms independent (Mazé-Guilmo et al., 2014).

Eventually, in other systems, resistance and tolerance have been found negatively correlated. For examples, inbred laboratory mouse strains lose weight upon infection with *Plasmodium chabaudi.* The extent of this impact on host health is negatively correlated with the peak number of parasites found in the blood (Råberg et al., 2007), meaning that mouse strains with higher resistance present lower tolerance. Similarly, infections of sea trout (*Salmo trutta trutta*) and Atlantic salmon (*Salmo salar*) with the trematode *Diplostomum pseudospathaceum* showed that resistance and tolerance were negatively correlated when assessing mean levels of both traits in different host populations (Klemme & Karvonen, 2016). This is interpreted as a result of trade-off between resistance and tolerance (Råberg et al., 2009; Restif & Koella, 2004; Sheldon & Verhulst, 1996).

We have seen that depending on the system studied resistance and tolerance can be (1) uncoupled (independent), (2) positively correlated (involving same genes and mechanisms), or (3) negatively correlated (traded-off). Theoretical models show that coupling between resistance and tolerance (or absence thereof) depends not only on the host but also on the parasite (Carval & Ferriere, 2010). This raises the following question: could there be differences in the resistance-tolerance coupling upon infection of one host type with two closely related parasite species? To answer this

question, we infected four inbred mouse strains and four groups of F1 hybrids representative of two house mouse subspecies, *M. m. domesticus* and *M. m. musculus*, with three parasite isolates representative of two naturally occuring parasite species, the protozoan parasite *Eimeria ferrisi* and *E. falciformis* (Jarquín-Díaz et al., 2019). *Eimeria* spp. are monoxenous parasites that expand asexually and reproduce sexually in intestinal epithelial cells, leading to malabsorption of nutrients, tissue damage and weight loss (Chapman et al., 2013). The evolutionary history of these different *Eimeria* species in the two house mouse subspecies is unknown and it is unclear whether subspecies-specific adaptation exists in one or the other.

We tested if coupling between resistance and tolerance differs between both parasite species and discussed the implication for parasite-host coevolution. As coevolving hosts and parasites can adapt to their local antagonist, we tested local adaptation of *E. ferrisi* to *Mus musculus*, using a parasite isolated in a *M. m. domesticus* host and one in a *M. m. musculus* host. Parasite local adaptation corresponds to a higher parasite fitness in sympatric than in allopatric host, and host local adaptation corresponds to a higher host fitness when infected with sympatric than allopatric parasite (Schulte et al., 2011). If found, local adaptation would be indirect evidence for coevolution of this parasite with *Mus musculus*.

Material and methods

1. Parasite isolates

The three parasite isolates used in this study were isolated from feces of three different *M. m. domesticus/M. m. musculus* hybrid mice captured in Brandenburg, Germany, in 2016 (capture permit No. 2347/35/2014). The parasite isolates belong to both the most prevalent *Eimeria* species in this area, namely *E. ferrisi* (isolates Brandenburg64 and Brandenburg139) and *E. falciformis* (isolate Brandenburg88)(Jarquín-Díaz et al., 2019). Isolate Brandenburg64 was isolated in a 92% *M. m. domesticus* individual (hybrid index (HI) = 0.08: Proportion of *M. m. musculus* alleles in a set of 14 diagnostic markers, see Balard et al. (2020)), isolate Brandenburg139 in a 85% *M. m. musculus* (HI=0.85) and isolate Brandenburg88 in a 80% *M. m. domesticus* (HI=0.2). Pre-patency and the peak day of parasite shedding for these isolates were estimated during infection in NMRI laboratory mice (Al-khlifeh et al., 2019) which were also used for serial passaging of the isolates. Parasite infective forms (oocysts) were recovered by flotation in saturated NaCl solution followed by washing and observation under light microscope (following the protocol described in Clerc et al. (2019)) and stored at room temperature in 1mL of 2% potassium dichromate for a maximum of 2 months before infection of the wild-derived mice. Oocysts were allowed to sporulate 10 days before infection in a water bath at 30°C.

2. Mouse groups

We used four wild-derived inbred mouse strains from which we generated four groups of F1 hybrids. Two parental strains represented *M. m. domesticus*: **SCHUNT**

(Locality: Schweben, Hessen, Germany [N: 50° 26', E: 9° 36'] (Martincová et al., 2019)) and **STRA** (Locality: Straas, Bavaria, Germany [N: 50° 11', E: 11° 46'] (Piálek et al., 2008), and two derived from *M. m. musculus*: **BUSNA** (Locality: Buškovice, Bohemia, Czech Republic [N: 50° 14', E: 13° 22'] (Piálek et al., 2008)) and **PWD** (Locality: Kunratice, Bohemia, Czech Republic [N: 50° 01', E: 14° 29'] (Gregorová & Forejt, 2000)). The four groups of F1 hybrids consisted of two intrasubspecific hybrids (**SCHUNTxSTRA** and **PWDxBUSNA**) and two intersubspecific hybrids (**STRAxBUSNA** and **SCHUNTxPWD**)(**Figure 1**). Age of the mice at the time of infection ranged between 5.6 and 21.4 weeks. All mouse strains and F1 hybrids were obtained from the Institute of Vertebrate Biology of the Czech Academy of Sciences in Studenec (licence number 61974/2017-MZE-17214; for further details on strains see https://housemice.cz/en).

Parasites of the *Eimeria* genus are known to induce host immune protection against reinfection (Rose et al., 1992; Smith & Hayday, 2000). To ensure that our mice were *Eimeria*-naive, mouse fecal samples were tested before infection for the presence of *Eimeria* spp. oocysts by flotation in saturated NaCl solution followed by washing and observation under light microscope.

3. Experimental infection

Mice were kept in individual cages during infection. Water and food (SNIFF, Rat/Mouse maintenance feed 10 mm) were provided *ad libitum* supplemented with 1 g of sunflower and barley seeds per day. Mice were orally infected with 150 sporulated oocysts of one *Eimeria* isolate suspended in 100μl phosphate-buffer saline (PBS) and monitored daily until their sacrifice by cervical dislocation at time of

155 regression of infection (reduction of oocyst output). Individuals presenting severe
156 health deficiency and/or a weight loss approaching 18% relative to their starting
157 weight were sacrificed earlier at defined humane end points (experiment license Reg.
158 0431/17). Weight was recorded and feces collected on a daily basis. Fecal pellets
159 were collected every day from each individual cage and suspended in 2% potassium
160 dichromate. Parasite oocysts were recovered using NaCl flotation (see above).

161 All individuals were negative for *Eimeria* at the beginning of our experiment (before
162 infection of first batch, as described in the next paragraph). In total, 168 mice were
163 infected. Mice were randomly allocated to experimental groups ensuring
164 homogeneous distribution of ages and sexes between groups. Our experiments were
165 conducted in four (partially overlapping) consecutive batches for logistical reasons.
166 The first two batches were infected with the two *E. ferrisi* isolates (Brandenburg64 and
167 Brandenburg139), the third and fourth by one *E. ferrisi* isolate (Brandenburg64) and
168 one *E. falciformis* isolate (Brandenburg88). Our experimental design is summarized in
169 **Table 1** (chronology of experimental batches can be scrutinized in **Supplementary**
170 **Table S1**).

171 Nematode infection is common in breeding facilities (Baker, 1998) and could interact
172 with *Eimeria* (Clerc et al., 2019). We surveyed for their presence and nematode eggs
173 were observed in flotated feces of mice belonging to all genotypes before the
174 experiment. Despite treatment of the first infection batch of mice (B1, 22 mice) with
175 anthelminthics (Profender®, Bayer AG, Levekusen, Germany) following the protocole
176 of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the
177 protocole of Floyd et al. (2005)) in randomly sampled fecal samples a week later. We

178 therefore decided not to treat mice of the following infection batches. Moreover, we
179 observed *Eimeria* oocysts in the feces of 28 mice belonging to the last experimental
180 batch (batch B4) at the day of infection, likely due to cross-contamination between
181 batches. For following statistical analyses, we considered along with the full data set
182 (N=168) a conservative data set in which cross-contaminated animals and animals
183 treated by anthelminthic were removed (N=118). Results obtained on the
184 conservative data set can be found in **Supplementary Material S2**. Despite
185 differences in significance due to a lower statistical power, the main conclusions of our
186 analyses were consistent with those obtained on the main data set.

187 # 4. Statistical analyses

188 ## 4.1. Choice of proxies for resistance, impact of parasite on host and tolerance

189 As resistance is the capacity of a host to reduce its parasite burden, it is usually
190 estimated by the inverse of infection intensity (Råberg et al., 2009). Pre-patency (the
191 time to shedding of infectious stages, so called oocysts) is longer for *E. falciformis* (7
192 days) than for *E. ferrisi* (5 days) (Al-khlifeh et al., 2019). Therefore, as a proxy of
193 (inverse of) resistance we used the number of oocysts per gram of feces (OPG) at the
194 day of maximal shedding. Using the Spearman's non-parametric rank correlation test,
195 we found this measurement to be tightly correlated with the sum of oocysts shed
196 throughout the experiment (Spearman's ρ=0.93, N=168, P<0.001). Due to the
197 aggregation characteristic of parasites (Shaw & Dobson, 1995), the appropriate
198 distribution for maximum number of OPG was found to be the negative binomial
199 distribution. This was confirmed based on log likelihood, AIC criteria and

200 goodness-of-fits plots (density, CDF, Q-Q, P-P plots; R packages MASS (Venables &
201 Ripley, 2002) and fitdistrplus (Delignette-Muller & Dutang, 2015)).

202 Both parasite species provoke inflammation, cellular infiltration, enteric lesions,
203 diarrhea, and ultimately weight loss (Al-khlifeh et al., 2019; Ankrom et al., 1975; Ehret
204 et al., 2017; Schito et al., 1996). Therefore, the impact of parasites on host health was
205 measured as the maximum relative weight loss compared to day 0 (body weight
206 measured at the start of the experimental infection). For mice sacrificed at humane
207 end points before the end of the experiment, last weight of the living animal was used.
208 This weight (loss) can be expected to be a very conservative estimate for our
209 analyses (rendering tolerance conservatively low for these animals, which might have
210 lost more weight if not sacrificed).

211 Tolerance is usually defined as a reaction norm, i.e. the regression slope of host fitness
212 (or health condition if that is the parameter of interest) on infection intensity per host
213 genotype (Råberg et al., 2009; Simms, 2000). Thus tolerance was assessed as the
214 slope of maximum relative weight loss compared to day 0 on number of OPG at the
215 day of maximal shedding, within each mouse group and for each parasite isolate. A
216 steep slope indicates a low tolerance (high weight lost for a given parasite burden).

217 **4.2. Statistical modelling**

218 Maximum OPG and relative weight loss were modelled separately as a response of
219 either mouse group, parasite isolate and their interaction. We used a negative binomial
220 generalised linear model for maximum OPG, and a linear model for relative weight loss.
221 For tolerance, we performed a linear regression with null intercept (as each mouse was

controlled against itself at start of the experiment, before losing weight or shedding parasite), modelling relative weight loss as a response of maximum OPG interacting either mouse group, parasite isolate and their interaction. To test the significance of the marginal contribution of each parameter to the full model, each parameter was removed from the full model, and the difference between full and reduced model was assessed using likelihood ratio tests (G).

For each of our model, we also asked within each parasite isolate if the response differed between mouse groups using likelihood ratio tests (G) as described above. Of note, four mice infected by*E. falciformis* isolate Brandenburg88 did not shed any oocysts as death occurred at or one day before the peak of oocysts shedding in other mice. For this reason, we modelled maximum OPG for mice infected with this parasite using a zero-inflated negative binomial (ZINB) generalised linear model, after verifying that it provided a better fit than the simple negative binomial based on log likelihood and AIC criteria.

4.3. Test of local adaptation

Local adaptation of *E. ferrisi* was tested using two isolates (the ”Western” Brandenburg64 and ”Eastern” Brandenburg139) and our four parental mouse strains (the two *M. m. domesticus* Western SCHUNT and STRA, and the two *M. m. musculus* Eastern BUSNA and PWD). We hypothesised a possible local adaptation of *E. ferrisi*, i.e. (1) a higher parasite fitness in sympatric than in allopatric host, or (2) a higher host fitness when infected with sympatric than allopatric parasite. The prediction drawn from (1) would be that the Eastern parasite (*E. ferrisi* isolate Brandenburg139)

reproduces better in the matching Eastern mouse subspecies (*M. m. musculus*) than in the allopatric one (*M. m. musculus*), and similarly the Western parasite (*E. ferrisi* isolate Brandenburg64) reproduce better in *M. m. domesticus* than in *M. m. musculus.* According to hypothesis (2), a higher tolerance of each host infected by its matching parasite despite similar parasite reproductive output could indicate increased host fitness, and host local adaptation.

4.4. Test of coupling between resistance and tolerance

We tested coupling between resistance and tolerance for *E. ferrisi* and *E. falciformis* using the isolates Brandenburg64 and Brandenburg88 and our eight mouse groups. To test such coupling, one can assess the strength of correlation between measure of resistance and measure of tolerance (Råberg et al., 2007). Of note, tolerance (in absolute value) is measured as the slope α of the linear regression of parasite load (x) on maximum relative weight loss (y) of equation $y = \alpha x + \beta$ (α being the slope and β the intercept, 0 in our case). Therefore, tolerance is expressed as $\alpha = y/x - \beta/x$. As x and y/x are by definition not independent, testing the correlation between resistance and tolerance can lead to spurious correlation (Brett, 2004). To alleviate the dangers of this statistical artifact, we additionally tested differences in resistance, impact on health and tolerance between mouse groups separately and also the underlying correlation between mean parasite load (x) and mean relative weight loss (y). We use the terminology ”coupling” (between resistance and tolerance) to describe genotype-level correlation between tolerance and resistance additionally supported by the absence of positive correlation between health-effect and resistance. Correlations were tested using Spearman’s rank correlation.

All analyses were performed using R version 3.5.2 (R Development Core Team, 2013)(negative binomial: function glm.nb from R package MASS (Venables & Ripley, 2002); ZIBN: function zeroinfl from R package pscl (Jackman, 2020; Zeileis et al., 2008); linear model: function lm from R core package stats; mean and 95% confidence intervals: function ggpredict from R package ggeffect (Lüdecke, 2018)). Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled using the free software inkscape (https://inkscape.org). Code and data used for this article can be found at: https://github.com/alicebalard/Article_RelatedParasitesResTol

Results

1. General

Parasites of all isolates successfully infected all mouse groups (at the exception of 5 individuals infected by *E. falciformis* isolate Brandenburg88 that died or had to be sacrificed due to a strong weight loss before the peak of shedding for this parasite), meaning that no ”qualitative infection resistance” (*sensu* Gandon and Michalakis (2000)) was detected. For *E. ferrisi* (both isolates Brandenburg139 and Brandenburg64), the pre-patent period was 5 days post infection (dpi) and the median day of maximal oocyst shedding was 6 dpi (standard deviation sd=0.7 and 0.9, respectively). The median day of maximum weight loss was 5 dpi for both isolates (sd=2.1 and 1.7 respectively). For *E. falciformis* (isolate Brandenburg88) pre-patency was 7 dpi, median day of maximal shedding was 8 dpi (sd=1.3) and median day of maximal weight loss 9 dpi (sd=1.6)(**Figure 2**). Of note a considerable number of mice infected with this isolate (13 out of 56 = 23%) died or had to be sacrificed at humane end points less than 3 days after the oocysts shedding peak for the group, all

belonging to *M. m. musculus* subspecies (PWD, BUSNA, or their F1 PWDxBUSNA; 5 died at dpi 8, 5 at dpi 9, 3 at dpi 10). *E. falciformis* isolate Brandenburg88 was more lethal for the *M. m. musculus* mice strains than for the other strains (χ^2_7= 31.96, P<0.001; **Table 2**).

2. No indication of local adaptation of *E. ferrisi*

We tested if our proxies for resistance, impact on weight and tolerance were different between the four parental mouse strains and between both *E. ferrisi* infection isolates (isolate Brandenburg64 and Brandenburg139). Maximum parasite load differed between mouse strains (LRT: G=25.5, df=6, P<0.001), but the interaction term mouse strain-parasite isolate was non significant (LRT: G=4.1, df=3, P=0.25). A similar result was found for maximum relative weight loss (LRT: mouse strain: G=16.8, df=6, P=0.01; interaction mouse strain-parasite isolate: G=4.1, df=3, P=0.25). This indicates that when resistance and impact on weight vary between host strains, they do so independently of the parasite isolate. Eventually, the variables mouse strain, parasite isolate and their interaction were found non significant at the 0.05 threshold for the slope of the linear regression between the two, indicating that differences of tolerance could not be detected between mouse strains or parasite isolates (**Figure 3**). Our results do not indicate either (1) an increased reproduction of each parasite in its matching host or (2) a higher tolerance of host infected by its matching parasite despite similar parasite reproductive output. Thus they do not support the hypothesis of local adaptation between *E. ferrisi* and its host.

3. Resistance and tolerance to *E. ferrisi* isolate Brandenburg64 are uncoupled

We tested coupling between resistance and tolerance for *E. ferrisi* isolate Brandenburg64 in our eight mouse groups. First, we tested whether our proxies for resistance, impact on weight and tolerance were different between the mouse groups. We found the maximum number of OPG and relative weight loss to be statistically different between mouse groups (LRT: maximum number of OPG: G=26.6, df=7, P<0.001; **Figure 4A**; maximum relative weight loss: G=21.5, df=7, P<0.01; **Figure 4B**). Tolerance was not found to significantly differ between mouse groups for this parasite isolate (LRT: G=6.8, df=7, P=0.45; **Figure 4C**).

We found a non significant positive correlation between resistance (inverse of maximum number of OPG) and impact on health (maximum weight loss) (Spearman's ρ=0.69, P=0.07, N=8; **Figure 4D**). Eventually, we did not find a correlation between resistance (inverse of maximum number of OPG) and tolerance (inverse of slope of maximum weight loss on maximum OPG) (Spearman's ρ=0, P=1, N=8; **Figure 4E**).

In conclusion, we did not find indications of resistance-tolerance coupling for *E. ferrisi* isolate Brandenburg64, the different mouse groups infected by this parasite presenting a similar level of tolerance while showing an effect of quantitative resistance on health.

4. Coupling between resistance and tolerance to *E. falciformis*

We then tested coupling between resistance and tolerance for *E. falciformis* isolate Brandenburg88 in our eight mouse groups. First, we tested if our proxies for

resistance, impact on weight and tolerance were different between the mouse groups. We found the maximum number of OPG and relative weight loss to be statistically different between mouse groups (LRT: maximum number of OPG: G=28.6, df=14, P=0.012; **Figure 5A**; maximum relative weight loss: G=21, df=7, P<0.01; **Figure 5B**). Finally, contrary to our results on *E. ferrisi* isolate Brandenburg64, the tolerance slopes for *E. falciformis* isolate Brandenburg88 were different between mouse groups (LRT: G=13.9, df=7, P=0.05; **Figure 5C**).

We detected a strong negative correlation between (inverse of) resistance (maximum number of OPG) and tolerance (inverse of slope of maximum weight loss on maximum OPG) (Spearman's ρ=-0.95, P=0.001; **Figure 5E**). We conclude that this correlation is unlikely a statistical artifact, as (1) mouse groups present statistically different values of resistance and tolerance and (2) we found a (non significant) negative correlation between resistance (inverse of maximum number of OPG) and impact on health (maximum weight loss) (Spearman's ρ=-0.5, P=0.22; **Figure 5D**), indicating that mouse groups losing more weight also shed less parasites.

We conclude that our results indicate the presence of negative resistance-tolerance coupling for *E. falciformis* isolate Brandenburg88.

Discussion

In this study, we assessed resistance and tolerance to two closely related parasites, *E. ferrisi* (two isolates) and *E. falciformis* (one isolate), in four mouse strains and their intra-and intersubspecific hybrids. Understanding this coupling has two major implications.

From a practical ”measurement” perspective we can ask whether tolerance can be predicted from resistance, as the latter is easier to measure (e.g. in field sampling). Many studies assess the impact of parasites on host fitness based on resistance. If, as we found in the present study, resistance and tolerance are decoupled this can be missleading. In our host system, the house mice, for example, it has been shown that hybrids between *M. m. domesticus* and *M. m. musculus* are more resistant to parasites (Baird et al., 2012), including *Eimeria*, but tolerance could not be measured under natural conditions (Balard et al., 2020). The effect of parasites on host fitness in the evolution of the house mouse hybrid zone is thus still rather ambiguous (Baird & Goüy de Bellocq, 2019). We show that careful distinction between parasite species is necessary when analysing parasite host interaction (see also Jarquín-Díaz et al., 2019) and that it is indispensable to measure both resistance and tolerance in *Eimeria* infections of house mice.

More generally, in a evolutionary perspective, coupling between resistance and tolerance might determine whether coevolution between host and parasite can be expected. As such, coevolution in host-parasite systems is often assumed but rarely proven (Woolhouse et al., 2002). Janzen (1980) notes that not all parasite-host systems are coevolving. The presence of efficient host defences against a given parasite is not necessarily produced in response to this parasite specifically and the parasite does not necessarily respond specifically. In the mouse-*E. ferrisi* system, where resistance and tolerance are decoupled, host and parasite fitness might be decoupled as a result, making host-parasite coevolution less likely. In the mouse-*E. falciformis* system we found a negative coupling between tolerance and resistance, making coevolution between host and parasite more likely.

Differences between parasite species could explain the evolution of different strategies: *E. ferrisi* commits to sexual reproduction after a relatively short time with few cycles of asexual expansion (Al-khlifeh et al., 2019; Ankrom et al., 1975), while *E. falciformis* has a relatively longer life cycle (Al-khlifeh et al., 2019; Haberkorn, 1970). As *E. ferrisi* infections do not reach extremely high intensities, high tolerance might be the optimal strategy for both house mouse subspecies. Resistance could then evolve relatively freely without any major impact of the parasite on the hosts' health. Moreover, our results did not support local adaptation of *E. ferrisi*, which might be explained by the absence of host-parasite coevolution caused by uncoupling of parasite and host fitness. In the case of *E. falciformis*, the long life cycle might lead to high tissue load. Tissue damage is observed during sexual reproduction for this parasite (Ehret et al., 2017) and might mean that a certain level of resistance is required. On the other hand, immunopathology has been observed in advanced *E. falciformis* infections (Stange et al., 2012). These intrinsic characteristics of *E. falciformis* might lead to multiple different optima for resistance and tolerance, leading to a trade-off.

In addition, we could speculate on two related alternative explanations. Firstly, *E. falciformis* could originally be a *M. m. domesticus* parasite dissipated into *M. m. musculus* territory by a spillover through the hybrid zone. Secondly, the particular *E. falciformis* isolate employed here was collected from a predominantly *M. m. domesticus* mouse (hybrid index 0.2). The isolate could hence be locally adapted to *M. m. domesticus*. Experiments with additional *E. falciformis* isolates from *M. m. musculus* are needed to test whether host subspecies adaptation can lead to high tolerance and low resistance in matching pairs of *E. falciformis* isolates and

mouse subspecies. This seems plausible, as the coupling between resistance and tolerance links host and parasite fitness, making coevolution and hence local adaptation more likely. Interestingly, this parasite-host coevolution wouldn't be antagonistic but rather mutualistic with regards to tolerance and parasite reproduction (that is, the inverse of resistance) (Little et al., 2010; Råberg et al., 2009). Alternatively, though descriptive, molecular-genetic analyses of diagnostic marker can be used to infer coevolutionary pathways between host and their parasites (e.g. Goüy de Bellocq et al., 2018; Kváč et al., 2013).

In conclusion, we argue that the difference between resistance and tolerance coupling in two different parasites can guide research in the house mouse system: if the effects of host hybridisation should be studied independently of potential host-parasite coadaptation, the prevalent *E. ferrisi* might be the most suitable parasite. If coevolution between hosts and parasites should be studied, the pathogenic *E. falciformis* is a more plausible target. Generally, the coupling between resistance and tolerance can differ between closely related parasite species and we argue that this trait of a host-parasite system determines the questions to be best approached with a particular parasite.

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574 # Tables

Mouse		*Eimeria*		
group	**subspecies**	***E. ferrisi* Brandenburg139**	***E. ferrisi* Brandenburg64**	***E. falciformis* Brandenburg88**
SCHUNT	*M.m.domesticus*	7 (5M / 2F)	14 (6M / 8F)	6 (3M / 3F)
STRA	*M.m.domesticus*	6 (2M / 4F)	15 (8M / 7F)	7 (4M /3F)
SCHUNTxSTRA	*F1 M.m.domesticus*		6 (2M / 4F)	8 (5M / 3F)
STRAxBUSNA	*F1 hybrid*		8 (5M / 3F)	8 (3M /5F)
SCHUNTxPWD	*F1 hybrid*		8 (3M / 5F)	6 (4M / 2F)
PWDxBUSNA	*F1 M.m.musculus*		9 (4M / 5F)	7 (4M /3F)
BUSNA	*M.m.musculus*	6 (2M / 4F)	14 (8M / 6F)	7 (3M /4F)
PWD	*M.m.musculus*	6 (3M / 3F)	13 (10M / 3F)	7 (1M / 6F)

Table 1. Infection experiment design.

Mouse subspecies	group	status at dpi 11	
		alive	**dead**
Mmd	SCHUNT	6	0
Mmd	STRA	7	0
Mmd	SCHUNTxSTRA	8	0
Mmd-Mmm	STRAxBUSNA	8	0
Mmd-Mmm	SCHUNTxPWD	6	0
Mmm	PWDxBUSNA	4	3
Mmm	BUSNA	3	4
Mmm	PWD	1	6
	total	**43**	**13**

Table 2. Contingency table: number of mice and status at dpi 11 for each mouse group upon infection with *E. falciformis* isolate Brandenburg88.

Figures legends

Figure 1. Parasite isolates and mouse wild-derived strains. (A) Map showing locations at which mice were collected for breeding of mouse strains and isolation of parasites. The purple line is an estimation of the center of the house mouse hybrid zone between *M. m. domesticus* and *M. m. musculus* based on sampling and genotyping of mice in this area (Balard et al., 2020; Ďureje et al., 2012; Macholán et al., 2019). (B) The eight mouse groups (parents and F1s) used in our experimental infections.

Figure 2. Parasite density (A) and relative weight (B) during *Eimeria* infection. Parasite density is calculated as number of oocysts detected (in millions) per gram of feces, relative weight is calculated as the percentage of weight compared to day 0. Mean and 95% CI are plotted for each parasite isolate. All mouse groups are pooled

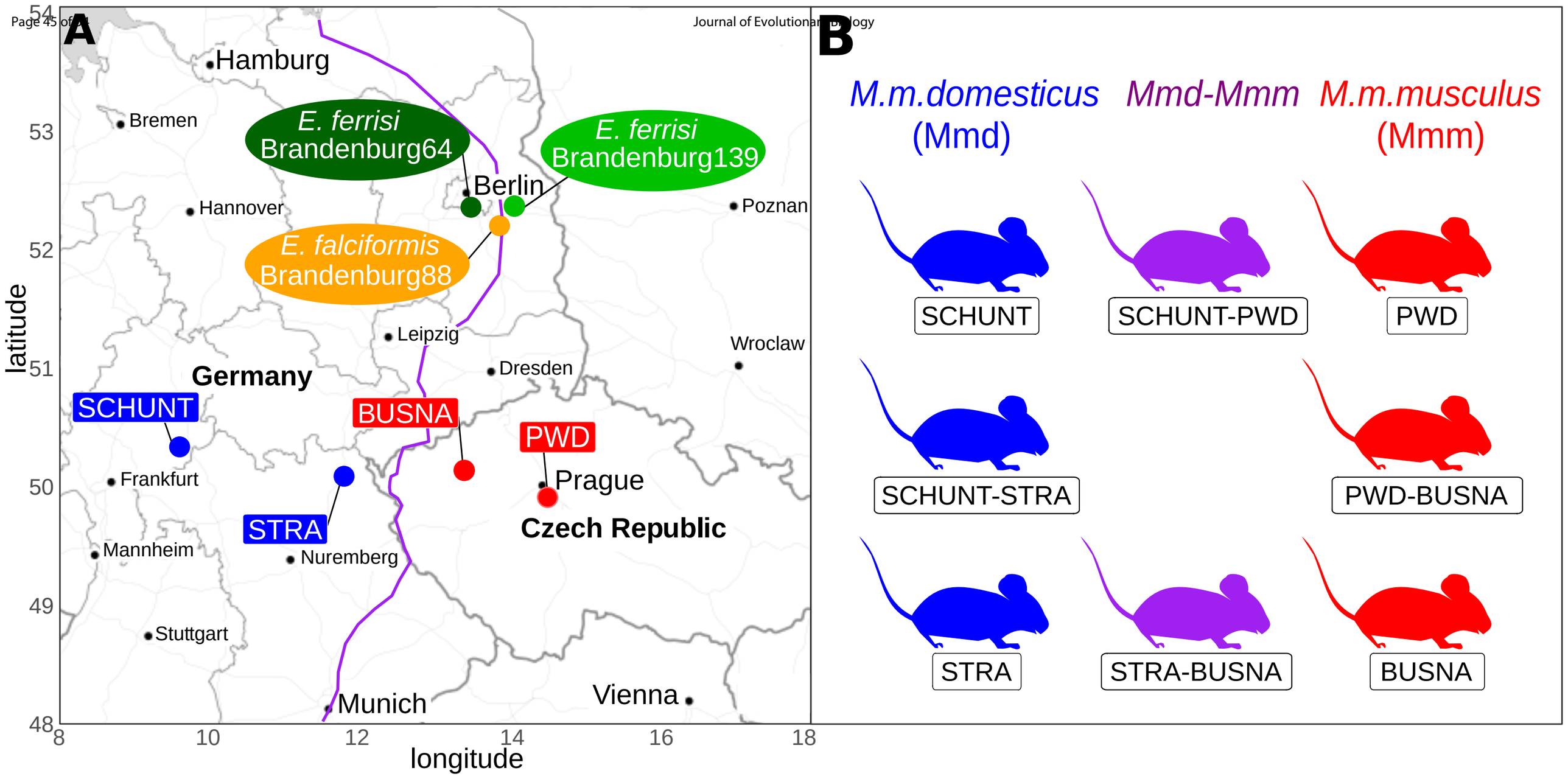
587 together.

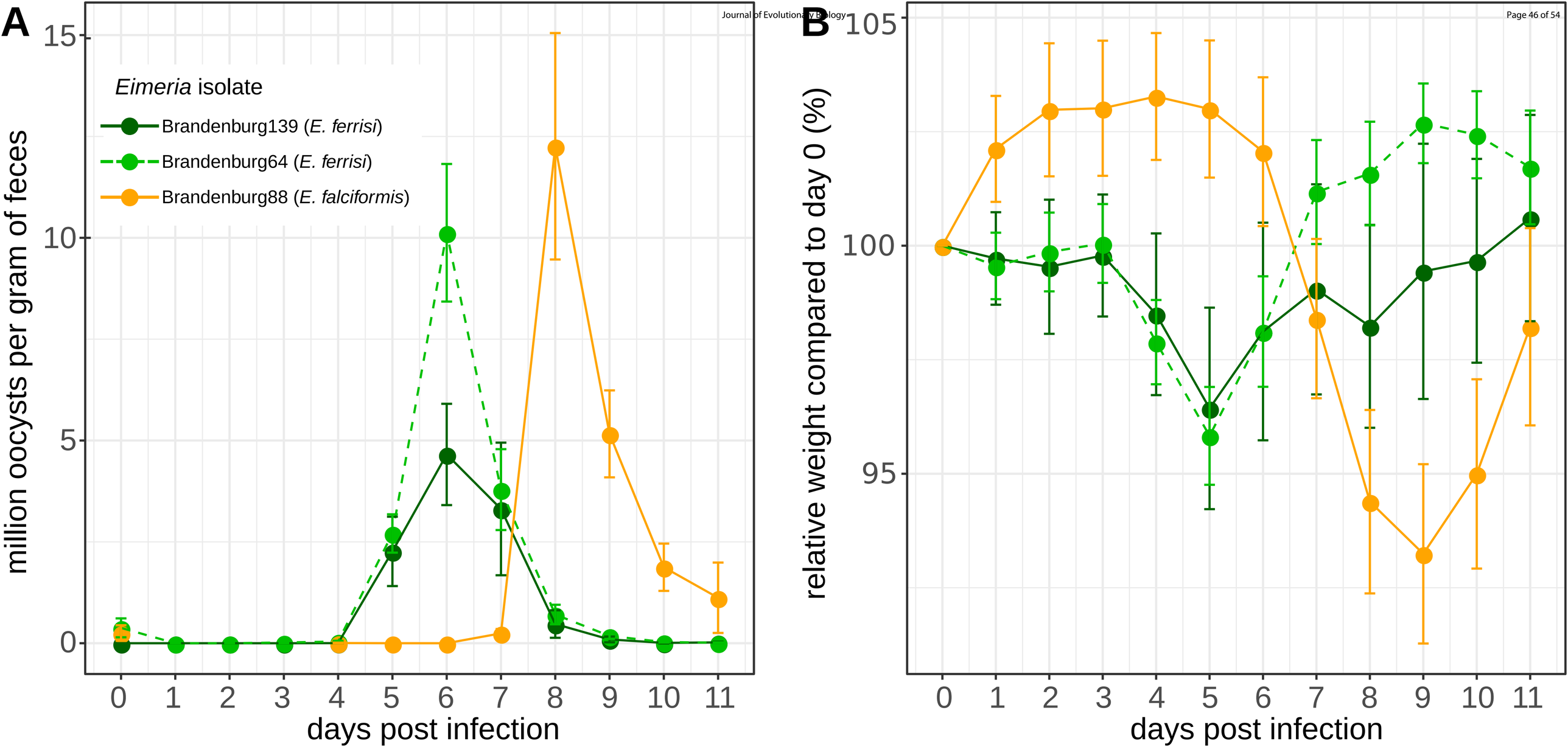
588 **Figure 3. Comparison of resistance, impact on weight and tolerance between**
589 **mouse strains for both *Eimeria ferrisi* isolates.** (A) Maximum oocysts per gram of
590 feces used as a proxy for (inverse of) resistance; (B) Impact on host health measured
591 as the maximum weight loss during patent period relative to starting weight (%); (C)
592 Tolerance estimated by the slope of the linear regression with null intercept modelling
593 maximum relative weight loss as a response of maximum oocysts per gram of feces. A
594 steep slope corresponds to a low tolerance. We did not detect (A) either higher parasite
595 shedding of the Eastern parasite isolate in Eastern mouse strains and vice versa or (C)
596 higher tolerance of Eastern hosts infected by Eastern parasite isolate and vice versa,
597 thus our results do not support the hypothesis of local adaptation between *E. ferrisi* and
598 its host.

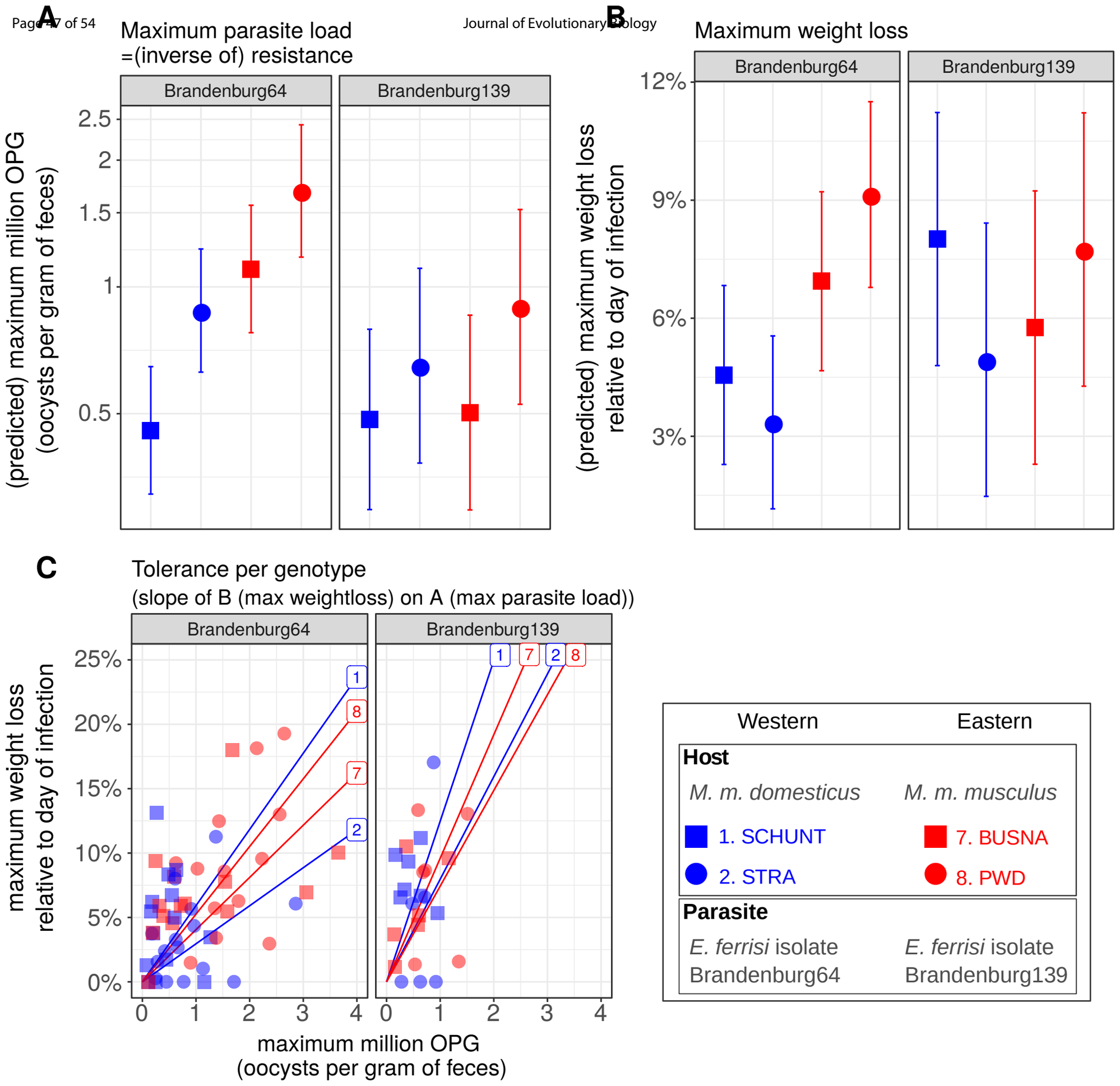
599 **Figure 4. No indication of resistance-tolerance coupling for *E. ferrisi* isolate**
600 **Brandenburg64.** Colors represent mouse subspecies (blue: *M. m. domesticus*, red:
601 *M. m. musculus*, purple: Mmd-Mmm). Left side: comparison of maximum oocysts per
602 gram of feces used as a proxy for (inverse of) resistance (A), impact on weight
603 measured as the maximum weight loss during patent period relative to starting weight
604 (B) and tolerance between mouse groups estimated by the slope of the linear
605 regression with null intercept modelling maximum relative weight loss as a response
606 of maximum oocysts per gram of feces, a steep slope corresponding to a low
607 tolerance (C). Maximum number of OPG and relative weight loss differ between
608 mouse groups, but tolerance is similar. Right side: non significant positive correlation
609 between mean maximum oocysts per gram of feces and mean relative weight loss (D)

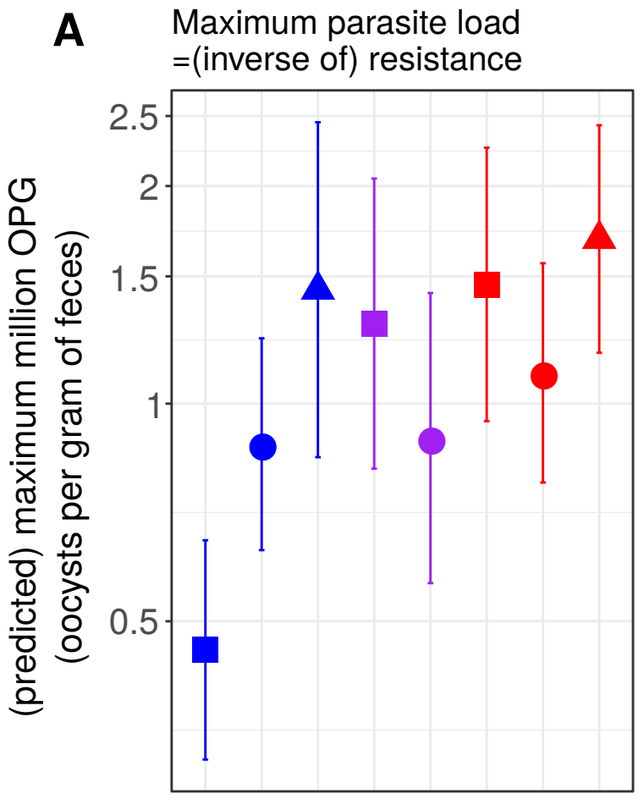
and absence of correlation between maximum oocysts per gram of feces used as a proxy for (inverse of) resistance and tolerance (E); Grey error bars represent 95% confidence intervals. Our results do not support coupling between resistance and tolerance *E. ferrisi* isolate Brandenburg64.

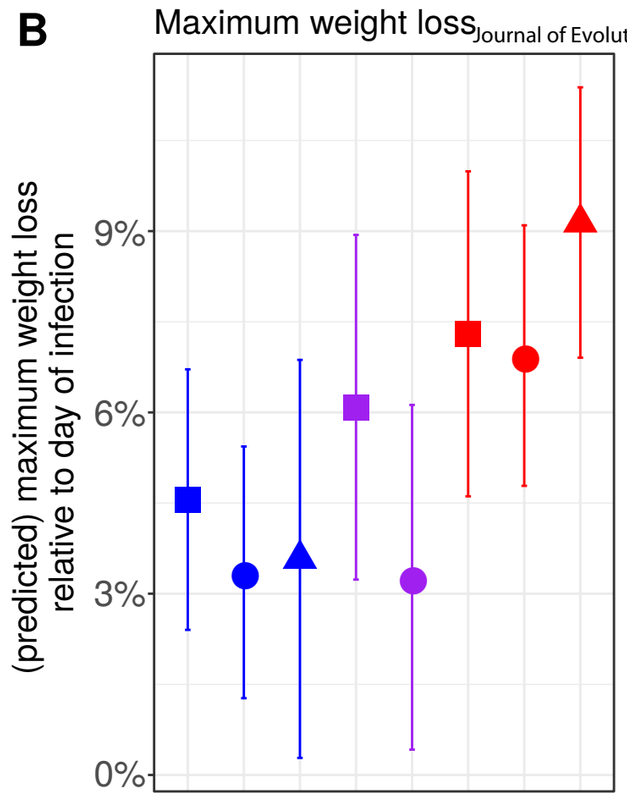
Figure 5. Coupling between resistance and tolerance for *E. falciformis* isolate Brandenburg88. Colors represent mouse subspecies (blue: *M. m. domesticus*, red: *M. m. musculus*, purple: Mmd-Mmm). Left side: comparison of maximum oocysts per gram of feces used as a proxy for (inverse of) resistance (A), impact on weight measured as the maximum weight loss during patent period relative to starting weight (B) and tolerance between mouse groups estimated by the slope of the linear regression with null intercept modelling maximum relative weight loss as a response of maximum oocysts per gram of feces, a steep slope corresponding to a low tolerance (C). Maximum number of OPG, relative weight loss and tolerance differ between mouse groups. Right side: non significant negative correlation between mean maximum oocysts per gram of feces and mean relative weight loss (D) and strong negative correlation between maximum oocysts per gram of feces used as a proxy for (inverse of) resistance and tolerance (E); Grey error bars represent 95% confidence intervals. Our results support coupling between resistance and tolerance *E. falciformis* isolate Brandenburg88.

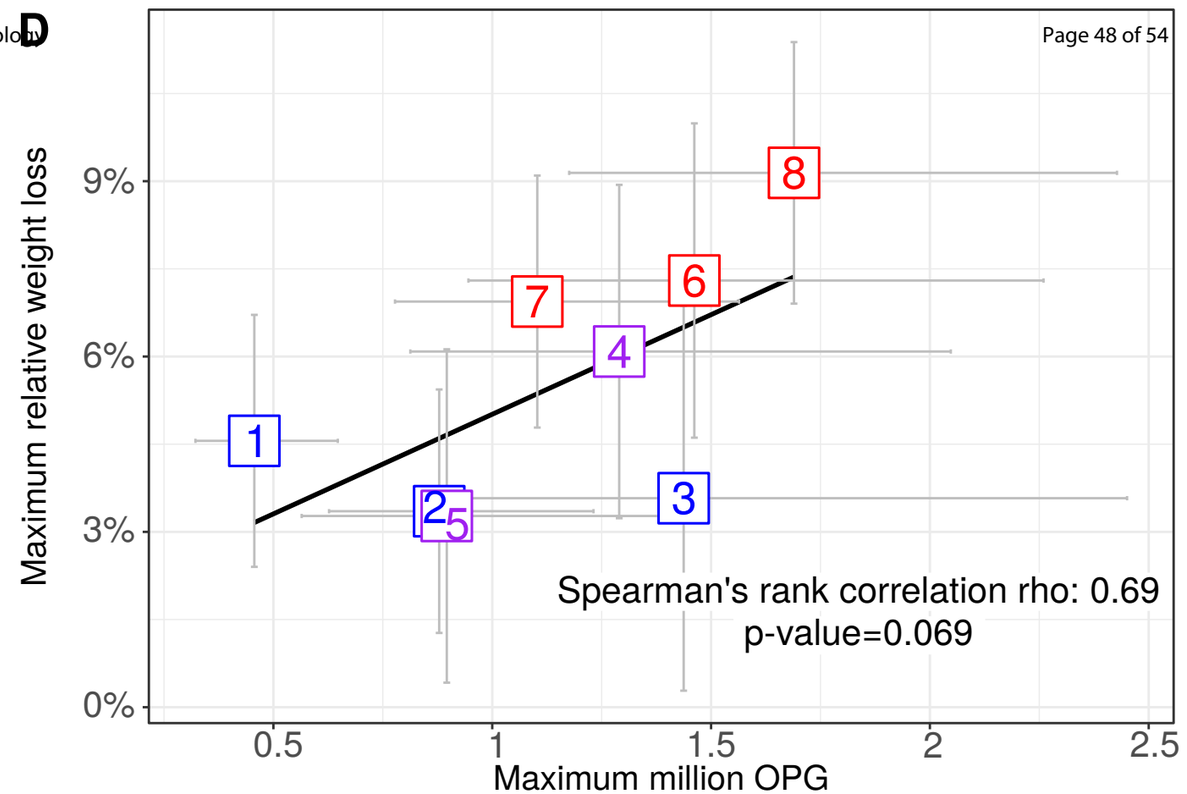

A
Hamburg
Bremen
E. ferrisi
Brandenburg64
E. ferrisi
Brandenburg139
Berlin
Hannover
Poznan
E. falciformis
Brandenburg88
Leipzig
Wroclaw
Germany
Dresden
SCHUNT
BUSNA
PWD
Frankfurt
Prague
STRA
Czech Republic
Mannheim
Nuremberg
Stuttgart
Munich
Vienna
latitude
longitude
54
53
52
51
50
49
48
8
10
12
14
16
18
B
M.m.domesticus
(Mmd)
Mmd-Mmm
M.m.musculus
(Mmm)
SCHUNT
SCHUNT-PWD
PWD
SCHUNT-STRA
PWD-BUSNA
STRA
STRA-BUSNA
BUSNA

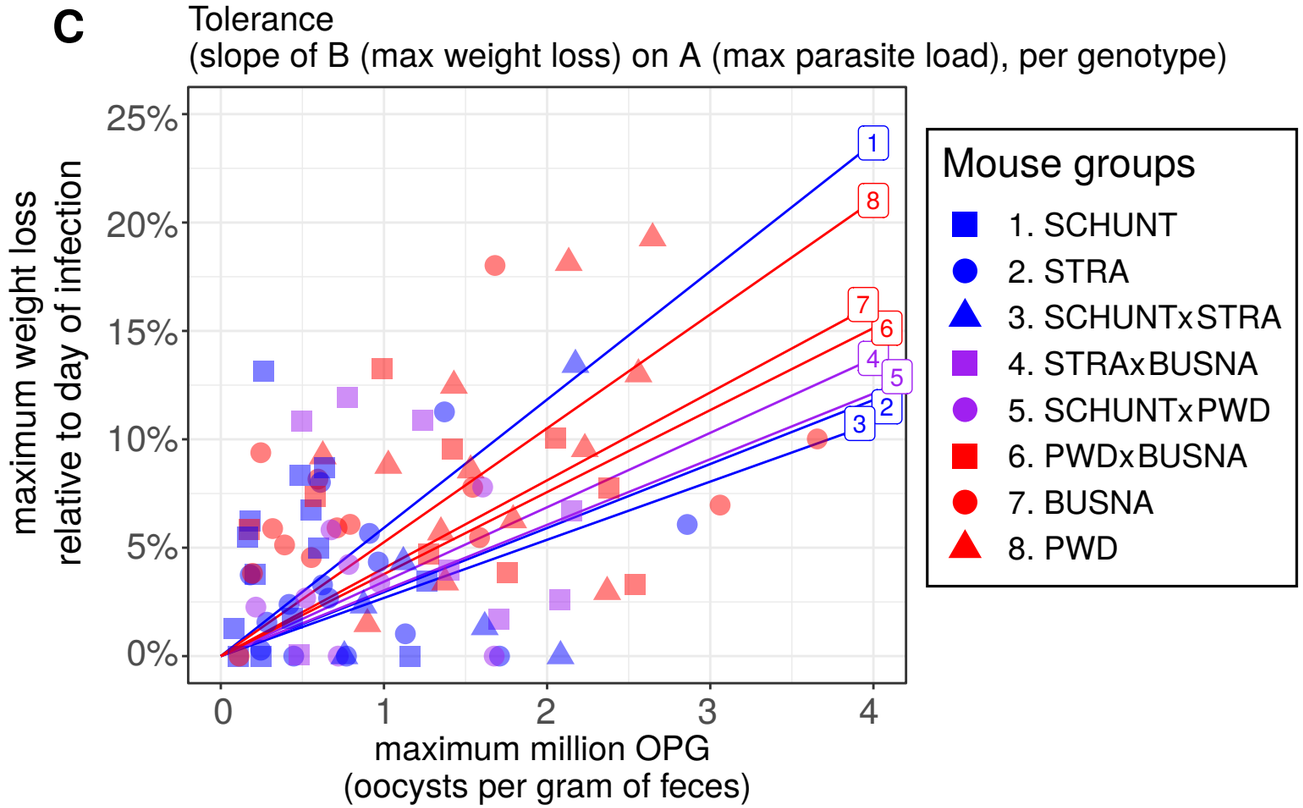

A
B
Eimeria isolate
Brandenburg139 (E. ferrisi)
Brandenburg64 (E. ferrisi)
Brandenburg88 (E. falciformis)
million oocysts per gram of feces
relative weight compared to day 0 (%)
days post infection
days post infection
0
5
10
15
95
100
105
0 1 2 3 4 5 6 7 8 9 10 11

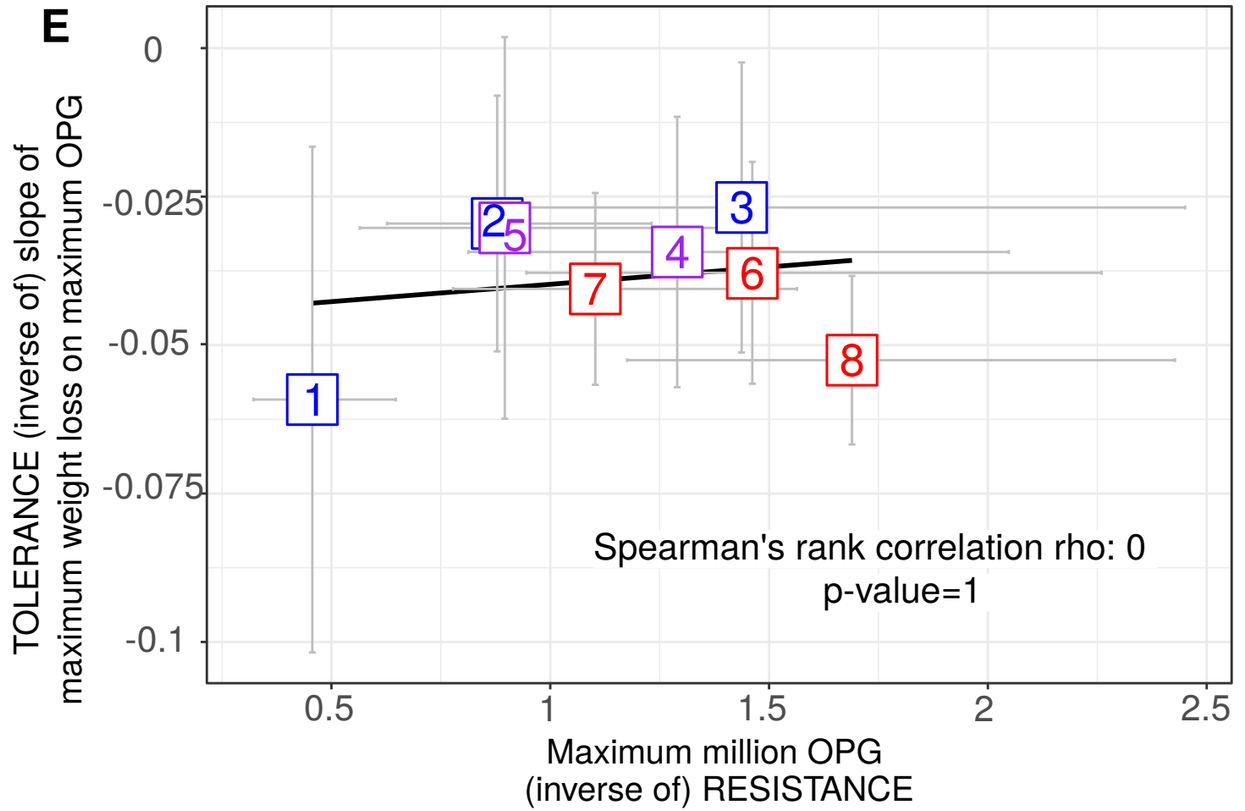

A
Maximum parasite load
=(inverse of) resistance
Brandenburg64
Brandenburg139
(predicted) maximum million OPG
(oocysts per gram of feces)
2.5
2
1.5
1
0.5
B
Maximum weight loss
Brandenburg64
Brandenburg139
(predicted) maximum weight loss
relative to day of infection
12%
9%
6%
3%
C
Tolerance per genotype
(slope of B (max weightloss) on A (max parasite load))
Brandenburg64
Brandenburg139
maximum weight loss
relative to day of infection
25%
20%
15%
10%
5%
0%
1
8
7
2
0
1
2
3
4
maximum million OPG
(oocysts per gram of feces)
Western
Eastern
Host
M. m. domesticus
M. m. musculus
1. SCHUNT
7. BUSNA
2. STRA
8. PWD
Parasite
E. ferrisi isolate
Brandenburg64
E. ferrisi isolate
Brandenburg139

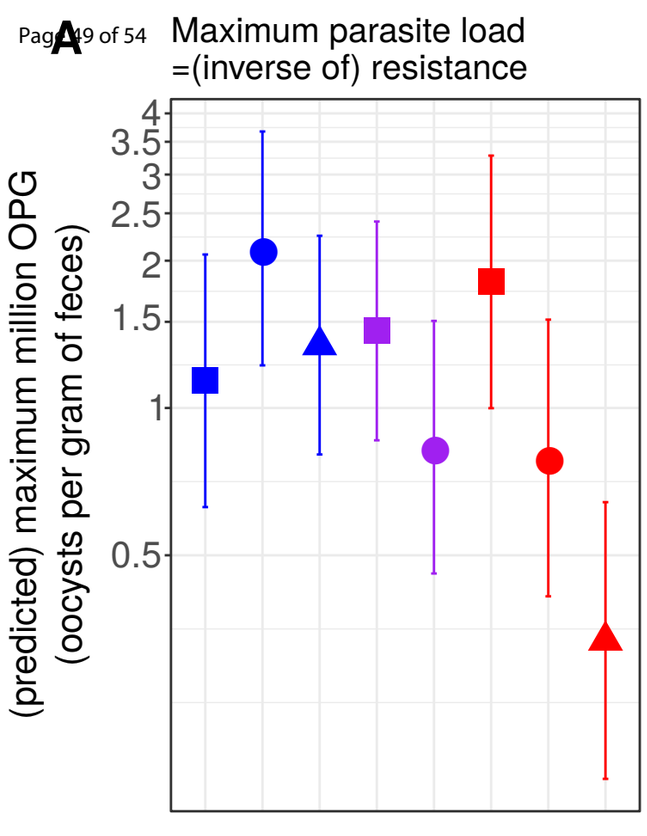

A
Maximum parasite load
=(inverse of) resistance
(predicted) maximum million OPG
(oocysts per gram of feces)
2.5
2
1.5
1
0.5

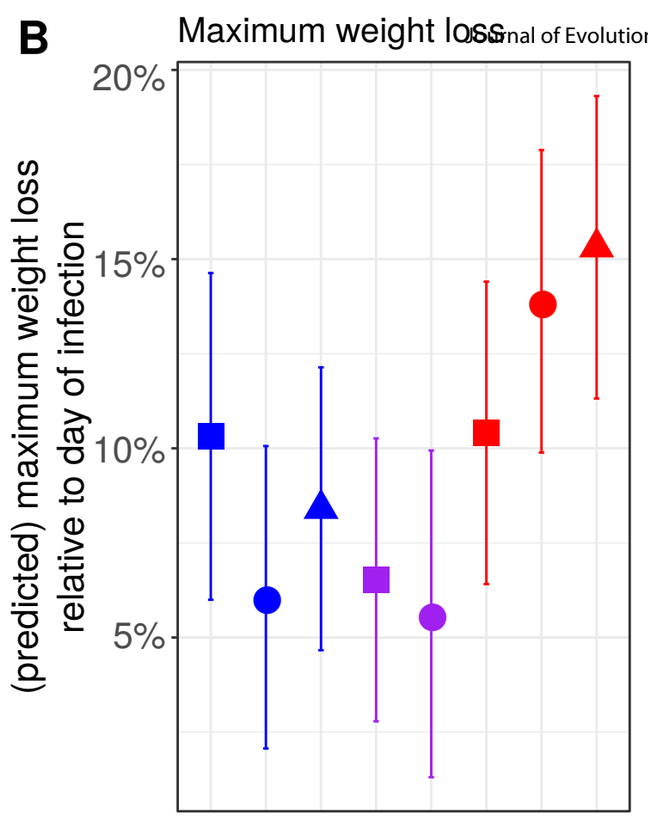

B
Maximum weight loss
(predicted) maximum weight loss
relative to day of infection
9%
6%
3%
0%

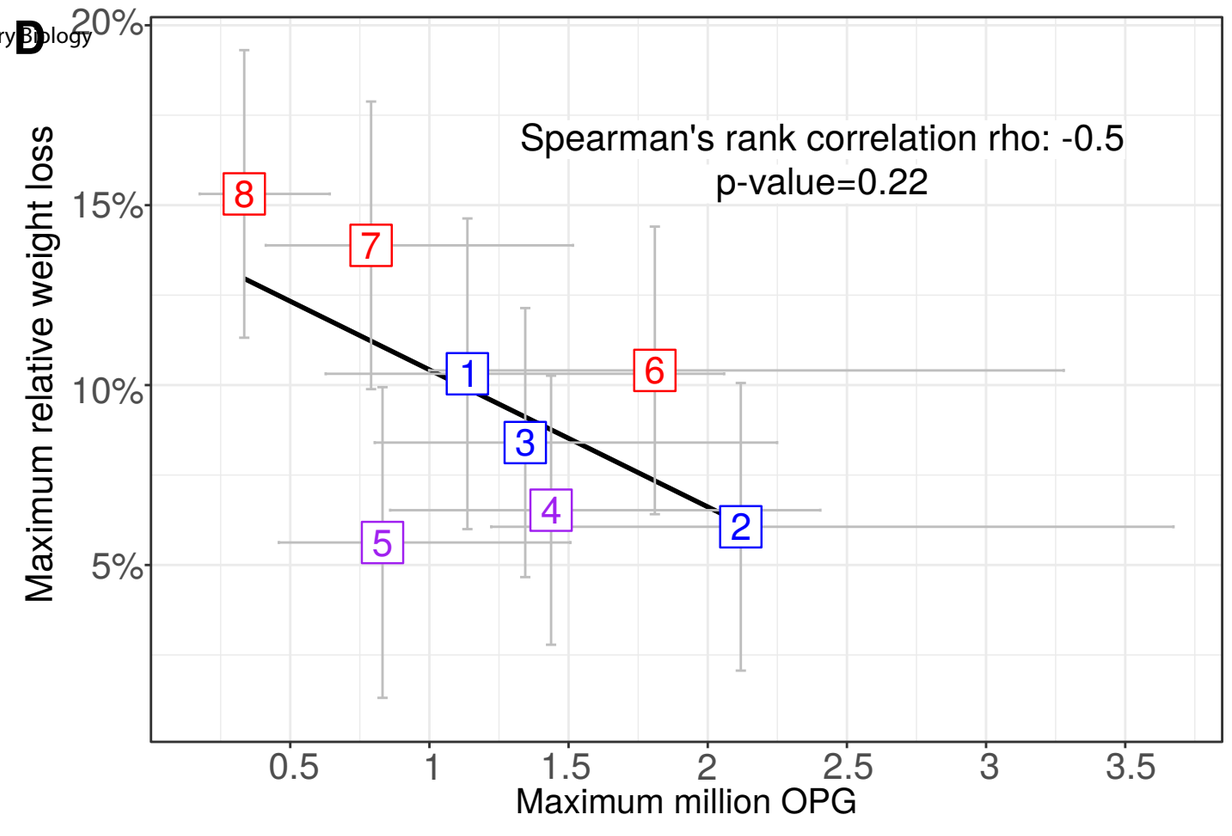

D
Maximum relative weight loss
9%
6%
3%
0%
0.5
1
1.5
2
2.5
Maximum million OPG
1
2
5
3
4
7
6
8
Spearman's rank correlation rho: 0.69
p-value=0.069

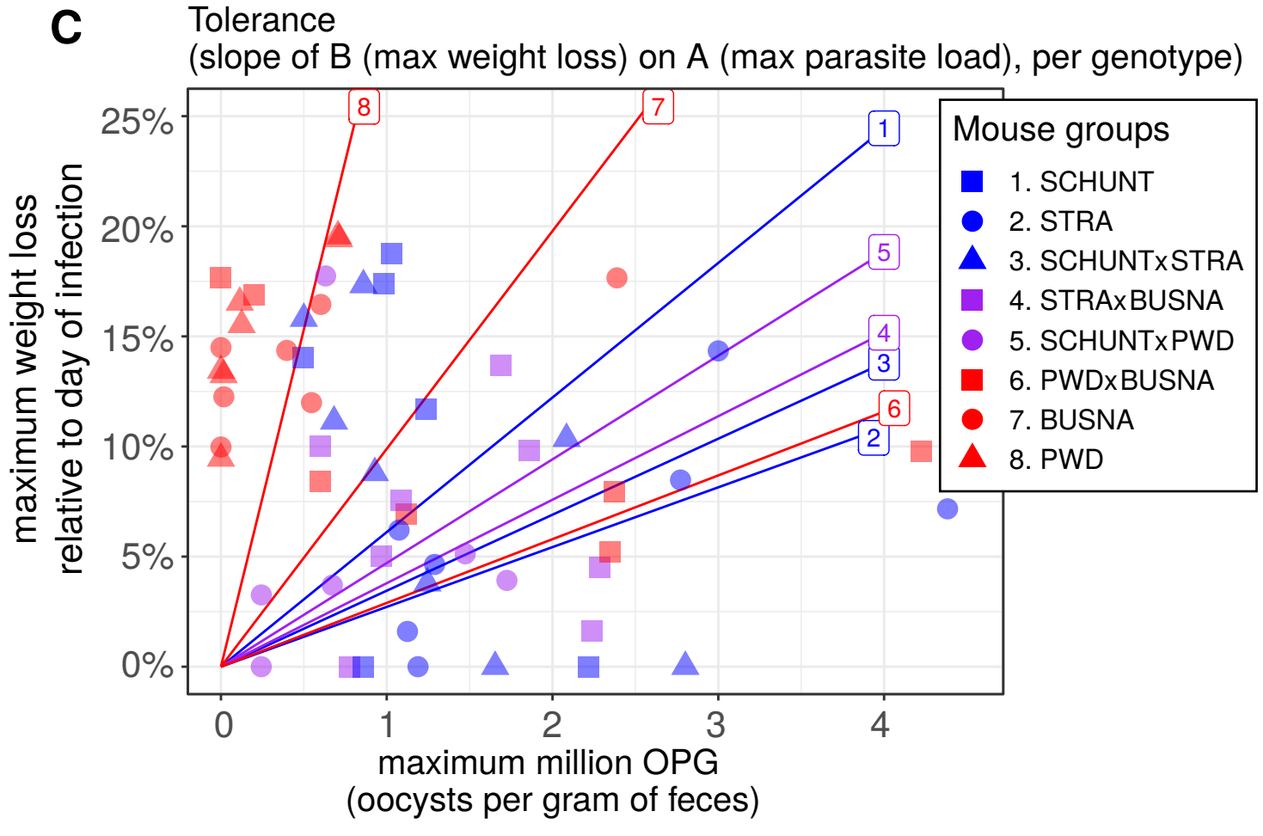

C
Tolerance
(slope of B (max weight loss) on A (max parasite load), per genotype)
maximum weight loss
relative to day of infection
25%
20%
15%
10%
5%
0%
0
1
2
3
4
maximum million OPG
(oocysts per gram of feces)
1
8
7
6
4
5
2
3
Mouse groups
1. SCHUNT
2. STRA
3. SCHUNTxSTRA
4. STRAxBUSNA
5. SCHUNTxPWD
6. PWDxBUSNA
7. BUSNA
8. PWD

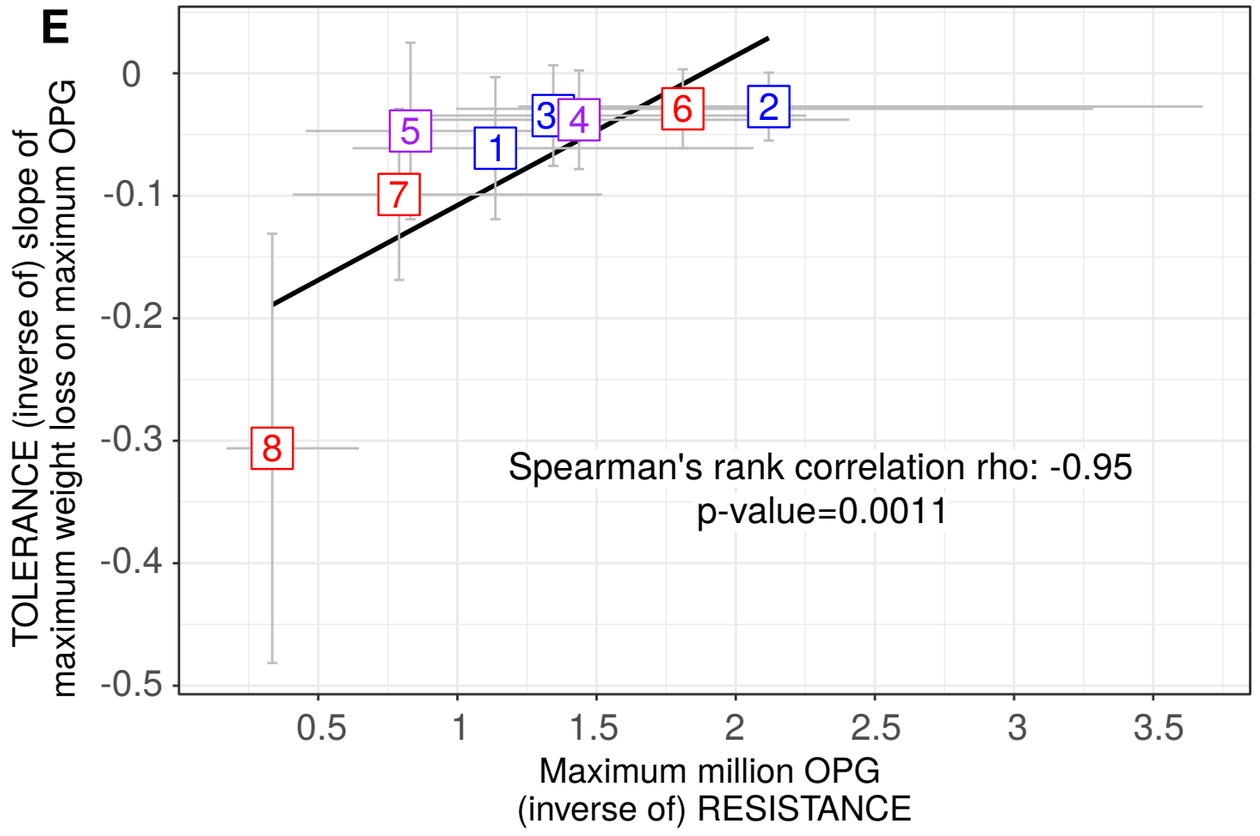

E
TOLERANCE (inverse of) slope of
maximum weight loss on maximum OPG
0
-0.025
-0.05
-0.075
-0.1
0.5
1
1.5
2
2.5
Maximum million OPG
(inverse of) RESISTANCE
2
5
3
4
7
6
1
8
Spearman's rank correlation rho: 0
p-value=1


A
Maximum parasite load
=(inverse of) resistance
(predicted) maximum million OPG
(oocysts per gram of feces)
4
3.5
3
2.5
2
1.5
1
0.5


B
Maximum weight loss
(predicted) maximum weight loss
relative to day of infection
20%
15%
10%
5%


D
Maximum relative weight loss
20%
15%
10%
5%
Spearman's rank correlation rho: -0.5
p-value=0.22
8
7
1
6
3
4
5
2
0.5
1
1.5
2
2.5
3
3.5
Maximum million OPG


C
Tolerance
(slope of B (max weight loss) on A (max parasite load), per genotype)
maximum weight loss
relative to day of infection
25%
20%
15%
10%
5%
0%
8
7
1
5
4
3
6
2
Mouse groups
1. SCHUNT
2. STRA
3. SCHUNTxSTRA
4. STRAxBUSNA
5. SCHUNTxPWD
6. PWDxBUSNA
7. BUSNA
8. PWD
0
1
2
3
4
maximum million OPG
(oocysts per gram of feces)


E
TOLERANCE (inverse of) slope of
maximum weight loss on maximum OPG
0
-0.1
-0.2
-0.3
-0.4
-0.5
5
1
3
4
6
2
7
8
Spearman's rank correlation rho: -0.95
p-value=0.0011
0.5
1
1.5
2
2.5
3
3.5
Maximum million OPG
(inverse of) RESISTANCE

Batch	Mouse strain (species)								*Eimeria* isolate (species)
	SCHUNT (Mmd)	STRA (Mmd)	SCHUNTx STRA (Mmd)	STRAx BUSNA (Mmd-Mmm)	SCHUNTx PWD (Mmd-Mmm)	PWDx BUSNA (Mmd)	BUSNA (Mmm)	PWD (Mmm)	
B1	2	3					2	3	Brandenburg139 (*E. ferrisi*)
	3	4					2	3	Brandenburg64 (*E. ferrisi*)
B2	5	3					4	3	Brandenburg139 (*E. ferrisi*)
	4	4					5	3	Brandenburg64 (*E. ferrisi*)
B3	3	3	2	2	3	3	3	3	Brandenburg64 (*E. ferrisi*)
	3	3	3	4	3	3	3	3	Brandenburg88 (*E. falciformis*)
B4	4	4	4	6	5	6	4	4	Brandenburg64 (*E. ferrisi*)
	3	4	5	4	3	4	4	4	Brandenburg88 (*E. falciformis*)

Supplementary material S2. Conservative dataset (N=118)

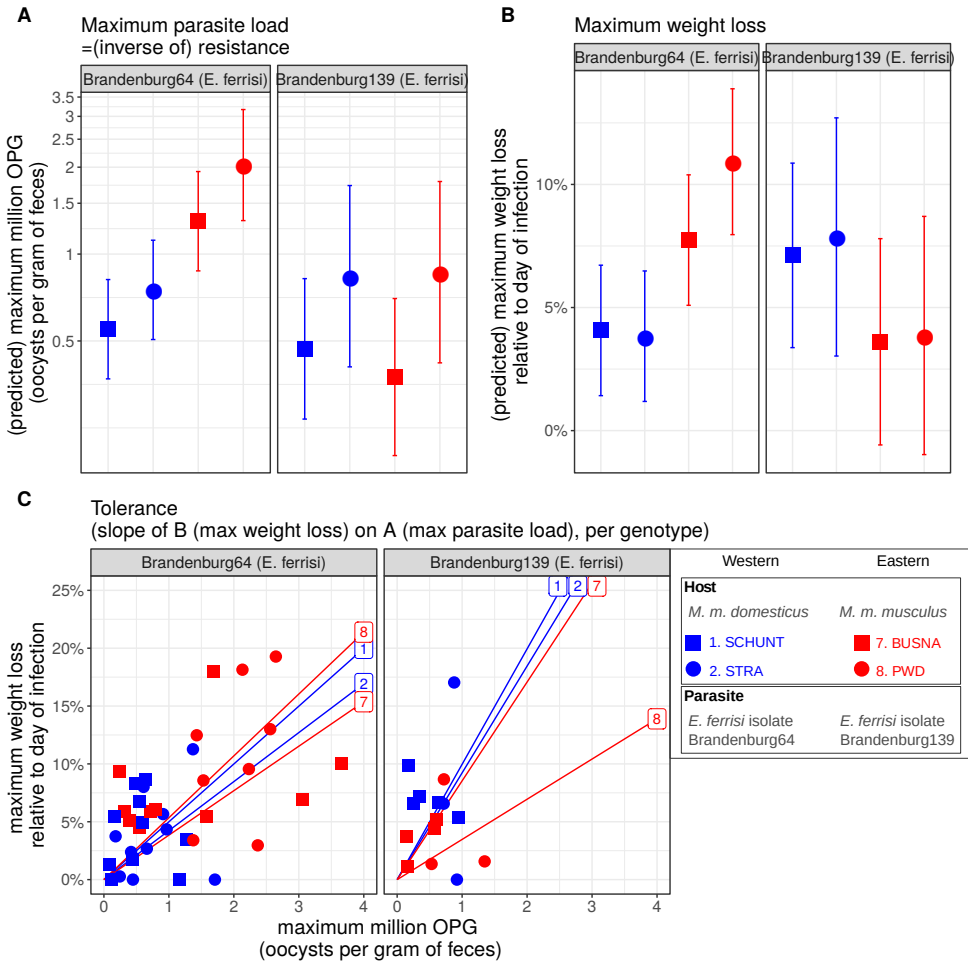


Figure S2.1. Comparison of resistance, impact on weight and tolerance between mouse strains for both *Eimeria ferrisi* isolates. (A) Maximum oocysts per gram of feces used as a proxy for (inverse of) resistance; (B) Impact on host health measured as the maximum weight loss during patent period relative to starting weight (%); (C) Tolerance estimated by the slope of the linear regression with null intercept modelling maximum relative weight loss as a response of maximum oocysts per gram of feces. A steep slope corresponds to a low tolerance. We did not detect (A) either higher parasite shedding of the Eastern parasite isolate in Eastern mouse strains and *vice versa* (LRT interaction factor mouse strain-parasite isolate: G=6.9, df=3, P=0.74) or (C) higher tolerance of Eastern hosts infected by Eastern parasite isolate and *vice versa* (LRT interaction factor mouse strain-parasite isolate: G=3.1, df=3, p=0.38), thus our results do not support the hypothesis of local adaptation between *E. ferrisi* and its host.

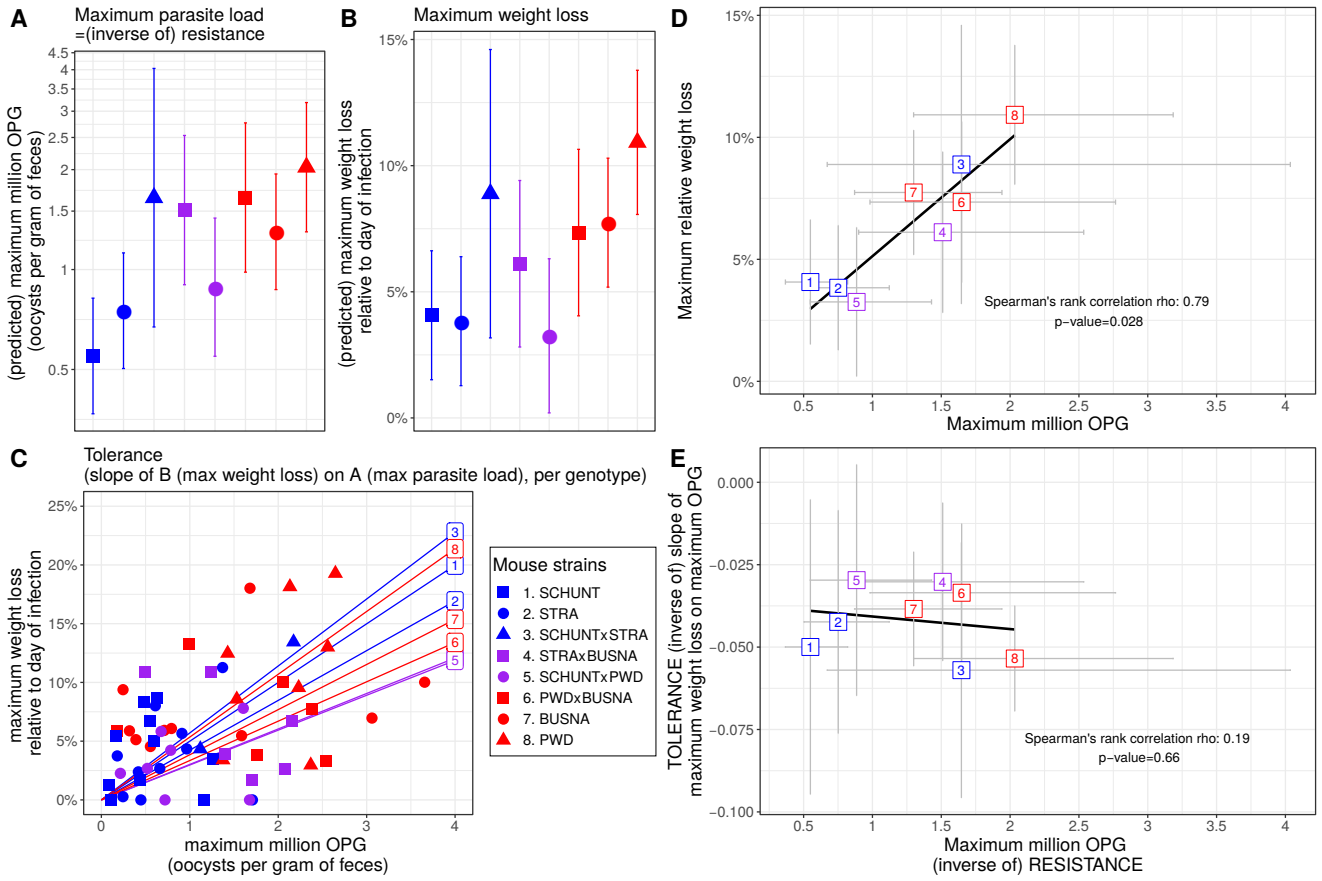


Figure S2.2. No indication of resistance-tolerance coupling for *E. ferrisi* isolate Brandenburg64. Colors represent mouse subspecies (blue: Mmd, red: Mmm, purple: Mmd-Mmm). Left side: comparison of maximum oocysts per gram of feces used as a proxy for (inverse of) resistance (A), impact on weight measured as the maximum weight loss during patent period relative to starting weight (B) and tolerance between mouse groups estimated by the slope of the linear regression with null intercept modelling maximum relative weight loss as a response of maximum oocysts per gram of feces, a steep slope corresponding to a low tolerance (C). Maximum number of OPG and relative weight loss differ between mouse groups (LRT: maximum number of OPG: G=22.6, df=7, p=0.002; maximum relative weight loss: G=21.7, df=7, p=0.0028), but tolerance is similar (LRT: G=5.4, df=7, p=0.62). Right side: non significant positive correlation between mean maximum oocysts per gram of feces and mean relative weight loss (D) and absence of correlation between maximum oocysts per gram of feces used as a proxy for (inverse of) resistance and tolerance (E); Grey error bars represent 95% confidence intervals. Our results do not support coupling between resistance and tolerance *E. ferrisi* isolate Brandenburg64.

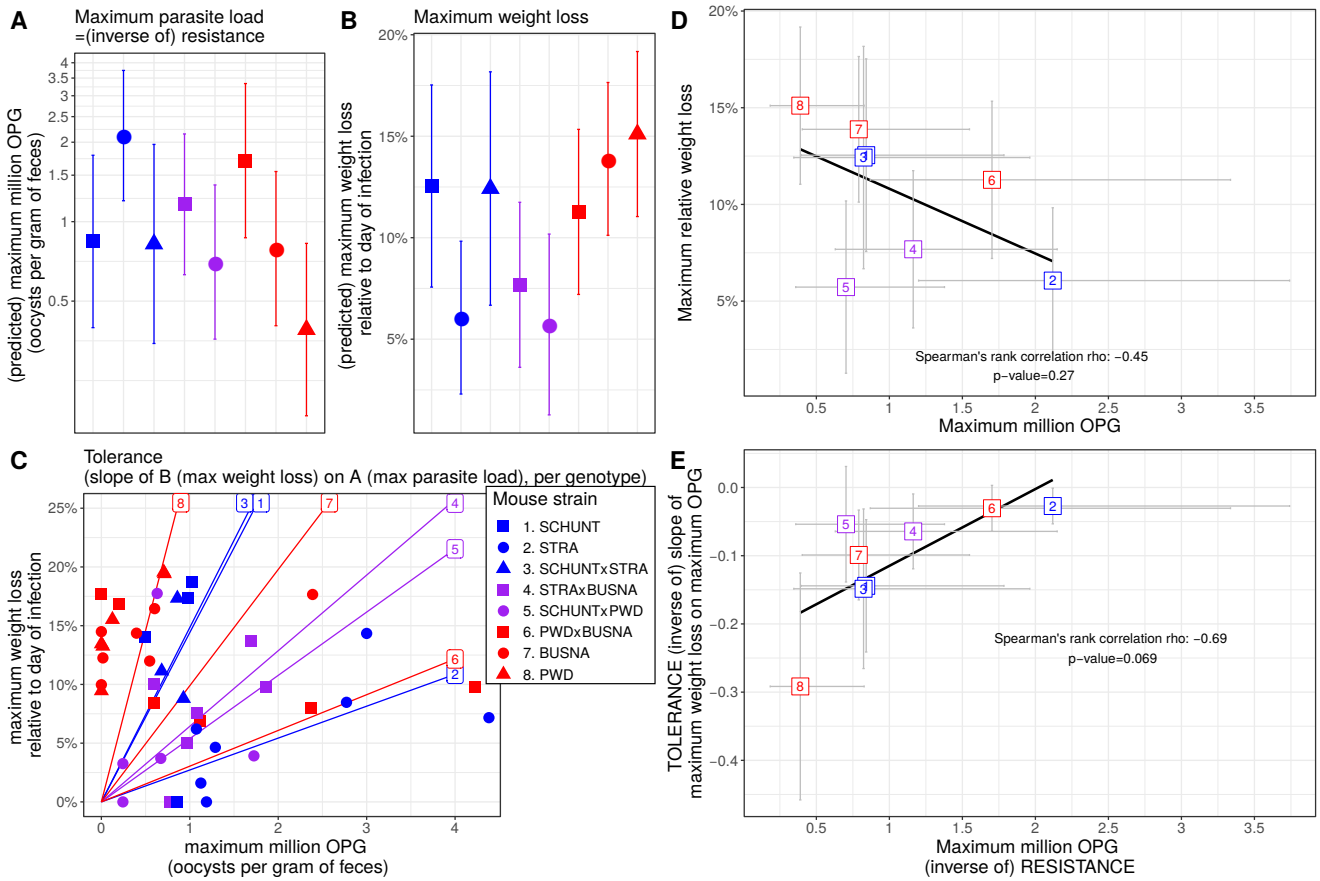
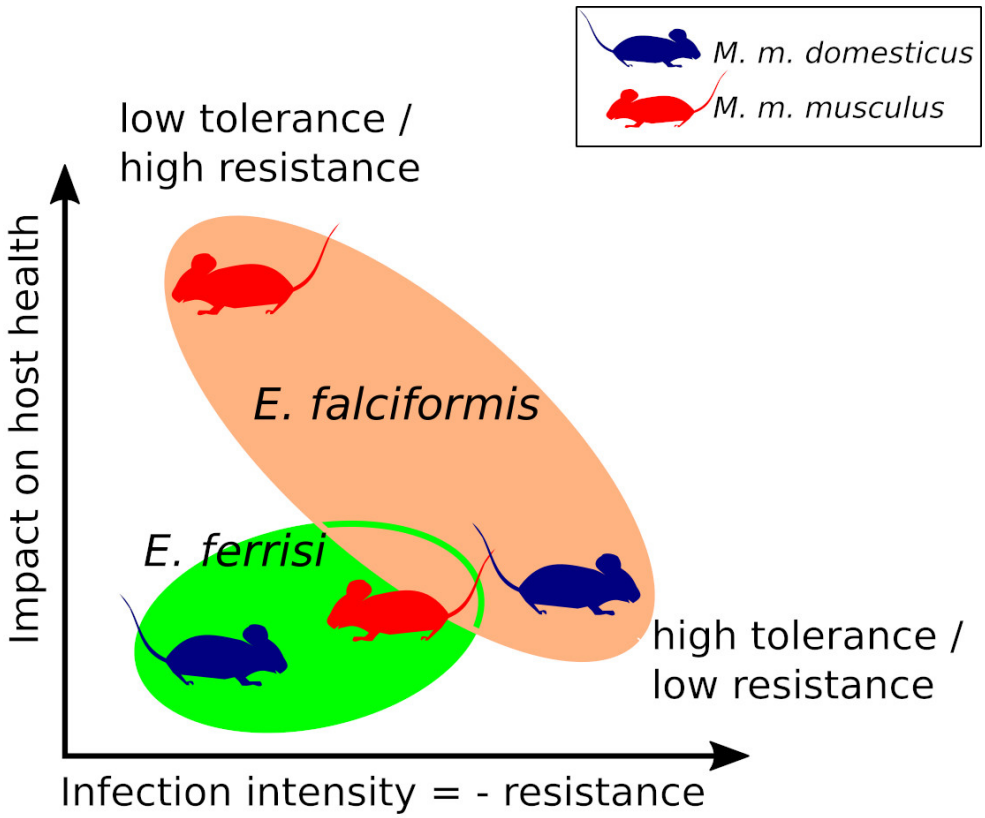


Figure S2.3. Coupling between resistance and tolerance for *E. falciformis* isolate Brandenburg88. Colors represent mouse subspecies (blue: Mmd, red: Mmm, purple: Mmd-Mmm). Left side: comparison of maximum oocysts per gram of feces used as a proxy for (inverse of) resistance (A), impact on weight measured as the maximum weight loss during patent period relative to starting weight (B) and tolerance between mouse groups estimated by the slope of the linear regression with null intercept modelling maximum relative weight loss as a response of maximum oocysts per gram of feces, a steep slope corresponding to a low tolerance (C). Maximum number of OPG, relative weight loss and tolerance differ between mouse groups (LRT: maximum number of OPG: G=24, df=14, p=0.046; maximum relative weight loss: G=20.1, df=7, p=0.005; tolerance: G=20.2, df=7, p=0.0051). Right side: non significant negative correlation between mean maximum oocysts per gram of feces and mean relative weight loss (D) and non significant negative correlation between maximum oocysts per gram of feces used as a proxy for (inverse of) resistance and tolerance (E); Grey error bars represent 95% confidence intervals. Our results present indications of coupling between resistance and tolerance *E. falciformis* isolate Brandenburg88, with lower support than the full dataset likely due to the lower statistical power.



Coupling between resistance and tolerance for two different *Eimeria* species: trade-off between resistance and tolerance between each mouse subspecies upon infection with *E. falciformis*, absent in the case of *E. ferrisi*.