

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

Abstract

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

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

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

26 **Introduction**

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

38 Disease tolerance (not to be confused from "immunological tolerance",
39 unresponsiveness to self antigens; Medzhitov et al., 2012) is the ability of the host to
40 limit the impact of parasite on its fitness (Kutzer & Armitage, 2016; Råberg et al.,
41 2009; Vale & Little, 2012). By potentially providing a longer-living niche, this defence
42 mechanism improves, or at least does not deteriorate, the fitness of the parasite.
43 Tolerance alleles are thus predicted by theoretical models to evolve to fixation due to
44 positive feedback loops (Boots et al., 2008; Restif & Koella, 2004; Roy & Kirchner,

45 2000). From a mechanistic perspective tolerance alleviates direct or indirect damage
46 (e.g. excessive immune response underlying resistance against parasites, called
47 immunopathology; Graham et al., 2005) caused by parasites (Råberg et al., 2009).
48 Tolerance mechanisms include modulation of inflammatory response (Ayres &
49 Schneider, 2012), tissue repair (stress response, damage repair and cellular
50 regeneration mechanisms; Soares et al., 2017), and compensation of
51 parasite-induced damage by increase of reproductive effort (Baucom & de Roode,
52 2011). The resulting metabolic costs of resistance and tolerance, with and without
53 parasite infection, determine the optimal (steady state and infection inducible) extent
54 and of both immune defences (Sheldon & Verhulst, 1996).

55 Resistance and tolerance can be positively associated if they involve the same
56 metabolic pathway, as was shown in the plant model *Arabidopsis thaliana* in response
57 against herbivory (Mesa et al., 2017). In animals, genetic association studies of
58 resistance and tolerance of *Drosophila melanogaster* against the bacterium
59 *Providencia rettgeri* have shown positively correlated genetic effects, as the same loci
60 were associated with changes of both traits in the same direction (Howick & Lazzaro,
61 2017).

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

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

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

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

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

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

Material and methods

1. Parasite isolates

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

2. Mouse groups

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

132 (Locality: Schweben, Hessen, Germany [N: 50° 26', E: 9° 36'] (Martincová et al.,
133 2019)) and **STRA** (Locality: Straas, Bavaria, Germany [N: 50° 11', E: 11° 46'] (Piálek
134 et al., 2008), and two derived from *M. m. musculus*: **BUSNA** (Locality: Buškovice,
135 Bohemia, Czech Republic [N: 50° 14', E: 13° 22'] (Piálek et al., 2008)) and **PWD**
136 (Locality: Kunratice, Bohemia, Czech Republic [N: 50° 01', E: 14° 29'] (Gregorová &
137 Forejt, 2000)). The four groups of F1 hybrids consisted of two intrasubspecific hybrids
138 (**SCHUNTxSTRA** and **PWDxBUSNA**) and two intersubspecific hybrids
139 (**STRAxBUSNA** and **SCHUNTxPWD**)(Figure 1). Age of the mice at the time of
140 infection ranged between 5.6 and 21.4 weeks. All mouse strains and F1 hybrids were
141 obtained from the Institute of Vertebrate Biology of the Czech Academy of Sciences in
142 Studenec (licence number 61974/2017-MZE-17214; for further details on strains see
143 <https://housemice.cz/en>).

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

149 3. Experimental infection

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

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

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

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

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

187 **4. Statistical analyses**

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

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

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

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

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

217 **4.2. Statistical modelling**

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

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

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

236 **4.3. Test of local adaptation**

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

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

4.4. Test of coupling between resistance and tolerance

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

267 All analyses were performed using R version 3.5.2 (R Development Core Team,
268 2013)(negative binomial: function glm.nb from R package MASS (Venables & Ripley,
269 2002); ZIBN: function zeroinfl from R package pscl (Jackman, 2020; Zeileis et al.,
270 2008); linear model: function lm from R core package stats; mean and 95%
271 confidence intervals: function ggpredict from R package ggeffect (Lüdecke, 2018)).
272 Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled
273 using the free software inkscape (<https://inkscape.org>). Code and data used for this
274 article can be found at: https://github.com/alicebalard/Article_RelatedParasitesResTol

275 Results

276 1. General

277 Parasites of all isolates successfully infected all mouse groups (at the exception of 5
278 individuals infected by *E. falciformis* isolate Brandenburg88 that died or had to be
279 sacrificed due to a strong weight loss before the peak of shedding for this parasite),
280 meaning that no "qualitative infection resistance" (*sensu* Gandon and Michalakis
281 (2000)) was detected. For *E. ferrisi* (both isolates Brandenburg139 and
282 Brandenburg64), the pre-patent period was 5 days post infection (dpi) and the median
283 day of maximal oocyst shedding was 6 dpi (standard deviation sd=0.7 and 0.9,
284 respectively). The median day of maximum weight loss was 5 dpi for both isolates
285 (sd=2.1 and 1.7 respectively). For *E. falciformis* (isolate Brandenburg88) pre-patency
286 was 7 dpi, median day of maximal shedding was 8 dpi (sd=1.3) and median day of
287 maximal weight loss 9 dpi (sd=1.6)(**Figure 2**). Of note a considerable number of mice
288 infected with this isolate (13 out of 56 = 23%) died or had to be sacrificed at humane
289 end points less than 3 days after the oocysts shedding peak for the group, all

290 belonging to *M. m. musculus* subspecies (PWD, BUSNA, or their F1 PWDxBUSNA; 5
291 died at dpi 8, 5 at dpi 9, 3 at dpi 10). *E. falciformis* isolate Brandenburg88 was more
292 lethal for the *M. m. musculus* mice strains than for the other strains ($\chi^2_7 = 31.96$,
293 $P < 0.001$; **Table 2**).

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

295 We tested if our proxies for resistance, impact on weight and tolerance were different
296 between the four parental mouse strains and between both *E. ferrisi* infection isolates
297 (isolate Brandenburg64 and Brandenburg139). Maximum parasite load differed
298 between mouse strains (LRT: $G=25.5$, $df=6$, $P<0.001$), but the interaction term mouse
299 strain-parasite isolate was non significant (LRT: $G=4.1$, $df=3$, $P=0.25$). A similar result
300 was found for maximum relative weight loss (LRT: mouse strain: $G=16.8$, $df=6$,
301 $P=0.01$; interaction mouse strain-parasite isolate: $G=4.1$, $df=3$, $P=0.25$). This
302 indicates that when resistance and impact on weight vary between host strains, they
303 do so independently of the parasite isolate. Eventually, the variables mouse strain,
304 parasite isolate and their interaction were found non significant at the 0.05 threshold
305 for the slope of the linear regression between the two, indicating that differences of
306 tolerance could not be detected between mouse strains or parasite isolates (**Figure**
307 **3**). Our results do not indicate either (1) an increased reproduction of each parasite in
308 its matching host or (2) a higher tolerance of host infected by its matching parasite
309 despite similar parasite reproductive output. Thus they do not support the hypothesis
310 of local adaptation between *E. ferrisi* and its host.

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

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

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

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

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

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

332 resistance, impact on weight and tolerance were different between the mouse groups.
333 We found the maximum number of OPG and relative weight loss to be statistically
334 different between mouse groups (LRT: maximum number of OPG: $G=28.6$, $df=14$,
335 $P=0.012$; **Figure 5A**; maximum relative weight loss: $G=21$, $df=7$, $P<0.01$; **Figure 5B**).
336 Finally, contrary to our results on *E. ferrisi* isolate Brandenburg64, the tolerance
337 slopes for *E. falciformis* isolate Brandenburg88 were different between mouse groups
338 (LRT: $G=13.9$, $df=7$, $P=0.05$; **Figure 5C**).

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

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

349 Discussion

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

354 From a practical "measurement" perspective we can ask whether tolerance can be
355 predicted from resistance, as the latter is easier to measure (e.g. in field sampling).
356 Many studies assess the impact of parasites on host fitness based on resistance. If,
357 as we found in the present study, resistance and tolerance are decoupled this can be
358 misleading. In our host system, the house mice, for example, it has been shown that
359 hybrids between *M. m. domesticus* and *M. m. musculus* are more resistant to
360 parasites (Baird et al., 2012), including *Eimeria*, but tolerance could not be measured
361 under natural conditions (Balard et al., 2020). The effect of parasites on host fitness in
362 the evolution of the house mouse hybrid zone is thus still rather ambiguous (Baird &
363 Goüy de Bellocq, 2019). We show that careful distinction between parasite species is
364 necessary when analysing parasite host interaction (see also Jarquín-Díaz et al.,
365 2019) and that it is indispensable to measure both resistance and tolerance in *Eimeria*
366 infections of house mice.

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

378 Differences between parasite species could explain the evolution of different
379 strategies: *E. ferrisi* commits to sexual reproduction after a relatively short time with
380 few cycles of asexual expansion (Al-khlifeh et al., 2019; Ankrom et al., 1975), while
381 *E. falciformis* has a relatively longer life cycle (Al-khlifeh et al., 2019; Haberkorn,
382 1970). As *E. ferrisi* infections do not reach extremely high intensities, high tolerance
383 might be the optimal strategy for both house mouse subspecies. Resistance could
384 then evolve relatively freely without any major impact of the parasite on the hosts'
385 health. Moreover, our results did not support local adaptation of *E. ferrisi*, which might
386 be explained by the absence of host-parasite coevolution caused by uncoupling of
387 parasite and host fitness. In the case of *E. falciformis*, the long life cycle might lead to
388 high tissue load. Tissue damage is observed during sexual reproduction for this
389 parasite (Ehret et al., 2017) and might mean that a certain level of resistance is
390 required. On the other hand, immunopathology has been observed in advanced
391 *E. falciformis* infections (Stange et al., 2012). These intrinsic characteristics of
392 *E. falciformis* might lead to multiple different optima for resistance and tolerance,
393 leading to a trade-off.

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

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

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

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574 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			
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.

575 Figures legends

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

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

587 together.

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

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

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

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