Decoupling of resistance and tolerance against one of two related parasites (*Eimeria*) in mice

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**Abstract**

Resistance (the host's capacity to reduce parasite burden) and tolerance (the host's capacity to reduce impact on host health of a given parasite burden) manifest two different lines of immune defenses. In some host-parasite systems these two defenses are balanced against each other, while in others they are uncoupled. In hybrid hosts, resistance has sometimes been interpreted as having an effect on fitness without considering the modulatory effect of tolerance. Here, we used two closely related parasite species of genus *Eimeria* and measured proxies for resistance and tolerance in four wild-derived strains of inbred mice from two subspecies during controlled infection.

We found a negative correlation between resistance and tolerance against *E. falciformis*, while the two are uncoupled against *E. ferrisi*. This might be explained by trade-offs, as resistance limits infection load and thereby the scope of possible tolerance, and both resistance and tolerance can be costly in terms of resource allocation. Resistance can be assumed to be limited by immunopathogenicity, tolerance by carrying capacity of the host or energy drained by the parasite.

Findings of resistance in natural populations of hybrid mice have to be interpreted carefully in this context. Resistance and tolerance have to be studied in conjunction.
Introduction

Host defense mechanisms evolving in response to feedback between hosts and parasites can be categorised into two components: resistance and tolerance (Little, Shuker, Colegrave, Day, & Graham, 2010). Resistance (the ability of a host to reduce its parasite burden) results from defense against parasite infection or proliferation early after infection (Råberg, Graham, & Read, 2009). Resistance can be energetically costly and therefore limited by resource allocation as measured by a decrease of other fitness components (e.g. delayed maturity, lower fecundity) in the absence of infection (Langand, Jourdane, Coustau, Delay, & Morand, 1998; Sheldon & Verhulst, 1996; Vijendravarma, Kraaijeveld, & Godfray, 2009). Additionally, too strong immune response against pathogens can lead to a negative impact on health or immunopathology (Graham, Allen, & Read, 2005). Tolerance balances damage caused by parasites themselves and immunopathology (Medzhitov, Schneider, & Soares, 2012) through control mechanisms like stress response, damage repair and cellular regeneration (Soares, Teixeira, & Moita, 2017). This is why, just like resistance, tolerance can involve energetic costs (Simms & Triplett, 1994). In natural populations, costs of the two lines of defense against parasites predict that resistance and tolerance are negatively correlated (Råberg, 2014; Råberg, Sim, & Read, 2007). They can also be found uncoupled if they are at intermediate levels (Athanasiadou, Tolossa, Debela, Tolera, & Houdijk., 2015). As resistance alone is not an estimator of parasite impact on health, understanding how resistance and tolerance are coupled is necessary to conclude on health effects of parasitism.

The house mouse subspecies *Mus musculus musculus* and *M. m. domesticus* (hereafter Mmm and Mmd, respectively), whose genomes diverged some 0.5 million years ago, hybridize in a secondary contact zone running through Europe (Boursot, Auffray, Britton-Davidian, & Bonhomme, 1993; Duvaux, Belkhir, Boulesteix, & Boursot, 2011). Hybrids show
elevated resistance to parasites compared to both parental subspecies (Baird et al., 2012; Balard et al., 2019). Newly generated diversity in the immune system can result in novel interplay in immunological response; interpretations of these results in terms of health or even fitness effects, however, have been attempted (Sage, Heyneman, Lim, & Wilson, 1986) and criticised (Baird & Goüy de Bellocq, 2019).

The protozoan parasite *Eimeria ferrisi* has been found to be the most prevalent (17%) *Eimeria* species in the house mouse hybrid zone in Brandenburg (Germany), followed by *E. falciformis* (4%) (Jarquín-Díaz, Balard, Jost, et al., 2019). *Eimeria* spp. are monoxenous parasites that expand asexually and reproduce sexually in intestinal epithelial cells, leading to malabsorption of nutrients, tissue damage and weight loss (Chapman et al., 2013). They are generally considered to be host specific, and different species infect a wide range of animals including birds, mammals, reptiles, amphibians, and fish (Chapman et al., 2013; Jarquín-Díaz, Balard, Mácová, et al., 2019). *Eimeria ferrisi* and *E. falciformis* live in the cecum villar epithelial cells and cecum crypt cells, respectively (Schito, Barta, & Chobotar., 1996). Pre-patency (the time to shedding of infectious stages, so called oocysts) is longer for *E. falciformis* (7 days) than for *E. ferrisi* (5 days) (Al-khlifeh et al., 2019). While both species provoke inflammation, cellular infiltration, enteric lesions, diarrhea, and ultimately weight loss (Ankrom, Chobotar, & Ernst, 1975; Ehret, Spork, Dieterich, Lucius, & Heitlinger 2017; Schito et al., 1996), the symptoms are stronger for *E. falciformis* than for *E. ferrisi* infections (Al-khlifeh et al., 2019). The evolutionary history of these different *Eimeria* species in the two house mouse subspecies is unknown and is unclear whether subspecies-specific adaptation exists in one or the other.

Given differences in pathogenicity and prevalence between the two *Eimeria* species we suspected that coupling between resistance and tolerance might differ. We assessed this experimentally in controlled infections of Mmm and Mmd. We employed four wild-derived
inbred strains representing the two mouse subspecies and assessed the symptoms both at the level of host subspecies and inbred strains.

**Material and methods**

**Parasite strains**

The three parasite isolates used in this study were isolated from feces of mice captured in a house mouse hybrid zone (HMHZ) running through Brandenburg, Germany (Macholán et al. 2019), in 2016 (capture permit No. 2347/35/2014). They belong to both the most prevalent *Eimeria* species in the wild (Jarquín-Díaz, Balard, Mácová, et al., 2019), namely *E. ferrisi* (isolates Brandenburg64 and Brandenburg139) and *E. falciformis* (isolate Brandenburg88). Hybrid index (HI) of each individual wild-caught mouse was calculated to account for the admixture of mouse genomes across the HMHZ as a proportion of Mmm alleles in a set of 14 diagnostic markers (Balard et al., 2019). Isolate Brandenburg64 was isolated in a 92% Mmd individual (HI = 0.08), isolate Brandenburg139 in a 85% Mmm (HI = 0.85) and isolate Brandenburg88 in a 80% Mmd (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 all 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, Fenton, Babayan, & Pedersen, 2019) and stored at room temperature in 1mL of 2% potassium dichromate for a maximum of 2 months before infection of the wild-derived mouse strains. Oocysts were allowed to sporulate 10 days before infection in a water bath at 30°C.
Mouse strains

We used four wild-derived fully-inbred mouse strains: two representing Mmd: SCHUNT (Locality: Schweben, Hessen, Germany [N: 50° 26', E: 9° 36'] (Martincová, Ŏureje, Kreisinger, Macholán, & Piálek, 2019)) and STRA (Locality: Straas, Bavaria, Germany [N: 50° 11', E: 11° 46'] (Piálek et al., 2008), and two derived from Mmm: 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)) (Figure 1).

![Figure 1. Parasite isolates and mouse strains.](image) The map shows locations at which mice were collected for breeding of mouse strains and isolation of parasites. The purple line is an estimation of the center of the house mouse hybrid zone between Mmd and Mmm based on sampling and genotyping of mice in this area (Balard et al., 2019; Ŏureje, Macholán, Baird, & Piálek, 2012, Macholán et al. 2019).
Age of the mice at the time of infection ranged between 7.6 and 21.4 weeks. All mouse strains were maintained before infection in the Institute of Vertebrate Biology in Studenec (licence number 61974/2017-MZE-17214; for further details on strains see https://housemice.cz/en).

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

**Experimental infection**

Mice were kept in individual cages during infection. Water and food (SNIFF, Rat/Mouse maintenance feed 10 mm) were provided *ad libitum* supplemented with 1 g of sunflower and barley seeds per day. Mice were orally infected with 150 sporulated oocysts of one *Eimeria* isolate suspended in 100 µl phosphate-buffer saline (PBS) and monitored daily until their sacrifice by cervical dislocation at 11 days after infection (dpi) (experiment license Reg. 0431/17). Individuals presenting severe health deficiency and/or a weight loss approaching 18% relative to their starting weight were sacrificed earlier. Weight was recorded and feces collected on a daily basis. Fecal pellets were collected every day from each individual cage and suspended in 2% potassium dichromate. Parasite oocysts were recovered using NaCl flotation (see above). In total, 108 mice were infected. Mice were randomly allocated to experimental groups ensuring homogeneous distribution of ages and sexes between groups. Our experiments were conducted in four consecutive batches for easy handling. The first two groups were infected by the two *E. ferrisi* isolates (Brandenburg64 and Brandenburg139), the
two second by one *E. ferrisi* isolate (Brandenburg64) and one *E. falciformis* isolate (Brandenburg88). Summarised experiment design is shown in Table 1.

| Mouse | Eimeria species / isolate |
|-------|---------------------------|
| strains | 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) |
| 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.**

We observed *Eimeria* oocysts in the feces of 9 mice belonging to the last experimental batch at the day of infection, likely due to cross-contamination between batches. Moreover, before arrival to the infection facility, nematode eggs were observed in flotated feces of mice belonging to all genotypes. Nematode infection is common in breeding facilities (Baker, 1998). Despite treatment of the first infection batch of mice (22 mice) with anthelminthics (Profender®, Bayer AG, Leverkusen, Germany) following the protocol of Mehlhorn et al. (2005), nematodes were still detected with PCR (following the protocol of Floyd, Rogers, Lambshead, & Smith, 2005) in randomly sampled fecal samples a week later. We therefore decided not to treat mice of the following infection batches. For following statistical tests, we considered the full dataset and a conservative dataset in which cross-contaminated animals and animals treated by anthelminthic are removed (see below).
Choice of measurements for resistance and tolerance

Resistance is the capacity of a host to reduce its parasite burden, therefore it is usually estimated by the inverse of infection intensity (Råberg et al., 2009). As a proxy we used the number of oocysts per gram of feces (OPG) at the day of maximal shedding. This measure is tightly correlated with the sum of oocysts shed throughout the experiment (Pearson correlation coefficient 0.91).

Tolerance is usually defined as the slope of the regression of host fitness, approximated by health condition, on infection intensity (Råberg, 2014). The major measurable symptom in murine *Eimeria* infections is weight loss. Therefore, the impact of parasites on host health was measured as the maximum relative weight loss compared to day 0 (body weight taken at the start of the experimental infection).

We defined a tolerance index for each individual, describing how its health varied with infection intensity, between day 0 of infection (weight = 100%, parasite intensity = 0 oocyst per mouse gram) and highest impact (weight = maximum weight loss relative to day 0, parasite intensity = maximum parasite number per gram of feces). This index was then standardised by log10 transformation, after addition of 1e-8 to the ratio to avoid infinite values. The obtained log10 transformed ratio that ranged between -8 (high tolerant) and -5.6 (low tolerant) was divided by the negative constant -8 to obtained a final index positively correlated with tolerance:

\[
\text{Tolerance index} = \frac{\log_{10}(\text{maximum relative weight loss} / \text{maximum number of oocysts per gram of feces} + 1e-8)}{-8}
\]
Statistical analyses

Appropriate distribution for maximum number of oocysts per gram of feces, maximum weight loss relative to day 0, and tolerance index were selected based on log likelihood and AIC criteria and by comparing goodness-of-fits plots (density, CDF, Q-Q, P-P plots) between usual distributions (R packages MASS (Venables & Ripley, 2002) and fitdistrplus (Delignette-Muller & Dutang, 2015)). The negative binomial, Weibull, and normal distribution were the most adequate to describe the peak of oocysts per gram of feces, impact on host health, and tolerance index, respectively. For modelling the impact on host health, we added 0.01 to the raw value as the Weibull distribution regression requires positive values.

We tested the effects of mouse subspecies (Mmd or Mmm), Eimeria species (E. ferrisi or E. falciformis) and their interaction on our three response variables using (generalised) linear models. To assess the stability of our results across mouse strains and Eimeria isolates, we also tested the effects of mouse strain (N=4), parasite isolate (N=3) and their interaction separately using the same models. To test the significance of the marginal contribution to each parameter to the full model, each parameter (mouse subspecies, Eimeria species, and their interaction in the first case; mouse strain, Eimeria isolate, and their interaction in the second case) was removed from the full model, and the difference between full model and sub-model was assessed using likelihood ratio tests (G). Post-hoc multiple comparison tests (Tukey Multiple Comparisons of Means) were then performed to test the significant difference of each host-parasite combination against all others in the most detailed model with the mouse strains and the parasite isolates as predictors.

We then compared the coupling between proxies of resistance and tolerance between mouse subspecies. Using the resistance index and tolerance index defined above, we fitted a linear
model to explain the variation of tolerance with resistance, *Eimeria* species and their interaction.

To verify the absence of impact of both previous contamination by *Eimeria* and anthelminthic treatment on our results, we performed different analyses (modeling of resistance, impact on health, tolerance at the host subspecies/parasite species level, as well as linear regression of the tolerance index with the resistance index, see below) on a dataset excluding the 22 mice treated by anthelminthics and the 9 mice showing contaminant infections. The results obtained in this conservative dataset are congruent with the results revealed in all mice, thus we consider the influence of both factors negligible. (see Supplementary Material S1).

All analyses were performed using the R software version 3.5.2 (R Development Core Team, 2018). Graphics were produced using the R package ggplot2 (Wickham, 2016) and compiled using the free software inkscape (https://inkscape.org).

All codes and data used for this article can be found at: https://github.com/alicebalard/Article_RelatedParasitesResTol

**Results**

**General parasitology**

The life cycle of all isolates was successfully completed in all mouse strains (Figure 2). For *E. ferrisi* (both isolates), the pre-patent period was 5 dpi and the median day of maximal oocyst shedding was 6 dpi (standard deviation sd=0.73 and 0.61, respectively). The median day of maximum weight loss was 5 dpi for both isolates (sd=2.1 and 1.9 respectively). For *E. falciformis* (isolate Brandenburg88) pre-patency was 7 dpi, median day of maximal shedding was 8 dpi (sd=1.2) and median day of maximal weight loss 9 dpi (sd=1.5). All tested *Eimeria* isolates infected all individuals of the tested mouse strains.
A considerable number of Mmm mice (8/14; 5 of BUSNA and 3 of PWD) infected with *E. falciformis* (isolate Brandenburg88) died (or had to be sacrificed at humane end points specified in animal experimental procedures) before the peak of oocyst shedding. Moreover, one Mmd mouse (strain SCHUNT) infected by *E. ferrisi* isolate Brandenburg139 had liquid diarrhea in the peak shedding day, making its feces not collectable. These mice were assessed as missing data for both resistance and following tolerance measurements.

Figure 2. Parasite density (A) and relative weight loss (B) during *Eimeria* infection. Parasite density is calculated as number of oocysts detected (x10e6) per gram of feces, relative weight loss is calculated compared to day 0. Mean and 95% CI are plotted for each parasite isolate. All hosts strains are pooled together.
**M. m. domesticus** is less resistant to *E. falciformis* than to *E. ferrisi*

To establish differences between the two house mouse subspecies and between the parasite species, we analysed the maximum number of oocysts per gram of feces (OPG) as a measure of resistance after infection with both *Eimeria* species on the 99 mice alive by the time of median peak shedding. We found statistically significant effects of parasite species (LRT: $G = 18.9$, df = 2, $P < 0.001$), mouse subspecies (LRT: $G = 16.2$, df = 2, $P < 0.001$) as well as an interaction between parasite species and mouse subspecies (LRT: $G = 10.2$, df = 1, $P < 0.01$). Post-hoc multiple comparison tests showed than the subspecies Mmd shed more OPG at the peak of shedding when infected with *E. falciformis* than with *E. ferrisi*. Moreover, the Mmm subspecies shed more OPG at the peak of shedding than the Mmd subspecies when infected with *E. ferrisi* (Table 2; Figure 3A).

| Mouse subspecies | *Eimeria* species | *Eimeria ferrisi* | *Eimeria falciformis* | *Eimeria ferrisi* | *Eimeria falciformis* |
|------------------|------------------|-------------------|----------------------|-------------------|----------------------|
| *Mus musculus domesticus* (Mmd) | *Eimeria ferrisi* | z value:4.04, Pr(>|z|)< 0.001 | est:0.95, Std.Error:0.23 | est:0.6, Std.Error:0.16 | est:0.14, Std.Error:0.32 |
| *Eimeria falciformis* | z value:3.62, Pr(>|z|)< 0.01 | z value:-1.48, Pr(>|z|):0.43 | est:-0.35, Std.Error:0.24 | est:-0.81, Std.Error:0.36 |
| *Mus musculus musculus* (Mmm) | *Eimeria ferrisi* | z value:-2.23, Pr(>|z|):0.11 | z value:-1.43, Pr(>|z|):0.47 |

**Table 2.** Post-hoc statistical test for maximum oocysts per gram of feces (Tukey Multiple Comparisons of Means) between each mouse subspecies and parasite species. See Figure 3A.
Figure 3. Resistance, impact on host health and tolerance marginal effects for the two mice subspecies and two *Eimeria* species. Values under bars represent the number of animals for each group. (A) Maximum oocysts per gram of feces used as a proxy for (inverse of) resistance (see Table 2); (B) Impact on host health measured as the maximum weight loss during patent period relative to starting weight (%) (see Table 4); (C) Tolerance index measured as \( \log_{10}(\text{maximum relative weight loss} / \text{maximum number of oocysts per gram of feces + 1e-8}) / -8 \) (see Table 6)
We then tested the influence of mouse strain and parasite isolate on maximum number of OPG. We found statistically significant effects of parasite isolate (LRT: G = 35.5, df = 8, P < 0.001), mouse strain (LRT: G = 36.3, df = 9, P < 0.001) as well as an interaction between parasite isolate and mouse strain (LRT: G = 21.8, df = 6, P < 0.01).

Post-hoc multiple comparison tests showed that STRA (Mmd strain) shed less OPG when infected with Brandenburg139 (E. ferrisi isolate) than when infected with Brandenburg88 (E. falciformis isolate), and PWD (Mmm strain) shed more OPG when infected with Brandenburg64 (E. ferrisi isolate) than when infected with Brandenburg88 (E. falciformis isolate). Moreover, PWD (Mmm subspecies) shed more OPG at the peak of shedding than STRA (Mmd subspecies) when infected with Brandenburg88 (E. falciformis isolate); when infected with Brandenburg64 (E. ferrisi isolate), SCHUNT (Mmd subspecies) shed more OPG at the peak of shedding than both Mmm subspecies, PWD and BUSNA.

We found no significant difference between the strains of the same subspecies within a given parasite species infection, nor between the isolates of the same species within a given mouse strain (Table 3; Figure 4A).
| Mouse strains | Parasite isolates | SCHUNT (Mmd) | BRANDENBURG139 (E. fersisi) | BRANDENBURG64 (E. fersisi) | BRANDENBURG88 (E. fersisi) | STRA (Mmd) | BUSNA (Mmm) | PWD (Mmm) |
|---------------|-------------------|--------------|-----------------------------|-----------------------------|-----------------------------|------------|------------|-----------|
| BRANDENBURG139 (E. fersisi) | est.-0.11, Std.Error 0.32 | est.0.8, Std.Error 0.38 | est.0.24, Std.Error 0.38 | est.0.55, Std.Error 0.32 | est.1.43, Std.Error 0.37 | est.0.1, Std.Error 0.32 | est.0.77, Std.Error 0.32 | est.1.01, Std.Error 0.32 |
| BRANDENBURG64 (E. fersisi) | z value: -0.34, Pr(>|z|): 1 | z value: 0.91 | z value: 0.35, Std.Error 0.32 | z value: 0.24, Std.Error 0.32 | z value: 0.147, Pr(>|z|): 0.94 | z value: 0.26, Std.Error 0.32 | z value: 0.03, Std.Error 0.32 | est.1.18, Std.Error 0.32 |
| BRANDENBURG88 (E. fersisi) | z value: 2.11, Pr(>|z|): 0.59 | z value: 2.94, Pr(>|z|): 0.15 | est.0.56, Std.Error 0.32 | est.0.26, Std.Error 0.32 | est.0.62, Std.Error 0.32 | est.0.81, Std.Error 0.32 | est.0.03, Std.Error 0.32 | est.0.24, Std.Error 0.32 |
| STRA (Mmd) | z value: 0.64, Pr(>|z|): 0.89 | z value: 0.1, Pr(>|z|): 0.99 | z value: -1.47, Pr(>|z|): 0.94 | est.0.3, Std.Error 0.32 | est.1.18, Std.Error 0.32 | est.0.53, Std.Error 0.32 | est.0.3, Std.Error 0.32 | est.0.96, Std.Error 0.32 |
| BUSNA (Mmm) | z value: 1.72, Pr(>|z|): 0.36 | z value: 2.68, Pr(>|z|): 0.22 | z value: -0.81, Pr(>|z|): 1 | z value: 0.95, Pr(>|z|): 1 | z value: 0.88, Std.Error 0.32 | z value: 0.65, Std.Error 0.32 | z value: 0.23, Std.Error 0.32 | est.0.46, Std.Error 0.32 |
| PWD (Mmm) | z value: 3.9, Pr(>|z|): < 0.01 | z value: 0.04, Pr(>|z|): < 0.001 | z value: 1.7, Pr(>|z|): 0.96 | z value: 2.3, Pr(>|z|): 0.05 | z value: 2.92, Pr(>|z|): 0.12 | est.1.44, Std.Error 0.32 | est.0.65, Std.Error 0.32 | est.0.42, Std.Error 0.32 |
| BUSNA (Mmm) | z value: 0.39, Pr(>|z|): 1 | z value: 0.15, Pr(>|z|): 1 | z value: -0.67, Pr(>|z|): 1 | z value: 1.65, Pr(>|z|): 0.87 | z value: 1.65, Pr(>|z|): 1 | z value: 1.147, Pr(>|z|): 0.92 | z value: 1.3, Pr(>|z|): 0.94 | est.0.25, Std.Error 0.32 |
| PWD (Mmm) | z value: 2.41, Pr(>|z|): 0.38 | z value: 3.95, Pr(>|z|): 0.02 | z value: -0.9, Pr(>|z|): 1 | z value: 1.65, Pr(>|z|): 0.87 | z value: 2.14, Pr(>|z|): 0.93 | z value: 2.45, Pr(>|z|): 0.36 | est.0.25, Std.Error 0.32 | est.0.21, Std.Error 0.32 |
| BUSNA (Mmm) | z value: 1.87, Pr(>|z|): 0.76 | z value: 2.24, Pr(>|z|): 0.5 | z value: 0.38, Pr(>|z|): 1 | z value: 1.42, Pr(>|z|): 0.99 | z value: 0.8, Pr(>|z|): 1 | z value: 1.9, Pr(>|z|): 0.75 | z value: 0.47, Pr(>|z|): 1 | est.-0.44, Std.Error 0.32 |
| PWD (Mmm) | z value: 1.48, Pr(>|z|): 0.94 | z value: 2.1, Pr(>|z|): 0.6 | z value: -0.62, Pr(>|z|): 1 | z value: 0.85, Pr(>|z|): 0.87 | z value: 0.52, Pr(>|z|): 1 | z value: 0.92, Pr(>|z|): 0.42 | z value: 0.09, Pr(>|z|): 0.93 | est.0.17, Std.Error 0.32 |
| BUSNA (Mmm) | z value: 5.17, Pr(>|z|): 0.01 | z value: 1.22, Pr(>|z|): 0.99 | z value: 0.96, Pr(>|z|): 0.52 | z value: 0.28, Pr(>|z|): 0.27 | z value: 0.73, Pr(>|z|): 1 | z value: 1.68, Pr(>|z|): 0.87 | z value: 0.39, Pr(>|z|): 1 | est.0.17, Std.Error 0.32 |
| PWD (Mmm) | z value: 0.48, Pr(>|z|): 1 | z value: -0.26, Pr(>|z|): 1 | z value: -0.16, Pr(>|z|): 1 | z value: 0.65, Pr(>|z|): 0.65 | z value: 3.96, Pr(>|z|): 0.01 | z value: 0.46, Pr(>|z|): 1 | z value: 0.03, Pr(>|z|): 0.25 | est.0.13, Std.Error 0.32 |

Table 3. Post-hoc statistical test for maximum oocysts per gram of feces (Tukey Multiple Comparisons of Means) between each mouse strain and parasite isolate. See Figure 4A.
Figure 4. Resistance, impact on host health and tolerance marginal effects for four inbred mouse strains infected with three *Eimeria* isolates each. Values under bars represent the number of animals for each group. (A) Maximum oocysts per gram of feces used as a proxy for (inverse of) resistance (see Table 3); (B) Impact on host health measured as the maximum weight loss during patent period relative to starting weight (%); (C) Tolerance index measured as \( \log_{10}(\text{maximum relative weight loss} / \text{maximum number of oocysts per gram of feces} + 1e^{-8}) / -8 \) (see Table 7).
Health of *M. m. musculus* is more affected by *E. falciformis* than by *E. ferrisi*

Analysing the weight loss upon infection as a proxy for impact on host health of the full dataset (N = 108), we found statistically significant differences both between the mouse subspecies (LRT: G = 10, df = 2, P < 0.01) and between the parasite species (LRT: G = 18.6, df = 2, P < 0.001). Post-hoc multiple comparison tests showed that Mmd lost less weight than Mmm when infected by *E. falciformis* (9.3% vs 18.7%), and Mmm lost more weight when infected by *E. falciformis* than by *E. ferrisi* (Table 4, Figure 3B).

Then we modelled maximum weight loss separating *Eimeria* by isolates (instead of species) and mice by strains (instead of subspecies). We found differences between parasite isolates (LRT: G = 30.7, df = 8, P < 0.001) and mouse strains (LRT: G = 23, df = 9, P < 0.01). Notably, PWD (Mmm) mice infected with Brandenburg64 (*E. ferrisi*) lost significantly more weight than STRA mice (Mmd) infected with the same isolate, following the pattern described at the mouse subspecies-parasite species level (Mmd losing less weight than Mmm when infected by *E. ferrisi*). Overall, we did not find any significant difference between mouse strains of the same subspecies within a given parasite species infection or between parasite isolates of the same species within a given mouse strain (Table 5, Figure 4B).
Table 4. Post-hoc statistical test for maximum weight loss (Tukey Multiple Comparisons of Means) between each mouse subspecies and parasite species. See Figure 3B.

| Mouse subspecies | Eimeria species | Eimeria ferri | Eimeria falciformis | Est. | Standard Error | Est. | Standard Error | Est. | Standard Error | Est. | Standard Error |
|------------------|-----------------|--------------|-------------------|------|----------------|------|----------------|------|----------------|------|----------------|
| Mus musculus domesticus (Mmd) | Eimeria ferri | 0.38 | 0.2 | 0.27 | 0.14 | 1.03 | 0.2 |
| Eimeria falciformis | z value: 1.84 | Pr(|z|) = 0.25 | 1.01 | 0.25 | 1.76 | 0.2 |
| Mus musculus musculus (Mmm) | Eimeria ferri | 1.89 | 0.23 | 0.5 | 0.96 | 0.76 | 0.2 |
| Eimeria falciformis | z value: 5.12 | Pr(|z|) < 0.001 | 2.62 | 0.04 | 3.76 | 0.001 |

Table 5. Post-hoc statistical test for maximum weight loss (Tukey Multiple Comparisons of Means) between each mouse strain and parasite isolate. See Figure 4B.
**M. m. musculus** is less tolerant to *E. falciformis* than to *E. ferrisi*

Combining resistance and the impact on health, we assessed tolerance of the mouse subspecies to both *Eimeria* species on the 99 mice for which this index could be calculated. Tolerance index was found to differ significantly between the mouse subspecies (LRT: $G = 18.6$, df = 2, $P < 0.001$), between the two parasite species (LRT: $G = 14.9$, df = 2, $P < 0.001$). Significant were also interactions between mouse subspecies and parasite species (LRT: $G = 13.1$, df = 1, $P < 0.001$).

Post-hoc multiple comparison tests showed that tolerance index upon infection with *E. falciformis* was higher for Mmd than for Mmm. Within Mmm subspecies, animals had a lower tolerance index to *E. falciformis* than to *E. ferrisi* (**Table 6; Figure 3C**). Testing at the level of *Eimeria* isolates and mouse strains, we found between-parasite isolates differences (LRT: $G = 24.1$, df = 8, $P < 0.01$), between-mouse strains differences (LRT: $G = 35.1$, df = 9, $P < 0.001$), and interaction between the two factors (LRT: $G = 19.9$, df = 6, $P < 0.01$). Post-hoc multiple comparison tests showed statistically significant differences between PWD infected with Brandenburg88 against both Mmd strains infected with the same parasite isolate, and against animals of the same strain infected with Brandenburg64. Overall, we did not find any significant difference in tolerance between mouse strains of the same subspecies within a given parasite species infection, or between parasite isolates of the same species within a given mouse strain (**Table 7; Figure 4C**). These results indicate that the lower tolerance of Mmm to *E. falciformis* compared to Mmd is consistent at the strain level.
### Table 6. Post-hoc statistical test for tolerance index (Tukey Multiple Comparisons of Means) between each mouse subspecies and parasite species. See Figure 3C.

| Mouse subspecies | Eimeria species | Brandenburg39 (E. ferriš) | Brandenburg88 (E. fałciformis) | SCHUNT (Mmd) | STRA (Mmd) | BUSNA (Mmm) | PWD (Mmm) | Brandenburg39 (E. ferriš) | Brandenburg88 (E. fałciformis) | SCHUNT (Mmd) | STRA (Mmd) | BUSNA (Mmm) | PWD (Mmm) |
|------------------|-----------------|---------------------------|-------------------------------|--------------|------------|-------------|-----------|---------------------------|-----------------------------|--------------|------------|-------------|-----------|
| Mus musculus domesticus (Mmd) | Eimeria ferriš | est:0.02, Std.Error:0.02 | est:0.03, Std.Error:0.02 | est:-0.01, Std.Error:0.01 | est:-0.11, Std.Error:0.03 | |
| Eimeria fałciformis | z value: 1.02, Pr(>|z|): 0.72 | est:0.02, Std.Error:0.02 | |
| Mus musculus musculus (Mmm) | Eimeria ferriš | z value:-0.76, Pr(>|z|): 0.87 | |
| Eimeria fałciformis | z value:-1.55, Pr(>|z|): 0.4 | |

### Table 7. Post-hoc statistical test for tolerance index (Tukey Multiple Comparisons of Means) between each mouse strain and parasite isolate. See Figure 4C.

| Mouse strains | Parasite isolates | Brandenburg39 (E. ferriš) | Brandenburg88 (E. fałciformis) | SCHUNT (Mmd) | STRA (Mmd) | BUSNA (Mmm) | PWD (Mmm) | Brandenburg39 (E. ferriš) | Brandenburg88 (E. fałciformis) | SCHUNT (Mmd) | STRA (Mmd) | BUSNA (Mmm) | PWD (Mmm) |
|---------------|-------------------|---------------------------|-------------------------------|--------------|------------|-------------|-----------|---------------------------|-----------------------------|--------------|------------|-------------|-----------|
| SCHUNT (Mmd)  | Eimeria ferriš     | z value:-2.01, Pr(>|z|): 0.06 | z value:-3.01, Pr(>|z|): 0.07 | est:-0.02, Std.Error:0.02 | est:-0.03, Std.Error:0.02 | est:-0.01, Std.Error:0.01 | est:-0.11, Std.Error:0.03 | z value:-3.01, Pr(>|z|): 0.07 | z value:-2.01, Pr(>|z|): 0.06 | |
| Eimeria fałciformis | z value:-3.01, Pr(>|z|): 0.07 | z value:-2.01, Pr(>|z|): 0.06 | |
| STRA (Mmd)    | Eimeria ferriš     | z value:-1.52, Pr(>|z|): 0.07 | z value:-3.01, Pr(>|z|): 0.07 | est:-0.02, Std.Error:0.02 | est:-0.03, Std.Error:0.02 | est:-0.01, Std.Error:0.01 | est:-0.11, Std.Error:0.03 | z value:-3.01, Pr(>|z|): 0.07 | z value:-1.52, Pr(>|z|): 0.07 | |
| Eimeria fałciformis | z value:-2.01, Pr(>|z|): 0.06 | |
| BUSNA (Mmm)   | Eimeria ferriš     | z value:-1.52, Pr(>|z|): 0.07 | z value:-3.01, Pr(>|z|): 0.07 | est:-0.02, Std.Error:0.02 | est:-0.03, Std.Error:0.02 | est:-0.01, Std.Error:0.01 | est:-0.11, Std.Error:0.03 | z value:-3.01, Pr(>|z|): 0.07 | z value:-1.52, Pr(>|z|): 0.07 | |
| Eimeria fałciformis | z value:-2.01, Pr(>|z|): 0.06 | |
| PWD (Mmm)     | Eimeria ferriš     | z value:-1.52, Pr(>|z|): 0.07 | z value:-3.01, Pr(>|z|): 0.07 | est:-0.02, Std.Error:0.02 | est:-0.03, Std.Error:0.02 | est:-0.01, Std.Error:0.01 | est:-0.11, Std.Error:0.03 | z value:-3.01, Pr(>|z|): 0.07 | z value:-1.52, Pr(>|z|): 0.07 | |
| Eimeria fałciformis | z value:-2.01, Pr(>|z|): 0.06 | |
Coupling of resistance and tolerance differs between *Eimeria* species

To test coupling between resistance and tolerance of the mouse subspecies within each of the parasite species, we plotted the mean maximum relative weight loss on maximum oocysts per gram of feces (Figure 5). High tolerance to a given parasite species means that the weight is lowly affected even in case of high parasite load, which corresponds to the lower right corner of the plot, and inversely the upper left corner represents low tolerance. We see that for *E. falciformis*, there is a trade-off between resistance and tolerance, with high tolerance-low resistance for Mmd, and high resistance-low tolerance for Mmm. In the case of *E. ferrisi*, resistance varies between both mouse subspecies, but tolerance does not vary consequently, showing a lack of coupling between resistance and tolerance for this parasite.

Figure 5. Coupling between resistance and tolerance for two different *Eimeria* species. Resistance is approximated as the inverse of maximum oocysts per gram of feces, impact on host health as maximum relative weight loss. The upper left corner represents the low tolerance area (strong impact on health despite low parasite load), the lower right the high tolerance area. There is a trade-off between resistance and tolerance between each mouse subspecies upon infection with *E. falciformis*, absent in the case of *E. ferrisi.*
Discussion

In this study, we used a controlled infection experiment to test whether two closely related parasites differ in their impact on their hosts – house mice. For this purpose, we assessed resistance and tolerance to *E. ferrisi* and *E. falciformis* and their effect on health in four different wild-derived strains from two mouse subspecies hybridising in Europe. The Western mouse (*M. m. domesticus*, Mmd) is more resistant to *E. ferrisi* than to *E. falciformis*; The Eastern mouse (*M. m. musculus*, Mmm) is more tolerant to *E. ferrisi* than to *E. falciformis*. We found tolerance to be decoupled from resistance against *E. ferrisi*. The two types of responses against *E. falciformis* were negatively correlated, suggesting a trade-off between resistance and tolerance for this parasite. While resistance decreases parasite fitness and prevalence in natural populations, tolerance generally has no impact on parasite fitness and either increases or does not affect prevalence (Miller, White, & Boots, 2005; Roy & Kirchner, 2000). This allows speculation on host-parasite co-evolution for both *Eimeria* species.

*E. ferrisi* commits to sexual reproduction after a relatively short time with few cycles of asexual expansion (Al-khlifeh et al., 2019; Ankrom et al., 1975). As *E. ferrisi* infections do not reach extremely high intensities with this infection strategy, high tolerance might be the optimal strategy for both house mouse subspecies. Resistance could then evolve relatively freely without any major impact of the parasite on the hosts’ health. Enhanced virulence (reduction of host fitness upon infection e.g. due to prolonged asexual replication before commitment to sexual replication and transmission) might not evolve because the low resistance of the host already allows an optimal transmission rate, especially considering the fast production of transmission stages (Anderson & May, 1982). A global optimum of high tolerance might also be the reason why no subspecies-specific adaptation of Mmd or Mmm
infecting strains, i.e. increased tolerance of matching host-parasite pairs, could be detected in this parasite species.

*E. falciformis* has a relatively long life cycle (Al-khlifeh et al., 2019; Haberkorn, 1970). This means that parasites multiply asexually for a relative long time leading to potentially higher tissue loads and – once it starts to reproduce sexually – extremely high reproductive output in strongly impacted hosts. Therefore, tolerance of this parasite might, on the one hand, lead to prohibitively high intensities if the parasite is allowed to expand asexually and damage the tissue (Ehret et al., 2017) without enough resistance. On the other hand, immunopathology has been observed in advanced *E. falciformis* infections. For example, proinflammatory T cell mediators have been shown to decrease parasite load but increase body weight loss upon infection (Stange et al., 2012). This might lead to multiple different optima for resistance and tolerance (Råberg et al., 2007). In this context, two alternative response strategies against *E. falciformis* might have evolved and stabilised in the house mouse subspecies: while Mmm rather resists *E. falciformis*, Mmd tends to tolerate it.

Instead of such more or less stable optima in the two mouse subspecies we could speculate two related alternative explanations. Firstly, *E. falciformis* could originally be a Mmd parasite dissipated into Mmm territory by a spillover through the hybrid zone. As an argument against this explanation, no significant difference in *E. falciformis* prevalence at each side of the hybrid zone have be observed (unpublished data). Secondly, the *E. falciformis* isolate Brandenburg88 employed here was taken close to the hybrid zone center but from a predominantly Mmd mouse (hybrid index 0.2). The isolate could hence be adapted to Mmd. Experiments with an additional *E. falciformis* isolate from Mmm are needed to answer the question whether host subspecies adaptation can lead to tolerance in matching pairs of *E. falciformis* and mouse subspecies.
Resistance and tolerance to parasites are highly relevant to the house mouse hybrid zone. As a so-called tension zone, this zone is maintained by a balance between dispersal and endogenous selection against hybrids (Barton & Hewitt, 1985; Macholán et al., 2007; Payseur, Krenz, & Nachman, 2004; Raufaste et al., 2005). It has been shown that hybrid mice are more resistant not only to *Eimeria* but also to other parasites including pinworms (Baird et al., 2012; Balard et al., 2019). Impact on tolerance could not be measured under natural conditions (Balard et al., 2019). The effect of parasites on hosts' fitness in particular and the role they can play in the evolution of species barriers is thus still rather ambiguous. We here show that it is indispensable to measure both resistance and tolerance in *Eimeria* infections of house mice. Such measurements can be made in future laboratory experiments involving hybrid mice.

The contrast between two different *Eimeria* spp. invites future research on the relationship between infection intensity, parasite reproductive output, host health and immune response. This might allow us to better understand both the process and mechanisms of the evolution of tolerance and resistance in the context of hybrid hosts and beyond.

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