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

Alice Balard[1,2], Víctor Hugo Jarquín-Díaz[1,2], Jenny Jost[1], Vivian Mittné[1], Francisca Böhning[1], Ľudovít Ďureje[3], Jaroslav Piálek[3], and Emanuel Heitlinger[1,2]

[1]*Institute for Biology. Department of Molecular Parasitology. Humboldt University Berlin (HU).*

Philippstr. 13, Haus 14, 10115, Berlin, Germany

[2]*Leibniz-Institut für Zoo- und Wildtierforschung (IZW) im Forschungsverbund Berlin e.V..*

Alfred-Kowalke-Straße 17, 10315, Berlin, Germany

[3]*Research Facility Studenec, Institute of Vertebrate Biology, Czech Academy of Sciences,*

Květná 8, 603 65 Brno, Czech Republic

Corresponding author: Alice Balard, alice.cam.balard@gmail.com

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

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 &

4

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 occuring parasite species, the protozoan parasite *Eimeria ferrisi* and

6

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).

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

138 infection ranged between 5.6 and 21.4 weeks. All mouse strains and F1 hybrids were

139 obtained from the Institute of Vertebrate Biology of the Czech Academy of Sciences in

140 Studenec (licence number 61974/2017-MZE-17214; for further details on strains see

141 https://housemice.cz/en).

142 Parasites of the *Eimeria* genus are known to induce host immune protection against

143 reinfection (Rose et al., 1992; Smith & Hayday, 2000). To ensure that our mice were

144 *Eimeria*-naive, mouse fecal samples were tested before infection for the presence of

145 *Eimeria* spp. oocysts by flotation in saturated NaCl solution followed by washing and

146 observation under light microscope.

3. Experimental infection

148 Mice were kept in individual cages during infection. Water and food (SNIFF,

149 Rat/Mouse maintenance feed 10 mm) were provided *ad libitum* supplemented with 1

150 g of sunflower and barley seeds per day. Mice were orally infected with 150

151 sporulated oocysts of one *Eimeria* isolate suspended in $100\mu l$ phosphate-buffer saline

152 (PBS) and monitored daily until their sacrifice by cervical dislocation at time of

153 regression of infection (reduction of oocyst output). Individuals presenting severe

154 health deficiency and/or a weight loss approaching 18% relative to their starting

155 weight were sacrificed earlier at defined humane end points (experiment license Reg.

156 0431/17). Weight was recorded and feces collected on a daily basis. Fecal pellets

157 were collected every day from each individual cage and suspended in 2% potassium

158 dichromate. Parasite oocysts were recovered using NaCl flotation (see above).

159 All individuals were negative for *Eimeria* at the beginning of our experiment (before

160 infection of first batch, as described in the next paragraph). In total, 168 mice were

161 infected. Mice were randomly allocated to experimental groups ensuring

162 homogeneous distribution of ages and sexes between groups. Our experiments were

163 conducted in four (partially overlapping) consecutive batches for logistical reasons.

164 The first two batches were infected with the two *E. ferrisi* isolates (Brandenburg64 and

165 Brandenburg139), the third and fourth by one *E. ferrisi* isolate (Brandenburg64) and

166 one *E. falciformis* isolate (Brandenburg88). Our experimental design is summarized in

167 **Table 1** (chronology of experimental batches can be scrutinized in **Supplementary**

168 **Table S1**).

169 Nematode infection is common in breeding facilities (Baker, 1998) and could interact

170 with *Eimeria* (Clerc et al., 2019). We surveyed for their presence and nematode eggs

171 were observed in flotated feces of mice belonging to all genotypes before the

172 experiment. Despite treatment of the first infection batch of mice (B1, 22 mice) with

173 anthelminthics (Profender®, Bayer AG, Levekusen, Germany) following the protocole

174 of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the

175 protocole of Floyd et al. (2005)) in randomly sampled fecal samples a week later. We

176 therefore decided not to treat mice of the following infection batches. Moreover, we

177 observed *Eimeria* oocysts in the feces of 28 mice belonging to the last experimental

178 batch (batch B4) at the day of infection, likely due to cross-contamination between

179 batches. For following statistical analyses, we considered along with the full data set

180 (N=168) a conservative data set in which cross-contaminated animals and animals

181 treated by anthelminthic were removed (N=118). Results obtained on the

182 conservative data set can be found in **Supplementary Material S2**. Despite

183 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.

4. Statistical analyses

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 ρ=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 = α x + β (α being the slope and β

253 the intercept, 0 in our case). Therefore, tolerance is expressed as α = y/x − β/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 (Lüdecke, 2018)).

268 Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled

269 using the free software inkscape (https://inkscape.org).

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_7^2= 31.96, P<0.001; **Table 2**).

2.   No indication of host 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 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 ρ=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 ρ=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 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 misleading. 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; Balard et al., 2020), 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

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 litterature

363 (Fineblum & Rausher, 1995) and later on transfered 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 meaningfull 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 approch did not only consist in calculing 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 a 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.

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, a parasite species leading to uncoupling between resistance and tolerance of the host (e.g. *E. ferrisi*) might be the most suitable parasite. If coevolution between hosts and parasites should be studied, a parasite species for which resistance and tolerance of the host are negatively correlated (e.g. *E. falciformis*) would be a more plausible target. Generally, we showed that 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.

References

Al-khlifeh, E., Balard, A., Jarquín-Díaz, V. H., Weyrich, A., Wibbelt, G. & Heitlinger, E. (2019). *Eimeria falciformis* BayerHaberkorn1970 and novel wild derived isolates from house mice: Differences in parasite lifecycle, pathogenicity and host immune reactions. *bioRxiv*, 611277. doi:10.1101/611277

Ankrom, S. L., Chobotar, B. & Ernst, J. V. (1975). Life cycle of *Eimeria ferrisi* Levine & Ivens, 1965 in the mouse, *Mus musculus*. *The Journal of Protozoology*, *22*, 317–323. doi:10.1111/j.1550-7408.1975.tb05177.x

Ayres, J. S. & Schneider, D. S. (2012). Tolerance of infections. *Annual Review of Immunology*, *30*, 271–294. doi:10.1146/annurev-immunol-020711-075030

Baird, S. J. E. & Goüy de Bellocq, J. (2019). Shifting paradigms for studying parasitism in hybridising hosts: Response to Theodosopoulos, Hund, and Taylor. *Trends in ecology & evolution*, *34*, 387–389. doi:doi.org/10.1016/j.tree.2019.01.011

Baird, S. J. E., Ribas, A., Macholán, M., Albrecht, T., Piálek, J. & Goüy de Bellocq, J. (2012). Where are the wormy mice? A reexamination of hybrid parasitism in the European house mouse hybrid zone. *Evolution*, *66*, 2757–2772. doi:10.1111/j.1558-5646.2012.01633.x

431 Balard, A., Jarquín-Díaz, V. H., Jost, J., Martincová, I., Ďureje, Ľ., Piálek, J., Macholán, M., de Bellocq,

432   J. G., Baird, S. J. E. & Heitlinger, E. (2020). Intensity of infection with intracellular *Eimeria* spp.

433   and pinworms is reduced in hybrid mice compared to parental subspecies. *Journal of Evolutionary*

434   *Biology*, *33*, 435–448. doi:10.1111/jeb.13578

435 Baucom, R. S. & de Roode, J. C. (2011). Ecological immunology and tolerance in plants and animals.

436   *Functional Ecology*, *25*, 18–28. doi:10.1111/j.1365-2435.2010.01742.x

437 Boots, M., Best, A., Miller, M. R. & White, A. (2008). The role of ecological feedbacks in the evolution

438   of host defence: What does theory tell us? *Philosophical Transactions of the Royal Society B:*

439   *Biological Sciences*, *364*, 27–36. doi:10.1098/rstb.2008.0160

440 Brett, M. T. (2004). When is a correlation between non-independent variables "spurious"? *Oikos*, *105*,

441   647–656. doi:10.1111/j.0030-1299.2004.12777.x

442 Carval, D. & Ferriere, R. (2010). A unified model for the coevolution of resistance, tolerance, and

443   virulence. *Evolution*, *64*, 2988–3009. doi:10.1111/j.1558-5646.2010.01035.x

444 Chapman, H. D., Barta, J. R., Blake, D., Gruber, A., Jenkins, M., Smith, N. C., Suo, X. & Tomley, F. M.

445   (2013). Chapter two - a selective review of advances in coccidiosis research. *83*, 93–171. doi:10.

446   1016/B978-0-12-407705-8.00002-1

447 Clerc, M., Fenton, A., Babayan, S. A. & Pedersen, A. B. (2019). Parasitic nematodes simultaneously

448   suppress and benefit from coccidian coinfection in their natural mouse host. *Parasitology*, *146*,

449   1096–1106. doi:10.1017/S0031182019000192

450 Delignette-Muller, M. L. & Dutang, C. (2015). Fitdistrplus: An r package for fitting distributions. *Journal of*

451   *Statistical Software*, *64*, 1–34. doi:10.18637/jss.v064.i04

452 Ďureje, Ľ., Macholán, M., Baird, S. J. E. & Piálek, J. (2012). The mouse hybrid zone in central europe:

453   From morphology to molecules. *Journal of Vertebrate Biology*, *61*, 308–318. doi:10.25225/fozo.

454   v61.i3.a13.2012

455 Ehret, T., Spork, S., Dieterich, C., Lucius, R. & Heitlinger, E. (2017). Dual RNA-seq reveals no plastic

456   transcriptional response of the coccidian parasite *Eimeria falciformis* to host immune defenses.

457   *BMC Genomics*, *18*, 686. doi:10.1186/s12864-017-4095-6

458 Fineblum, W. L. & Rausher, M. D. (1995). Tradeoff between resistance and tolerance to herbivore

459   damage in a morning glory. *Nature*, *377*, 517–520. doi:10.1038/377517a0

460 Floyd, R. M., Rogers, A. D., Lambshead, P. J. D. & Smith, C. R. (2005). Nematode-specific PCR primers

461   for the 18S small subunit rRNA gene. *Molecular Ecology Notes*, *5*, 611–612. doi:10.1111/j.1471-

462   8286.2005.01009.x

463 Gandon, S. & Michalakis, Y. (2000). Evolution of parasite virulence against qualitative or quantitative

464   host resistance. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *267*,

465   985–990. doi:10.1098/rspb.2000.1100

466 Graham, A. L., Allen, J. E. & Read, A. F. (2005). Evolutionary causes and consequences of

467   immunopathology. *Annual Review of Ecology, Evolution, and Systematics*, *36*, 373–397.

468   doi:10.1146/annurev.ecolsys.36.102003.152622

469 Gregorová, S. & Forejt, J. (2000). PWD/Ph and PWK/Ph inbred mouse strains of *Mus m. musculus*

470   subspecies–a valuable resource of phenotypic variations and genomic polymorphisms. *Folia*

471   *Biologica*, *46*, 31–41.

472 Haberkorn, A. (1970). Die Entwicklung von *Eimeria falciformis* (Eimer 1870) in der weißen Maus

473   (*Mus musculus*). *Zeitschrift für Parasitenkunde*, *34*, 49–67. doi:10.1007/BF00629179

474 Howick, V. M. & Lazzaro, B. P. (2017). The genetic architecture of defence as resistance to and tolerance

475   of bacterial infection in *Drosophila melanogaster*. *Molecular Ecology*, *26*, 1533–1546. doi:10.1111/

476   mec.14017

477 Jackman, S. (2020). *pscl: Classes and methods for R developed in the political science computational*

478   *laboratory*. United States Studies Centre, University of Sydney. Sydney, New South Wales,

479   Australia.

480 Janzen, D. H. (1980). When is it coevolution? *Evolution*, *34*, 611–612. doi:10.1111/j.1558-5646.1980.

481   tb04849.x

482 Jarquín-Díaz, V. H., Balard, A., Jost, J., Kraft, J., Dikmen, M. N., Kvičerová, J. & Heitlinger, E. (2019).

483   Detection and quantification of house mouse *Eimeria* at the species level – Challenges and

484   solutions for the assessment of coccidia in wildlife. *International Journal for Parasitology:*

485   *Parasites and Wildlife*, *10*, 29–40. doi:10.1016/j.ijppaw.2019.07.004

486 Klemme, I. & Karvonen, A. (2016). Vertebrate defense against parasites: Interactions between

487   avoidance, resistance, and tolerance. *Ecology and Evolution*, *7*, 561–571.

488   doi:10.1002/ece3.2645

489 Kutzer, M. A. M. & Armitage, S. A. O. (2016). Maximising fitness in the face of parasites: A review of host

490   tolerance. *Zoology*, *119*, 281–289. doi:10.1016/j.zool.2016.05.011

491 Lefèvre, T., Williams, A. J. & de Roode, J. C. (2010). Genetic variation in resistance, but not tolerance,

492   to a protozoan parasite in the monarch butterfly. *Proceedings of the Royal Society B: Biological*

493   *Sciences*, *278*, 751–759. doi:10.1098/rspb.2010.1479

494 Lüdecke, D. (2018). Ggeffects: Tidy data frames of marginal effects from regression models. *Journal of*

495   *Open Source Software*, *3*, 772. doi:10.21105/joss.00772

496 Macholán, M., Baird, S. J. E., Fornůsková, A., Martincová, I., Rubík, P., Ďureje, Ľ., Heitlinger, E. & Piálek,

497   J. (2019). Widespread introgression of the *Mus musculus musculus* Y chromosome in Central

498   Europe. *bioRxiv*. doi:10.1101/2019.12.23.887471

499 Martincová, I., Ďureje, Ľ., Kreisinger, J., Macholán, M. & Piálek, J. (2019). Phenotypic effects of the

500   Y chromosome are variable and structured in hybrids among house mouse recombinant lines.

501   *Ecology and Evolution*, *9*, 6124–6137. doi:10.1002/ece3.5196

502 Mazé-Guilmo, E., Loot, G., Páez, D. J., Lefèvre, T. & Blanchet, S. (2014). Heritable variation in host

503   tolerance and resistance inferred from a wild host–parasite system. *Proceedings of the Royal*

504   *Society B: Biological Sciences*, *281*, 20132567. doi:10.1098/rspb.2013.2567

505 Medzhitov, R., Schneider, D. S. & Soares, M. P. (2012). Disease tolerance as a defense strategy. *Science*,

506   *335*, 936–941. doi:10.1126/science.1214935

507 Mesa, J. M., Scholes, D. R., Juvik, J. A. & Paige, K. N. (2017). Molecular constraints on resistance–

508   tolerance trade-offs. *Ecology*, *98*, 2528–2537. doi:10.1002/ecy.1948

509 Piálek, J., Vyskočilová, M., Bímová, B., Havelková, D., Piálková, J., Dufková, P., Bencová, V., Ďureje, L.,

510   Albrecht, T., Hauffe, H. C., Macholán, M., Munclinger, P., Storchová, R., Zajícová, A., Holáň, V.,

511   Gregorová, S. & Forejt, J. (2008). Development of unique house mouse resources suitable for

512   evolutionary studies of speciation. *Journal of Heredity*, *99*, 34–44. doi:10.1093/jhered/esm083

513 R Development Core Team. (2013). *R: A language and environment for statistical computing*.

514   http://www.R-project.org/. R Foundation for Statistical Computing. Vienna, Austria.

515 Råberg, L., Graham, A. L. & Read, A. F. (2009). Decomposing health: Tolerance and resistance to

516   parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences*,

517   *364*, 37–49. doi:10.1098/rstb.2008.0184

518 Råberg, L., Sim, D. & Read, A. F. (2007). Disentangling genetic variation for resistance and tolerance to
519   infectious diseases in animals. *Science*, *318*, 812–814. doi:10.1126/science.1148526

520 Restif, O. & Koella, J. C. (2004). Concurrent evolution of resistance and tolerance to pathogens. *The*
521   *American Naturalist*, *164*, E90–E102. doi:10.1086/423713

522 Rose, M. E., Hesketh, P. & Wakelin, D. (1992). Immune control of murine coccidiosis: CD4+ and CD8+
523   T lymphocytes contribute differentially in resistance to primary and secondary infections.
524   *Parasitology*, *105*, 349–354. doi:10.1017/S0031182000074515

525 Roy, B. A. & Kirchner, J. W. (2000). Evolutionary dynamics of pathogen resistance and tolerance.
526   *Evolution*, *54*, 51–63. doi:10.1111/j.0014-3820.2000.tb00007.x

527 Schito, M. L., Barta, J. R. & Chobotar, B. (1996). Comparison of four murine *Eimeria* species in
528   immunocompetent and immunodeficient mice. *The Journal of Parasitology*, *82*, 255–262.
529   doi:10.2307/3284157

530 Schmid-Hempel, P. (2013). *Evolutionary parasitology: The integrated study of infections, immunology,*
531   *ecology, and genetics*. Oxford University Press. doi:10.1093/acprof:oso/9780199229482.001.
532   0001

533 Shaw, D. J. & Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife
534   populations: A quantitative review. *Parasitology*, *111*, S111–S133.
535   doi:10.1017/S0031182000075855

536 Sheldon, B. C. & Verhulst, S. (1996). Ecological immunology: Costly parasite defences and trade-offs in
537   evolutionary ecology. *Trends in ecology & evolution*, *11*, 317–321.

538 Simms, E. L. (2000). Defining tolerance as a norm of reaction. *Evolutionary Ecology*, *14*, 563–570. doi:10.
539   1023/a:1010956716539

540 Smith, A. L. & Hayday, A. C. (2000). Genetic Dissection of primary and secondary responses to a
541   widespread natural pathogen of the gut, *Eimeria vermiformis*. *Infection and Immunity*, *68*,
542   6273–6280. doi:10.1128/IAI.68.11.6273-6280.2000

543 Soares, M. P., Teixeira, L. & Moita, L. F. (2017). Disease tolerance and immunity in host protection against
544   infection. *Nature Reviews Immunology*, *17*, 83–96. doi:10.1038/nri.2016.136

545 Stange, J., Hepworth, M. R., Rausch, S., Zajic, L., Kühl, A. A., Uyttenhove, C., Renauld, J.-C., Hartmann,
546   S. & Lucius, R. (2012). IL-22 mediates host defense against an intestinal intracellular parasite in

547   the absence of IFN-γ at the cost of Th17-driven immunopathology. *Journal of Immunology*, *188*,

548   2410–2418. doi:10.4049/jimmunol.1102062

549 Vale, P. F. & Little, T. J. (2012). Fecundity compensation and tolerance to a sterilizing pathogen in daphnia.

550   *Journal of Evolutionary Biology*, *25*, 1888–1896. doi:10.1111/j.1420-9101.2012.02579.x

551 Venables, W. N. & Ripley, B. D. (2002). *Modern Applied Statistics with S* (4th ed.). New York, NY: Springer.

552   doi:10.1007/978-0-387-21706-2

553 Wickham, H. (2016). *Ggplot2: Elegant graphics for data analysis (second edition)*. New York, NY:

554   Springer. doi:10.1007/978-0-387-98141-3

555 Woolhouse, M. E. J., Webster, J. P., Domingo, E., Charlesworth, B. & Levin, B. R. (2002). Biological and

556   biomedical implications of the co-evolution of pathogens and their hosts. *Nature Genetics*, *32*,

557   569–577. doi:10.1038/ng1202-569

558 Zeileis, A., Kleiber, C. & Jackman, S. (2008). Regression models for count data in R. *Journal of Statistical*

559   *Software*, *27*. doi:10.18637/jss.v027.i08

560 **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

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
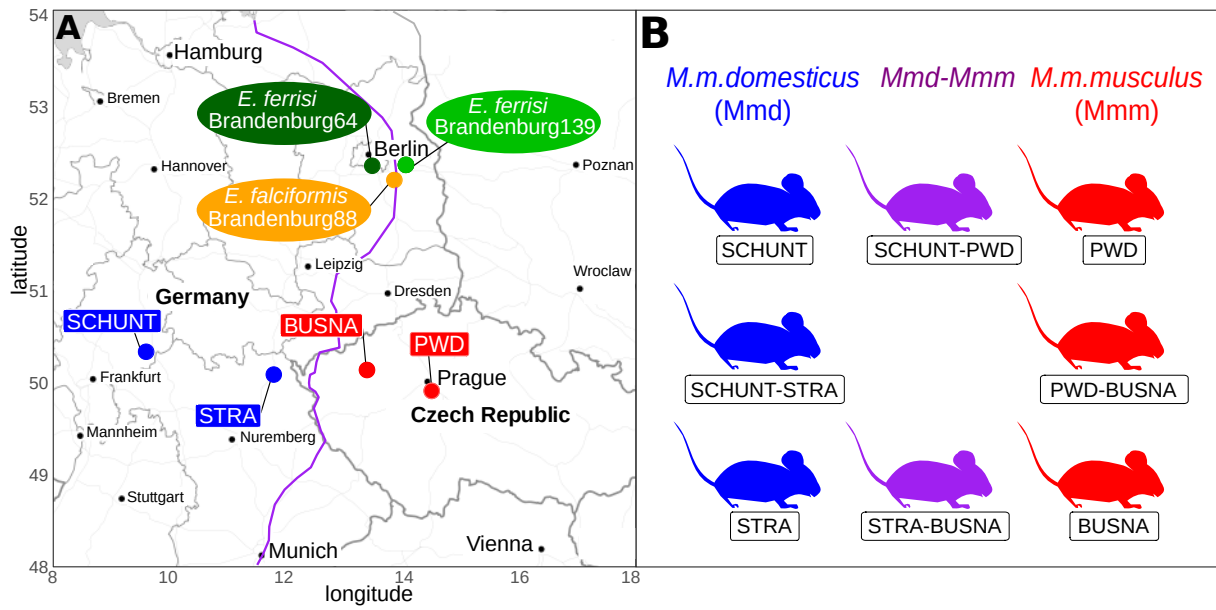
573 together.

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
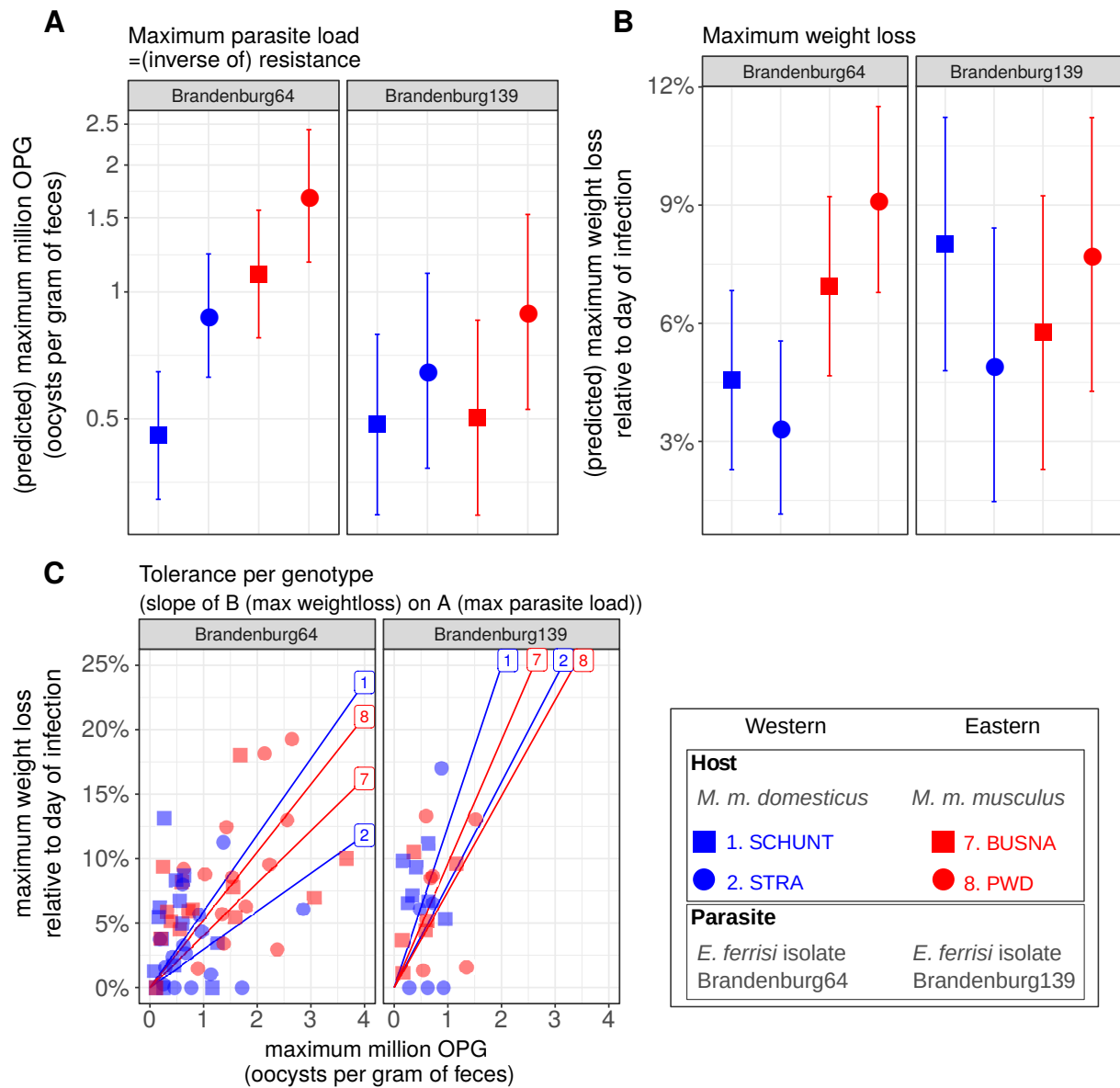
599 tolerance *E. ferrisi* isolate Brandenburg64.

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

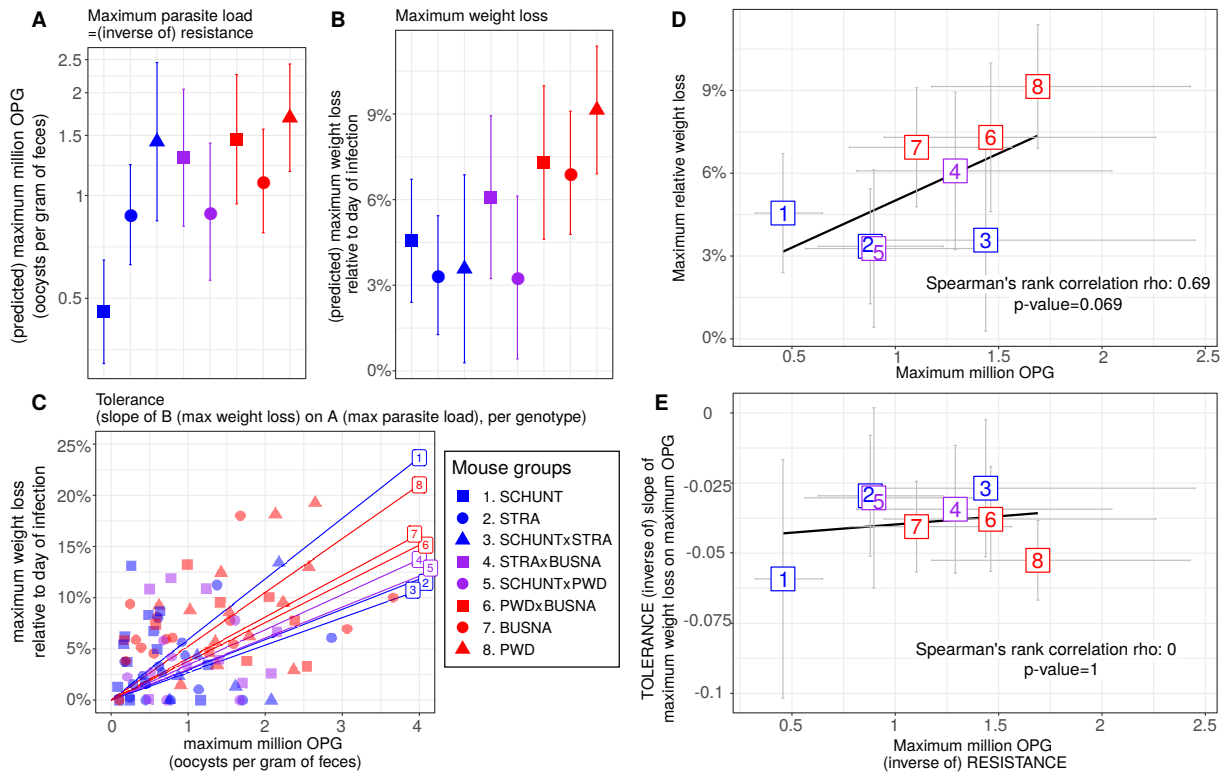614 *E. falciformis* isolate Brandenburg88.

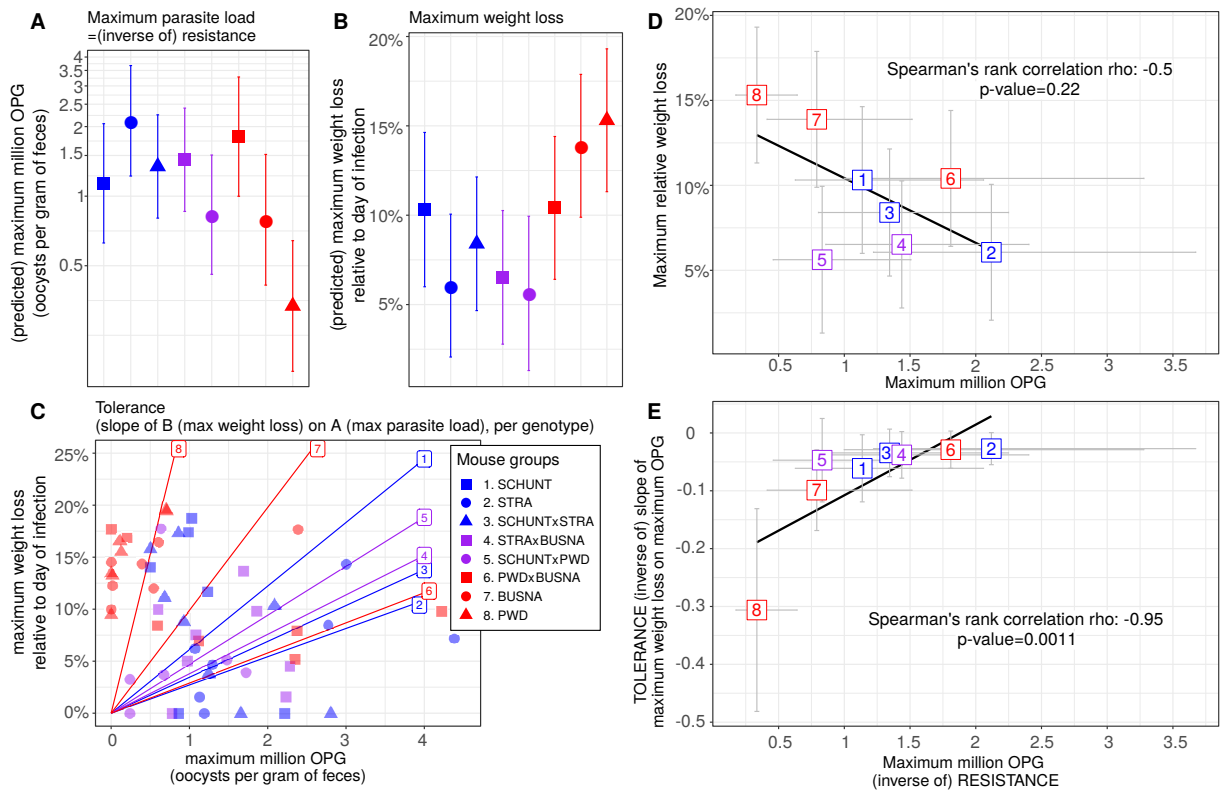**Figure 1:** Parasite isolates and mouse wild-derived strains.
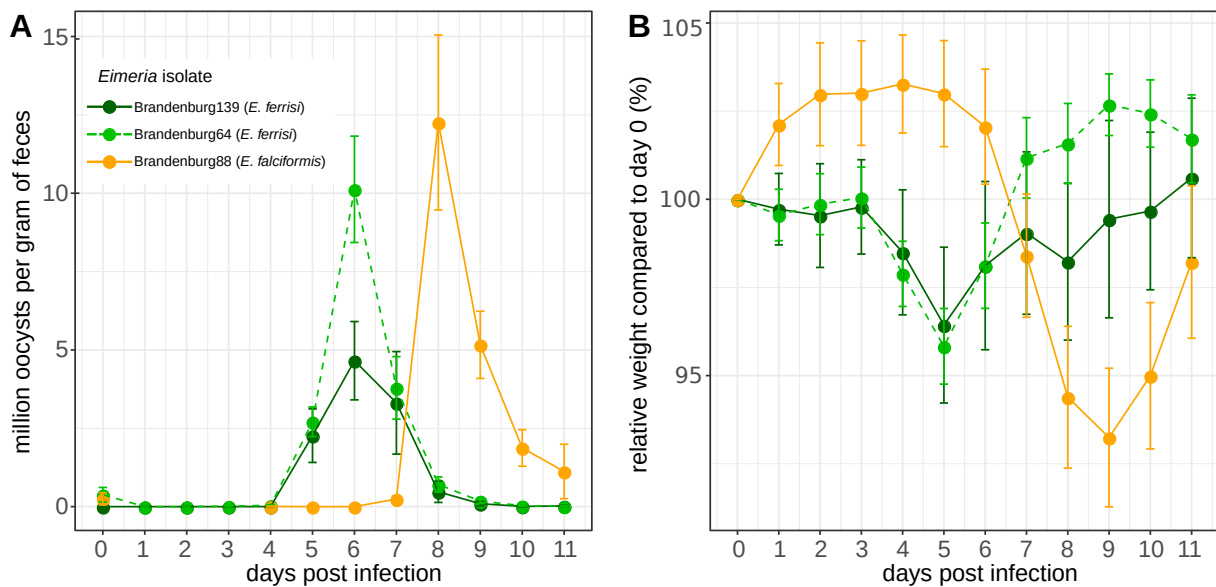
Figure 2: Parasite density (A) and relative weight (B) during *Eimeria* infection.

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.

Competing Interests Statement: 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.

Authors Contributions: AB, JP and EH designed the experiment and analysis. LD and JP provided the research material. AB, VHJD, JJ, VM and FB carried out the experiment. AB performed the analysis. AB and EH wrote the manuscript, with major contribution from JP and feedback from all the authors.