

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

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Cover letter

Dear Editorial team,

We wish to submit an original research article entitled “Coupling between tolerance and resistance differs between related *Eimeria* parasite species: implications for co-evolution with their mouse hosts” for consideration by Ecology and Evolution. 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 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. Thank you for your consideration of this manuscript.

Sincerely,

The authors

1 **Abstract**

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

23 **Keywords:** Resistance, Tolerance, *Eimeria*, Coevolution

24 Introduction

25 Host defence mechanisms evolve to alleviate the detrimental effect of parasites. They
26 can be categorised into two components: resistance and tolerance (Råberg et al.,
27 2009). Resistance is the ability of a host to reduce parasite burden, resulting from
28 defence against parasite infection or proliferation early after infection
29 (Schmid-Hempel, 2013). The negative effect of resistance on parasite fitness can
30 lead to antagonistic coevolution. According to theoretical models, fluctuating host and
31 parasite genotypes arise, and balancing selection maintains resistance alleles
32 polymorphic (Boots et al., 2008; Roy & Kirchner, 2000). Resistance has been the
33 classical "catch all" measure for host-parasite systems, but recently it has been shown
34 to be incomplete, especially with respect to potential fitness effects on the host
35 (Kutzer & Armitage, 2016; Råberg et al., 2009).

36 Disease tolerance (not to be confused from "immunological tolerance",
37 unresponsiveness to self antigens; Medzhitov et al., 2012) is the ability of the host to
38 limit the impact of parasite on its fitness (Kutzer & Armitage, 2016; Råberg et al.,
39 2009; Vale & Little, 2012). By potentially providing a longer-living niche, this defence
40 mechanism improves, or at least does not deteriorate, the fitness of the parasite.
41 Tolerance alleles are thus predicted by theoretical models to evolve to fixation due to
42 positive feedback loops (Boots et al., 2008; Restif & Koella, 2004; Roy & Kirchner,
43 2000). From a mechanistic perspective tolerance alleviates direct or indirect damage
44 (e.g. excessive immune response underlying resistance against parasites, called
45 immunopathology; Graham et al., 2005) caused by parasites (Råberg et al., 2009).
46 Tolerance mechanisms include modulation of inflammatory response (Ayres &

47 Schneider, 2012), tissue repair (stress response, damage repair and cellular
48 regeneration mechanisms; Soares et al., 2017), and compensation of
49 parasite-induced damage by increase of reproductive effort (Baucom & de Roode,
50 2011). The resulting metabolic costs of resistance and tolerance, with and without
51 parasite infection, determine the optimal (steady state and infection inducible) extent
52 and of both immune defences (Sheldon & Verhulst, 1996).

53 Resistance and tolerance can be positively associated if they involve the same
54 metabolic pathway, as was shown in the plant model *Arabidopsis thaliana* in response
55 against herbivory (Mesa et al., 2017). In animals, genetic association studies of
56 resistance and tolerance of *Drosophila melanogaster* against the bacterium
57 *Providencia rettgeri* have shown positively correlated genetic effects, as the same loci
58 were associated with changes of both traits in the same direction (Howick & Lazzaro,
59 2017).

60 Nevertheless, resistance and tolerance can also be genetically and physiologically
61 independent, involving different proximate mechanisms. Lack of correlation between
62 both defences was shown for example in monarch butterflies (*Danaus plexippus*)
63 infected by the protozoan parasite *Ophryocystis elektroscirrha*. This study found
64 genetic variation in resistance between butterflies families, but a fixed tolerance
65 (Lefèvre et al., 2010). Similarly, no correlation could be found between resistance and
66 tolerance for the fish *Leuciscus burdigalensis* in response to infection with its parasite
67 *Tracheliastes polycolpus*. The authors explain the decoupling of both defences by the
68 fact that, in this system, tolerance likely involves wound repair rather than immune
69 regulation, making resistance and tolerance mechanisms independent (Mazé-Guilmo

70 et al., [2014](#)).

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

82 We have seen that depending on the system studied resistance and tolerance can be
83 (1) uncoupled (independent), (2) positively correlated (involving same genes and
84 mechanisms), or (3) negatively correlated (traded-off). Theoretical models show that
85 coupling between resistance and tolerance (or absence thereof) could depend not
86 only on the host but also on the parasite (Carval & Ferriere, [2010](#)). Here we tested
87 this hypothesis. More precisely, we asked whether there could be differences in the
88 resistance-tolerance coupling upon infection of one host type with two closely related
89 parasite species. To answer this question, we infected four inbred mouse strains and
90 four groups of F1 hybrids representative of two house mouse subspecies,
91 *M. m. domesticus* and *M. m. musculus*, with three parasite isolates representative of
92 two naturally occurring parasite species, the protozoan parasite *Eimeria ferrisi* and

93 *E. falciformis* (Jarquín-Díaz et al., 2019). *Eimeria* spp. are monoxenous parasites that
94 expand asexually and reproduce sexually in intestinal epithelial cells, leading to
95 malabsorption of nutrients, tissue damage and weight loss (Chapman et al., 2013).
96 The evolutionary history of these different *Eimeria* species in the two house mouse
97 subspecies is unknown and it is unclear whether subspecies-specific adaptation
98 exists in one or the other.

99 We tested if coupling between resistance and tolerance differs between both parasite
100 species and discussed the implication for parasite-host coevolution. Additionally, as
101 coevolving hosts and parasites can adapt to their antagonist, we tested adaptation to
102 the host subspecies (hereafter "host adaptation") of *E. ferrisi* to *Mus musculus*, using
103 a parasite isolated in a *M. m. domesticus* host and one in a *M. m. musculus* host.
104 Higher parasite fitness of one isolate in one of the two hosts and inversely for the
105 second isolate, or higher host fitness upon infection with one of the two parasite
106 isolates and inversely for the second isolate, would be indirect evidence for
107 coevolution of this parasite with *Mus musculus*.

108 **Material and methods**

109 **1. Parasite isolates**

110 The three parasite isolates used in this study were isolated from feces of three different
111 *M. m. domesticus*/*M. m. musculus* hybrid mice captured in Brandenburg, Germany, in
112 2016 (capture permit No. 2347/35/2014). The parasite isolates belong to both the most
113 prevalent *Eimeria* species in this area, namely *E. ferrisi* (isolates Brandenburg64 and
114 Brandenburg139) and *E. falciformis* (isolate Brandenburg88)(Jarquín-Díaz et al., 2019).

115 Isolate Brandenburg64 was isolated in a 92% *M. m. domesticus* individual (hybrid index
116 (HI) = 0.08: Proportion of *M. m. musculus* alleles in a set of 14 diagnostic markers, see
117 Balard et al. (2020)), isolate Brandenburg139 in a 85% *M. m. musculus* (HI=0.85) and
118 isolate Brandenburg88 in a 80% *M. m. domesticus* (HI=0.2). Pre-patency and the peak
119 day of parasite shedding for these isolates were estimated during infection in NMRI
120 laboratory mice (Al-khlifeh et al., 2019) which were also used for serial passaging of
121 the isolates. Parasite infective forms (oocysts) were recovered by flotation in saturated
122 NaCl solution followed by washing and observation under light microscope (following
123 the protocol described in Clerc et al. (2019)) and stored at room temperature in 1mL
124 of 2% potassium dichromate for a maximum of 2 months before infection of the wild-
125 derived mice. Oocysts were allowed to sporulate 10 days before infection in a water
126 bath at 30°C.

127 2. Mouse groups

128 We used four wild-derived inbred mouse strains from which we generated four groups
129 of F1 hybrids. Two parental strains represented *M. m. domesticus*: **SCHUNT**
130 (Locality: Schweben, Hessen, Germany [N: 50° 26', E: 9° 36'] (Martincová et al.,
131 2019)) and **STRA** (Locality: Straas, Bavaria, Germany [N: 50° 11', E: 11° 46'] (Piálek
132 et al., 2008), and two derived from *M. m. musculus*: **BUSNA** (Locality: Buškovice,
133 Bohemia, Czech Republic [N: 50° 14', E: 13° 22'] (Piálek et al., 2008)) and **PWD**
134 (Locality: Kunratice, Bohemia, Czech Republic [N: 50° 01', E: 14° 29'] (Gregorová &
135 Forejt, 2000)). The four groups of F1 hybrids consisted of two intrasubspecific hybrids
136 (**SCHUNTxSTRA** and **PWDxBUSNA**) and two intersubspecific hybrids
137 (**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*-naïve, 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 100 μ l phosphate-buffer saline (PBS) and monitored daily until their sacrifice by cervical dislocation at time of regression of infection (reduction of oocyst output). Individuals presenting severe health deficiency and/or a weight loss approaching 18% relative to their starting weight were sacrificed earlier at defined humane end points (experiment license Reg. 0431/17). Weight was recorded and feces collected on a daily basis. Fecal pellets were collected every day from each individual cage and suspended in 2% potassium dichromate. Parasite oocysts were recovered using NaCl flotation (see above).

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 S1**).

Nematode infection is common in breeding facilities (Baker, 1998) and could interact with *Eimeria* (Clerc et al., 2019). We surveyed for their presence and 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 anthelmintics (Profender®, Bayer AG, Levekusen, Germany) following the protocole of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the protocole of Floyd et al. (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 anthelmintic 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

184 analyses were consistent with those obtained on the main data set.

185 **4. Statistical analyses**

186 **4.1. Choice of proxies for resistance, impact of parasite on host and tolerance**

187 As resistance is the capacity of a host to reduce its parasite burden, it is usually
188 estimated by the inverse of infection intensity (Råberg et al., 2009). Pre-patency (the
189 time to shedding of infectious stages, so called oocysts) is longer for *E. falciformis* (7
190 days) than for *E. ferrisi* (5 days) (Al-khlifeh et al., 2019). Therefore, as a proxy of
191 (inverse of) resistance we used the number of oocysts per gram of feces (OPG) at the
192 day of maximal shedding. Using the Spearman's non-parametric rank correlation test,
193 we found this measurement to be tightly correlated with the sum of oocysts shed
194 throughout the experiment (Spearman's $\rho=0.93$, $N=168$, $P<0.001$). Due to the
195 aggregation characteristic of parasites (Shaw & Dobson, 1995), the appropriate
196 distribution for maximum number of OPG was found to be the negative binomial
197 distribution. This was confirmed based on log likelihood, AIC criteria and
198 goodness-of-fits plots (density, CDF, Q-Q, P-P plots; R packages MASS (Venables &
199 Ripley, 2002) and fitdistrplus (Delignette-Muller & Dutang, 2015)).

200 Both parasite species provoke inflammation, cellular infiltration, enteric lesions,
201 diarrhea, and ultimately weight loss (Al-khlifeh et al., 2019; Ankrom et al., 1975; Ehret
202 et al., 2017; Schito et al., 1996). Therefore, the impact of parasites on host health was
203 measured as the maximum relative weight loss compared to day 0 (body weight
204 measured at the start of the experimental infection). For mice sacrificed at humane
205 end points before the end of the experiment, last weight of the living animal was used.

206 This weight (loss) can be expected to be a very conservative estimate for our
207 analyses (rendering tolerance conservatively low for these animals, which might have
208 lost more weight if not sacrificed).

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

215 **4.2. Statistical modelling**

216 Maximum OPG and relative weight loss were modelled separately as a response of
217 either mouse group, parasite isolate and their interaction. We used a negative binomial
218 generalised linear model for maximum OPG, and a linear model for relative weight loss.
219 For tolerance, we performed a linear regression with null intercept (as each mouse was
220 controlled against itself at start of the experiment, before losing weight or shedding
221 parasite), modelling relative weight loss as a response of maximum OPG interacting
222 either mouse group, parasite isolate and their interaction. To test the significance of
223 the marginal contribution of each parameter to the full model, each parameter was
224 removed from the full model, and the difference between full and reduced model was
225 assessed using likelihood ratio tests (G).

226 For each of our model, we also asked within each parasite isolate if the response
227 differed between mouse groups using likelihood ratio tests (G) as described above. Of

228 note, four mice infected by *E. falciformis* isolate Brandenburg88 did not shed any
229 oocysts as death occurred at or one day before the peak of oocysts shedding in other
230 mice. For this reason, we modelled maximum OPG for mice infected with this parasite
231 using a zero-inflated negative binomial (ZINB) generalised linear model, after verifying
232 that it provided a better fit than the simple negative binomial based on log likelihood
233 and AIC criteria.

234 **4.3. Test of host adaptation**

235 Host adaptation of *E. ferrisi* was tested using two isolates (the "Western"
236 Brandenburg64 and "Eastern" Brandenburg139) and our four parental mouse strains
237 (the two *M. m. domesticus* Western SCHUNT and STRA, and the two *M. m. musculus*
238 Eastern BUSNA and PWD). We hypothesised a possible host adaptation of *E. ferrisi*.
239 The prediction drawn from this would be that the Eastern parasite (*E. ferrisi* isolate
240 Brandenburg139) reproduces better in the matching Eastern mouse subspecies
241 (*M. m. musculus*) than in the Western one (*M. m. domesticus*), and similarly the
242 Western parasite (*E. ferrisi* isolate Brandenburg64) reproduce better in
243 *M. m. domesticus* than in *M. m. musculus*. Additionally, a higher tolerance of each
244 host infected by its matching parasite despite similar parasite reproductive output
245 could indicate increased host fitness, and host adaptation.

246 **4.4. Test of coupling between resistance and tolerance**

247 We tested coupling between resistance and tolerance for *E. ferrisi* and *E. falciformis*
248 using the isolates Brandenburg64 and Brandenburg88 and our eight mouse groups.

249 To test such coupling, one can assess the strength of correlation between measure of
250 resistance and measure of tolerance (Råberg et al., 2007). Of note, tolerance (in
251 absolute value) is measured as the slope α of the linear regression of parasite load (x)
252 on maximum relative weight loss (y) of equation $y = \alpha x + \beta$ (α being the slope and β
253 the intercept, 0 in our case). Therefore, tolerance is expressed as $\alpha = y/x - \beta/x$. As x
254 and y/x are by definition not independent, testing the correlation between resistance
255 and tolerance can lead to spurious correlation (Brett, 2004). To alleviate the dangers
256 of this statistical artifact, we additionally tested differences in resistance, impact on
257 health and tolerance between mouse groups separately and also the underlying
258 correlation between mean parasite load (x) and mean relative weight loss (y). We use
259 the terminology "coupling" (between resistance and tolerance) to describe
260 genotype-level correlation between tolerance and resistance additionally supported by
261 the absence of positive correlation between health-effect and resistance. Correlations
262 were tested using Spearman's rank correlation.

263 All analyses were performed using R version 3.5.2 (R Development Core Team,
264 2013)(negative binomial: function glm.nb from R package MASS (Venables & Ripley,
265 2002); ZIBN: function zeroinfl from R package pscl (Jackman, 2020; Zeileis et al.,
266 2008); linear model: function lm from R core package stats; mean and 95%
267 confidence intervals: function ggpredict from R package ggeffect (Lüdtke, 2018)).
268 Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled
269 using the free software inkscape (<https://inkscape.org>).

270 Results

271 1. General

272 Parasites of all isolates successfully infected all mouse groups (at the exception of 5
273 individuals infected by *E. falciformis* isolate Brandenburg88 that died or had to be
274 sacrificed due to a strong weight loss before the peak of shedding for this parasite),
275 meaning that no "qualitative infection resistance" (*sensu* Gandon and Michalakis
276 (2000)) was detected. For *E. ferrisi* (both isolates Brandenburg139 and
277 Brandenburg64), the pre-patent period was 5 days post infection (dpi) and the median
278 day of maximal oocyst shedding was 6 dpi (standard deviation sd=0.7 and 0.9,
279 respectively). The median day of maximum weight loss was 5 dpi for both isolates
280 (sd=2.1 and 1.7 respectively). For *E. falciformis* (isolate Brandenburg88) pre-patency
281 was 7 dpi, median day of maximal shedding was 8 dpi (sd=1.3) and median day of
282 maximal weight loss 9 dpi (sd=1.6)(**Figure 2**). Of note a considerable number of mice
283 infected with this isolate (13 out of 56 = 23%) died or had to be sacrificed at humane
284 end points less than 3 days after the oocysts shedding peak for the group, all
285 belonging to *M. m. musculus* subspecies (PWD, BUSNA, or their F1 PWDxBUSNA; 5
286 died at dpi 8, 5 at dpi 9, 3 at dpi 10). *E. falciformis* isolate Brandenburg88 was more
287 lethal for the *M. m. musculus* mice strains than for the other strains ($\chi^2_7 = 31.96$,
288 $P < 0.001$; **Table 2**).

289 2. No indication of host adaptation of *E. ferrisi*

290 We tested if our proxies for resistance, impact on weight and tolerance were different
291 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 host 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

315 parasite isolate (LRT: $G=6.8$, $df=7$, $P=0.45$; **Figure 4C**).

316 We found a non significant positive correlation between resistance (inverse of maximum
317 number of OPG) and impact on health (maximum weight loss) (Spearman's $\rho=0.69$,
318 $P=0.07$, $N=8$; **Figure 4D**). Eventually, we did not find a correlation between resistance
319 (inverse of maximum number of OPG) and tolerance (inverse of slope of maximum
320 weight loss on maximum OPG) (Spearman's $\rho=0$, $P=1$, $N=8$; **Figure 4E**).

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

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

325 We then tested coupling between resistance and tolerance for *E. falciformis* isolate
326 Brandenburg88 in our eight mouse groups. First, we tested if our proxies for
327 resistance, impact on weight and tolerance were different between the mouse groups.
328 We found the maximum number of OPG and relative weight loss to be statistically
329 different between mouse groups (LRT: maximum number of OPG: $G=28.6$, $df=14$,
330 $P=0.012$; **Figure 5A**; maximum relative weight loss: $G=21$, $df=7$, $P<0.01$; **Figure 5B**).
331 Finally, contrary to our results on *E. ferrisi* isolate Brandenburg64, the tolerance
332 slopes for *E. falciformis* isolate Brandenburg88 were different between mouse groups
333 (LRT: $G=13.9$, $df=7$, $P=0.05$; **Figure 5C**).

334 We detected a strong negative correlation between (inverse of) resistance (maximum
335 number of OPG) and tolerance (inverse of slope of maximum weight loss on
336 maximum OPG) (Spearman's $\rho=-0.95$, $P=0.001$; **Figure 5E**). We conclude that this

337 correlation is unlikely a statistical artifact, as (1) mouse groups present statistically
338 different values of resistance and tolerance and (2) we found a (non significant)
339 negative correlation between resistance (inverse of maximum number of OPG) and
340 impact on health (maximum weight loss) (Spearman's $\rho=-0.5$, $P=0.22$; **Figure 5D**),
341 indicating that mouse groups losing more weight also shed less parasites.

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

344 **Discussion**

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

349 From a practical "measurement" perspective we can ask whether tolerance can be
350 predicted from resistance, as the latter is easier to measure (e.g. in field sampling).
351 Many studies assess the impact of parasites on host fitness based on resistance. If,
352 as we found in the present study, resistance and tolerance are decoupled this can be
353 misleading. In our host system, the house mice, for example, it has been shown that
354 hybrids between *M. m. domesticus* and *M. m. musculus* are more resistant to parasites
355 (Baird et al., 2012; Balard et al., 2020), including *Eimeria*, but tolerance could not be
356 measured under natural conditions (Balard et al., 2020). The effect of parasites on host
357 fitness in the evolution of the house mouse hybrid zone is thus still rather ambiguous
358 (Baird & Goüy de Bellocq, 2019). We show that careful distinction between parasite

359 species is necessary when analysing parasite host interaction (see also Jarquín-Díaz
360 et al., 2019) and that it is indispensable to measure both resistance and tolerance in
361 *Eimeria* infections of house mice.

362 In this work we used the concept of tolerance as used originally in the plant literature
363 (Fineblum & Rausher, 1995) and later on transferred to animal studies
364 (Råberg et al., 2007). This concept of tolerance can be criticised, as it links
365 mathematically tolerance to resistance. Nevertheless, we argue that this view is
366 biologically meaningful considering resistance and tolerance as a step-wise defence
367 system, one step limiting the parasite multiplication, the other limiting the impact of
368 this multiplication on fitness-related traits. To limit the possible statistical artifact, our
369 approach did not only consist in calculating blindly correlations between resistance and
370 tolerance, but we also tested differences in resistance, impact on health and
371 tolerance. We additionally excluded the possibility of positive correlation between
372 mean health-effect and mean resistance of each host strains, which could indicate
373 some host strains having few parasites-few effects on health, and others more
374 parasites-more effects on health: this configuration would limit the possibility of
375 detecting an actual resistance-tolerance trade-off.

376 More generally, in an evolutionary perspective, coupling between resistance and
377 tolerance might help determine if coevolution between host and parasite can be
378 expected: a host-parasite system in which one finds negative coupling between
379 tolerance and resistance would be an especially promising system for studies of
380 host-parasite co-evolution. Indeed, coevolution in host-parasite systems is often
381 assumed but rarely proven (Woolhouse et al., 2002). Janzen (1980) notes that not all

382 parasite-host systems are coevolving. The presence of efficient host defences against
383 a given parasite is not necessarily produced in response to this parasite specifically
384 and the parasite does not necessarily respond specifically. In the mouse-*E. ferrisi*
385 system, where resistance and tolerance are decoupled, host and parasite fitness
386 might be decoupled as a result, making host-parasite coevolution less likely. In the
387 mouse-*E. falciformis* system we found a negative coupling between tolerance and
388 resistance, making coevolution between host and parasite more likely.

389 Differences between parasite species could explain the evolution of different
390 strategies: *E. ferrisi* commits to sexual reproduction after a relatively short time with
391 few cycles of asexual expansion (Al-khlifeh et al., 2019; Ankrom et al., 1975), while
392 *E. falciformis* has a relatively longer life cycle (Al-khlifeh et al., 2019; Haberkorn,
393 1970). As *E. ferrisi* infections do not reach extremely high intensities, high tolerance
394 might be the optimal strategy for both house mouse subspecies. Resistance could
395 then evolve relatively freely without any major impact of the parasite on the hosts'
396 health. Moreover, our results did not support host adaptation of *E. ferrisi*, which might
397 be explained by the absence of host-parasite coevolution caused by uncoupling of
398 parasite and host fitness. In the case of *E. falciformis*, the long life cycle might lead to
399 high tissue load. Tissue damage is observed during sexual reproduction for this
400 parasite (Ehret et al., 2017) and might mean that a certain level of resistance is
401 required. On the other hand, immunopathology has been observed in advanced
402 *E. falciformis* infections (Stange et al., 2012). These intrinsic characteristics of
403 *E. falciformis* might lead to multiple different optima for resistance and tolerance,
404 leading to a trade-off.

405 In conclusion, we argue that the difference between resistance and tolerance coupling
 406 in two different parasites can guide research in the house mouse system: if the effects
 407 of host hybridisation should be studied independently of potential host-parasite
 408 coadaptation, a parasite species leading to uncoupling between resistance and
 409 tolerance of the host (e.g. *E. ferrisi*) might be the most suitable parasite. If coevolution
 410 between hosts and parasites should be studied, a parasite species for which
 411 resistance and tolerance of the host are negatively correlated (e.g. *E. falciformis*)
 412 would be a more plausible target. Generally, we showed that the coupling between
 413 resistance and tolerance can differ between closely related parasite species and we
 414 argue that this trait of a host-parasite system determines the questions to be best
 415 approached with a particular parasite.

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560 Tables

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

Table 1. Infection experiment design.

Mouse		status at dpi 11	
subspecies	group	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.

561 Figures legends

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

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

573 together.

574 **Figure 3. Comparison of resistance, impact on weight and tolerance between**
575 **mouse strains for both *Eimeria ferrisi* isolates.** (A) Maximum oocysts per gram of
576 feces used as a proxy for (inverse of) resistance; (B) Impact on host health measured
577 as the maximum weight loss during patent period relative to starting weight (%); (C)
578 Tolerance estimated by the slope of the linear regression with null intercept modelling
579 maximum relative weight loss as a response of maximum oocysts per gram of feces. A
580 steep slope corresponds to a low tolerance. We did not detect (A) either higher parasite
581 shedding of the Eastern parasite isolate in Eastern mouse strains and vice versa or (C)
582 higher tolerance of Eastern hosts infected by Eastern parasite isolate and vice versa,
583 thus our results do not support the hypothesis of host adaptation between *E. ferrisi* and
584 its host.

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

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

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

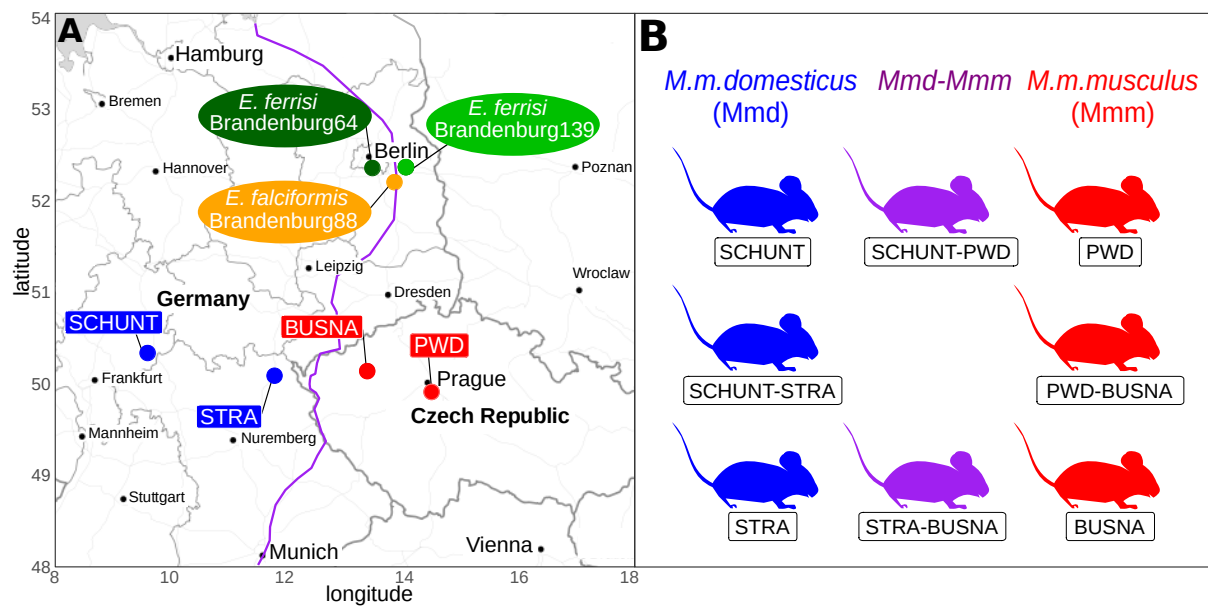


Figure 1: Parasite isolates and mouse wild-derived strains.

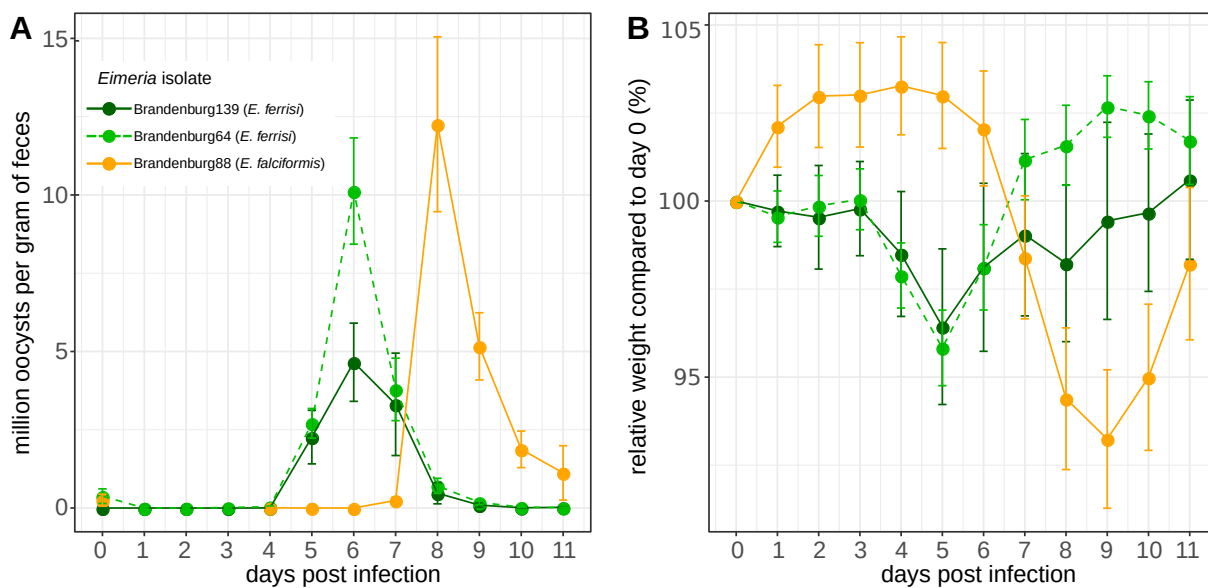


Figure 2: Parasite density (A) and relative weight (B) during *Eimeria* infection.

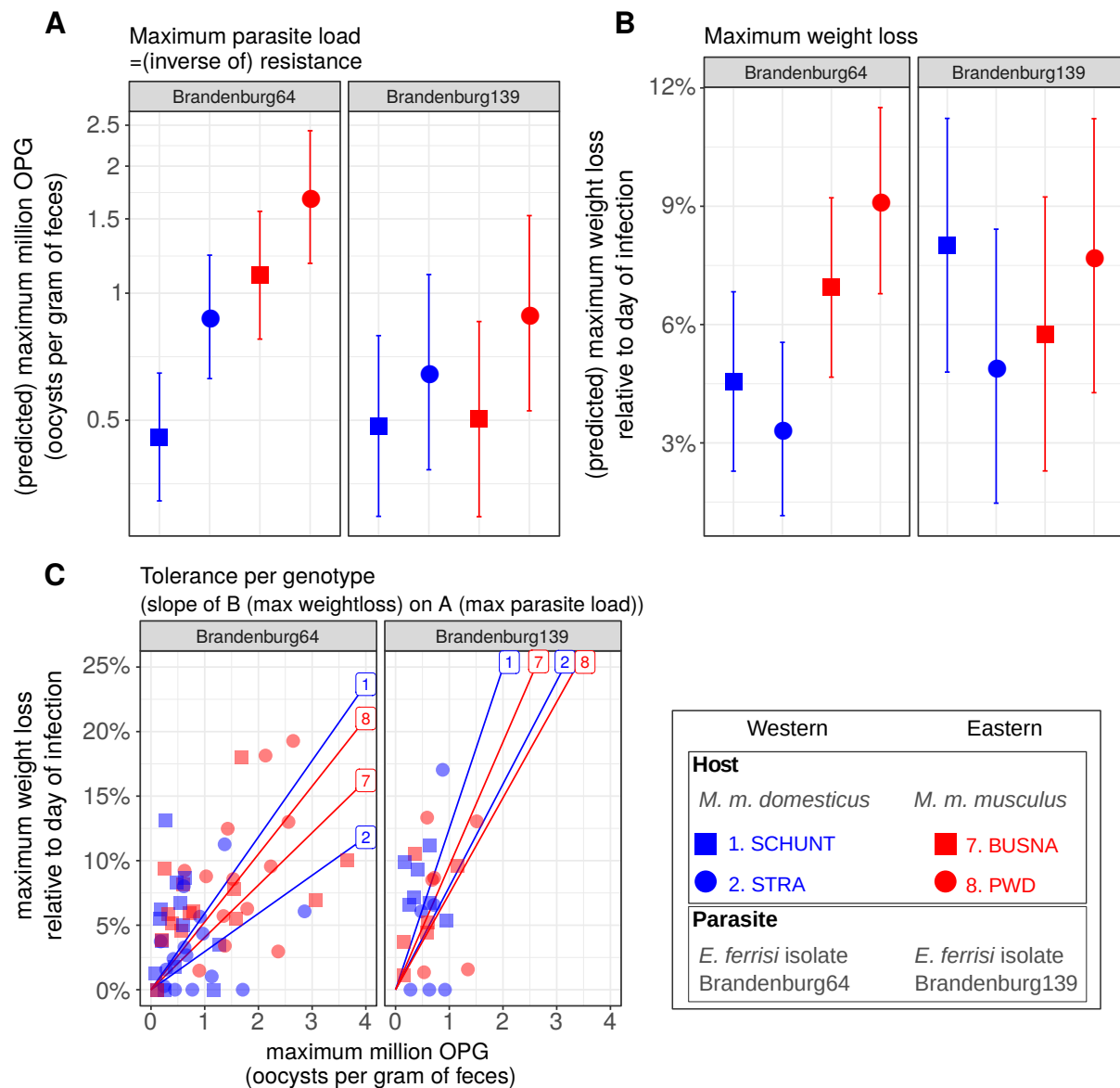


Figure 3: Comparison of resistance, impact on weight and tolerance between mouse strains for both *Eimeria ferrisi* isolates.

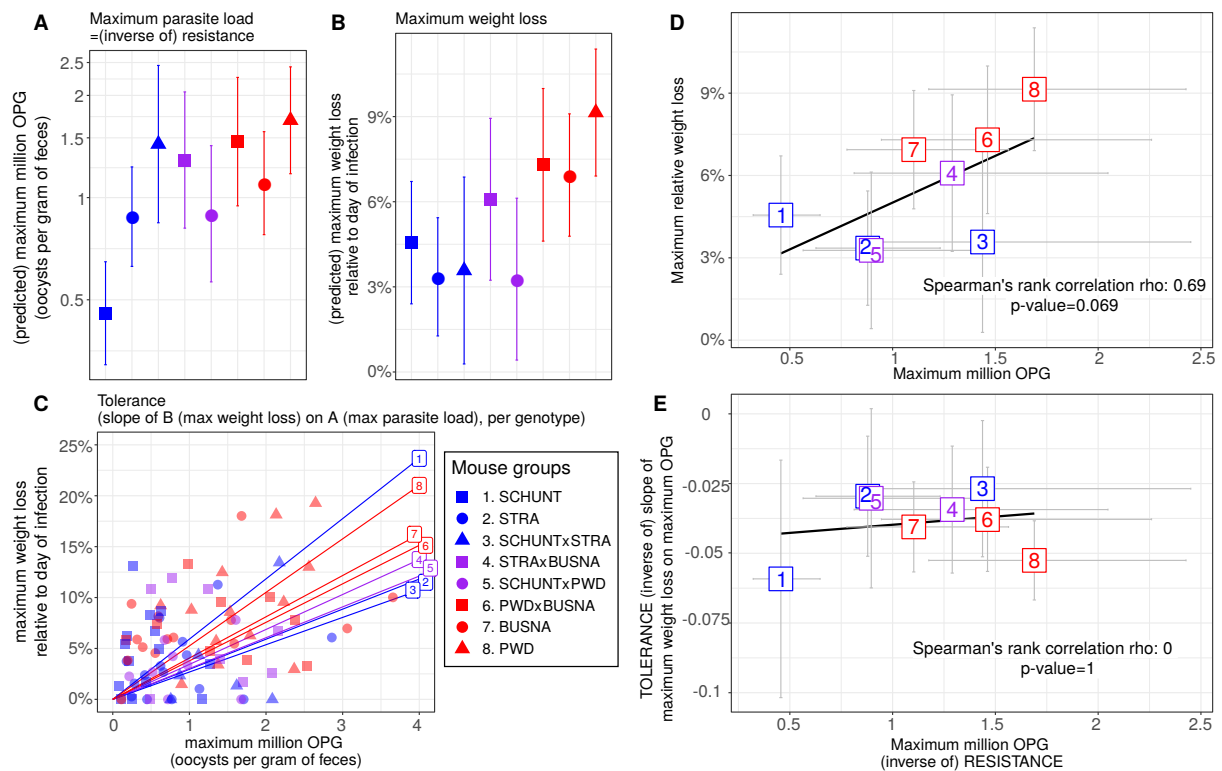


Figure 4: No indication of resistance-tolerance coupling for *E. ferrisi* isolate Brandenburg64.

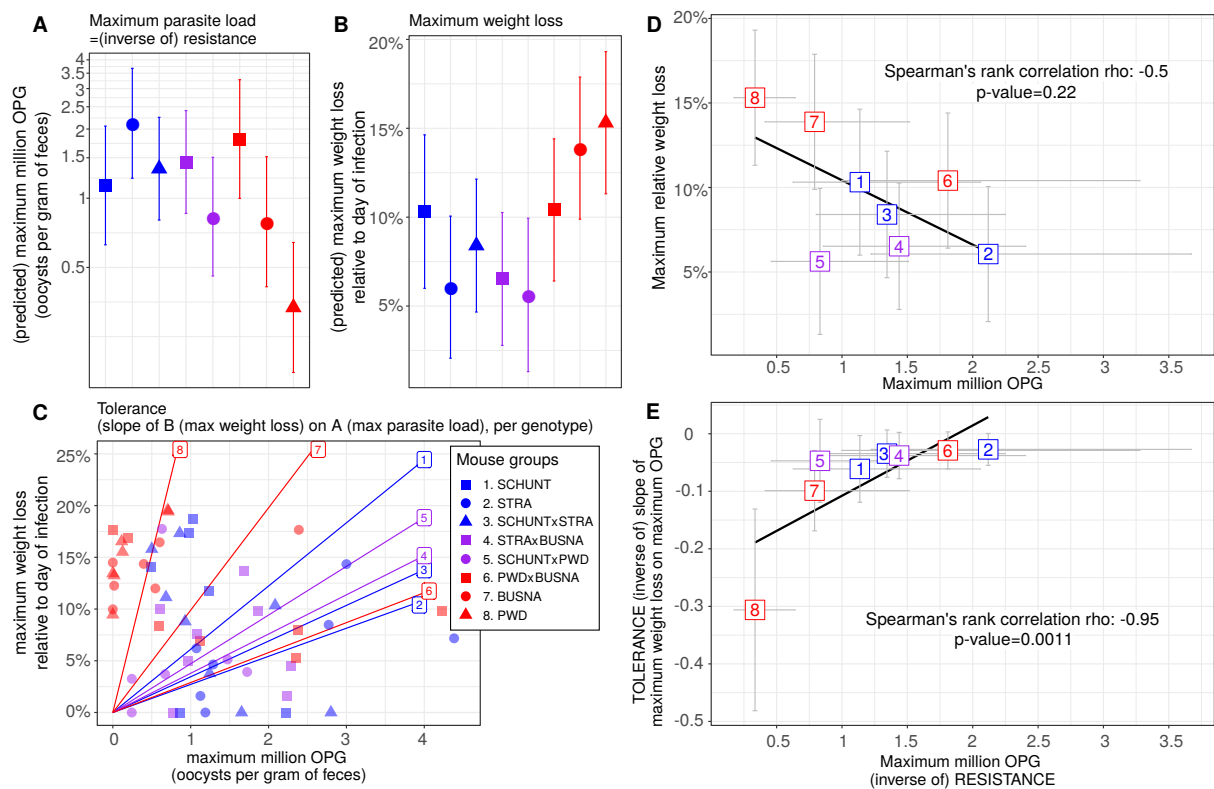


Figure 5: Coupling between resistance and tolerance for *E. falciformis* isolate Brandenburg88.

615 **Data Accessibility:** -Code and full data: Zenodo doi: 10.5281/zenodo.3911935

616 **Competing Interests Statement:** This work is original and has not been published
617 elsewhere, nor is it currently under consideration for publication elsewhere, we have
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622 and JP provided the research material. AB, VHJD, JJ, VM and FB carried out the
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