Hybrid resistance to pathogenic coccidia Eimeria spp. in the European House Mouse Hybrid Zone

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# Abstract

Parasite infections have been suggested to modulate the fitness of hybrid hosts, either limiting or facilitating hybridization. To test hybrid susceptibility vs. hybrid vigour, we assess intracellular infections by *Eimeria*, a parasite with high pathogenicity, and test its intensity in hybrids and pure *Mus musculus* subspecies in a novel transect of the European House Mouse Hybrid Zone. We find lower abundance of *Eimeria* in hybrid hosts. Additionally we show that parasite intensities in infected hybrid hosts are lower than in pure genotypes largely excluding ecological epidemiological factors. We thus show hybrid vigour against this parasite. Using the same approaches in our new transect we additionally reproduce findings of hybrid vigour against pinworm infections. Lower impact of parasites on hybrid hosts health could offset reductions in fertility in house mouse hybrids. Improved health could counterbalance reductions in other fitness components and thereby reduce the “net strength” of isolating speciation barriers.

# Introduction

The prevalence of hybridization and gene introgression between species might be underestimated by biologists using the -otherwise often immensely helpful- biological species concept of reproductively isolated populations (Mayr 1942). Mallet, however, suggested that hybridization occurs in more than 10% of animal species and 25% of plant species (Mallet 2005) and our own species hybridised recently (Green et al. 2010).

Hybrid zones, areas where individuals of different species meet and hybridise, can be studied over decades and allow to infer the impact of the different endogenous and exogenous forces at play in this process. The European House Mouse Hybrid Zone (HMHZ) is a tension zone characterized by selection against hybrids replaced by immigrating less admixed mice (Barton and Hewitt 1985). The two house mouse sub-species *Mus musculus domesticus* and *Mus musculus musculus* (hereafter Mmd and Mmm) have entered into this secondary contact around 5000 years ago as a result of their colonization of Europe during the Iron age following the spread of human agriculture (Boursot et al. 1993, Macholán et al. 2012). After 500 000 years of allopatric divergence house mice can be found nowadays separated by the 20 km-wide and 2500 km-long HMHZ, stretching from Scandinavia in the North, to the Black Sea in the South (Macholán et al. 2007, Jones et al. 2010). The HMHZ is barrier with limited permeability to gene flow between mouse taxa (Macholán et al. 2011). The main selective forces acting against hybrids are thought to be endogenous rather than ecological (Macholán et al. 2007), mainly acting on sperm-related traits (Albrechtová et al. 2012).

The HMHZ was one of the first animal hybrid zones studied for the role of parasitism on species barriers (Sage et al. 1986). Parasites by definition decrease their hosts’ fitness (Poulin 2006). One can thus suppose that parasitism has an influence on the hybridization process, if resistance to parasites differs between hybrid and pure hosts (Fritz et al. 1999).

Nevertheless the intensity of a particular parasite infection is not necessarily correlated with a fitness decrease. Indeed, animals tolerant for lowly pathogenic parasites might not see their fitness decrease with higher parasitemia. This could be the case if the parasite is beneficial for the host in interactions with other parasites (Heitlinger et al. 2017) or if immune reactions against it are costly in relation the harm it causes. Besides, as a foundation of the “Old Friend” (or “Hygiene”) hypothesis, the constant presence of helminths in (not only human) populations has lead to the evolution of a background basal release of regulatory cytokines (Rook 2009) which might in turn impact the outcome of more pathogenic infections. Having considered these cases, one can still wonder if parasitic infections are positively or negatively influencing the spread of hybrid genotypes. This question was studied for decades in the HMHZ as host model, initially considering helminths as parasite model.

Parasite infections were originally thought to prevent hybridization in house mice, as hybrid susceptibility to helminths was believed to be observed in field and experimental studies (Sage et al. 1986, Moulia et al. 1991, Moulia et al. 1993). Infection experiments using the protozoan *Sarcocystis muris* lead to similar conclusion (Derothe et al. 2001) of hybrid susceptibility. This phenomenon was explained by the perturbation of co-adapted host immune gene complexes in recombinant hybrids. These findings came to be questioned when laboratory experiments showed either no hybrid effect on helminth load, or even reduced load in hybrids compared to pure strains (Moulia et al. 1995, Derothe et al. 2004). Moreover, in 2012, Baird et al. provided evidence of hybrid vigour in the HMHZ in response to helminths infection (especially the pinworms *Aspiculuris tetraptera* and *Syphacia obvelata* and the whipworm *Trichuris muris* (Baird et al. 2012)). Design of field studies preceding Baird et al. 2012 was limited: low sample size and long maintenance of mice in laboratory before assessment of parasite burden (Baird et al. 2012) might have introduced bias. For parasites other than helminths, laboratory infections with the protozoan *Trypanosoma musculi* did not show significant difference of parasite load between hybrid mice and pure Mmd or Mmm (Derothe et al. 1999).

Several factors could explain these discrepancies. In 1999, Derothe et al. proposed the “parasite constraint” hypothesis (Derothe et al. 1999). It states that a parasite should exert sufficient selective pressure on the host to lead to host hybrid susceptibility, as co-adapted complexes would be selected in hosts on this basis.

Pinworms (oxyurids) have been shown to be the most prevalent helminths infecting house mice in the HMHZ (Baird et al. 2012). They are broadly distributed, can reinfect their host all along their lives and can be considered close to non-pathogenic (Taffs 1976). Following Derothe’s reasoning it is arguable whether pinworms would apply strong enough selection pressure on their hosts to induce hybrid susceptibility according to the parasite constraint hypothesis. Therefore it is questionable whether pinworms should be the only parasite to test the parasite constraint hypothesis in natural systems.

In an attempt to address the inconsistencies we selected a parasite model that is likely to exert selective pressure on its host, the intracellular coccidia from genus *Eimeria*. Different *Eimeria* spp. infect the intestinal epithelial cells of several vertebrates and provoke symptoms such as weight loss and diarrhoea in mice. Infection experiment in laboratory (NMRI) mice showed a weight loss up to 20% following infection with oocysts isolated from mice captured in the HMHZ (Al-Khlifeh et al. (under preparation)). *Eimeria* sp. are often considered host-specific, with several thousand species parasitising different vertebrates (Haberkorn 1970, Chapman et al. 2013). In the European HMHZ, three *Eimeria* species have been identified so far in mice: *E. ferrisi*, *E. falciformis* and *E. vermiformis*. The three species have a distribution throughout the HMHZ with a prevalence of 20 to 30% (Jarquin et al (under preparation)). In populations of bank voles, *Eimeria* spp. has been shown to reduce breeding success (Hakkarainen et al. 2007) and overwinter survival of deer mice (Fuller and Blaustein 1996) is affected. Moreover, *Eimeria* spp. intensity was found positively correlated with bank vole’s age, indicating re-infection throughout life (Winternitz et al. 2012). For all these reasons, we consider *Eimeria* a good candidate to question the parasite constraint hypothesis.

We assessed *Eimeria* infection in a novel transect of the HMHZ just outside of Berlin in the North-East of Germany. We test the impact of hybridization on both abundance and intensities of this parasite.

# Material & Methods

## Sampling

House mice (*Mus musculus*) were trapped in Brandenburg (Germany) in September from 2014 to 2017, using live traps placed into farms or houses. The considered transect ranges from 51.68 to 53.29 degrees of latitude (200 km) and from 12.52 to 14.32 degrees of longitude (140 km). At this period of the year it is possible to catch a high number of mice along the hybrid zone, and sampling at the same season every year reduces potential seasonal variability (Haukisalmi et al. 1988, Abu-Madi et al. 2000). Sampling was planned to maximize capture of the full range of mice genotypes. Mice individually isolated in cages were euthanized by isoflurane inhalation followed by cervical dislocation and dissected within 24 hours after capture (animal experiment permit No. 2347/35/2014). Mouse tissue samples (muscle and spleen) were collected in liquid nitrogen then stored at -80°C for host genotyping. Digestive tracts were dissected and ileum, cecum and colon tissues stored separately in liquid nitrogen followed by conservation at -80°C.

## Host genotyping

The strength of hybridization was estimated for each mouse as a value of hybrid index (HI). HI is calculated as proportion of Mmm alleles within a set of four to fourteen biallelic (ten or more for 92% of the mice) diagnostic markers fixed as either Mmd or Mmm. This set consists in one mitochondrial marker (mtBamH, restriction site in mitochondrial Nd1 gene which discriminates between mice subspecies (Munclinger et al. 2002, Božíková et al. 2005, Dureje et al. 2012)), one Y-linked marker (presences/absence of insertion in Zfy2 gene (Nagamine et al. 1992, Boissinot and Boursot 1997, Dureje et al. 2012)), six X-linked marker (three short interspersed nuclear elements, namely Btk, Tsx (Munclinger et al. 2003) and Syap1 (Macholán et al. 2007), and three SNPs, namely X332, X347 and X65 (Payseur et al. 2004, Dufková et al. 2011, Dureje et al. 2012)), and six autosomal markers (Es1, Gpd1, Idh1, Mpi, Np, Sod1) (Bonhomme et al. 1984, Munclinger et al. 2002, Macholán et al. 2007, Dureje et al. 2012). HIs range from 0 to 1, HI of 0 indicating a pure Mmd and HI of 1 a pure Mmm (Wang et al. 2011, Baird et al. 2012).

A map delimiting the probabilistic separation between both sub-species was computed using the program Geneland with some graphical modifications using a subset of six autosomal markers genotyped in all mice (Es1, Gpd1, Idh1, Mpi, Np, Sod1). Geneland uses a Markov chain Monte Carlo (MCMC) approach to combine both geographical and genetic information (Guillot et al. 2005). Number of clusters was set to 2, 10000 MCMC iterations were performed and saved every 100 iterations (100 iterations saved). First five iterations were discarded as burn-in, and the resolution of the map was set to 100 pixels for both x and y axes.

## Parasite loads estimation

Mice digestive tracts were dissected and inspected for helminth presence with a binocular microscope. Helminths were counted and stored in 70% ethanol for later identification by molecular analysis and in 3.5% formalin for later morphological comparison with species descriptions. In this study we considered only the most prevalent helminths:the oxyurids *Syphacia obvelata* and *Aspiculuris tetraptera*.

DNA was extracted from ileum and cecum tissues and quantitative PCR (qPCR) were completed to estimate *Eimeria* spp. load. DNA extraction was performed using the innuPREP DNA Mini Kit (Analytik Jena AG, Jena, Germany) following the instructions of the manufacturer with additional mechanical tissue disruption with liquid nitrogen in a mortar. Both quality and quantity of isolated DNA were measured by spectrophotometry in a NanoDrop 2000c (Thermo Scientific, Waltham, USA). *Eimeria* spp. presence was tested by qPCR to detect intracellular stages of the parasite as well as house mouse nuclear genome as internal reference. Primers used for *Eimeria* spp. detection targeted a short mitochondrial COI region (Eim\_COI\_qX-F: TGTCTATTCACTTGGGCTATTGT; Eim\_COI\_qX-R: GGATCACCGTTAAATGAGGCA), while *Mus musculus* primers targeted the CDC42 nuclear gene (Ms\_gDNA\_CDC42\_F: CTCTCCTCCCCTCTGTCTTG; Ms\_gDNA\_CDC42\_R: TCCTTTTGGGTTGAGTTTCC). Reactions were performed using 1X iTaqTM Universal SYBRⓇ Green Supermix (Bio-Rad Laboratories GmbH, München, Germany), 400 nM of each primer and 50 ng of DNA template in 20 µL final volume. Cycling amplification were carried out in a MastercyclerⓇ RealPlex 2 (Eppendorf, Hamburg, Germany) according to the following amplification program: 95°C initial denaturation (2 min) followed by 40 cycles of 95°C denaturation (15 s), 55°C annealing (15 s) and 68°C extension (20 s). Melting curve analyses were performed in order to detect primer dimer formation and unspecific amplification. ΔCt was calculated as difference of threshold cycle (Ct) between mouse and *Eimeria* spp. values. Samples with a ΔCt higher than -5 for at least one of the two intestinal tissue were considered positive (Jarquin et al (under preparation)), and in case of a detection in both tissues, the higher value was taken as a proxy of individual parasite load. This parasite load of intestinal tissue stage is expressed in “ΔCt Eimeria-host” throughout this paper. For statistical modelling 5 was added to “ΔCt Eimeria-host” values so that negative samples present a load of zero and positive samples a load > 0.

## General parasite assessment

For each parasite and detection method, abundance (mean parasite load of all hosts) and intensity (mean parasite load of infected hosts) were estimated (Rózsa et al. 2000). Bias-corrected and accelerated bootstrap confidence intervals as proposed by Efron and Tibshirani (Efron and Tibshirani 1993) were computed with 1000 bootstrap to account for skewness of the distribution of parasite loads. Prevalence (number of infected individuals amongst all tested individuals) was calculated and confidence intervals obtained with Sterne’s exact method (Sterne 1954, Reiczigel et al. 2010). Calculations were performed using the software R (R Development Core Team 2008) and the epiR package (Nunes et al. 2018).

## Statistical test of hybridization effect

Following the approach of Baird et al., we tested if hybrid mice had higher or lower parasite load than expected in case of load intermediate between that of pure hybridizing taxa (“additivity”, Baird et al. 2012). The hybridization effect on each individual is modelled by the degree at which new gene combinations are brought together compared to the pure sub-species. Following Barton’s approach (Szymura and Barton 1986, Macholán et al. 2011) this was estimated from the hybrid index using the function for expected heterozygosity:   
The estimated parasite load for one given HI was expressed as follows:

were L1 is the parasite load of pure Mmd, L2 the parasite load of pure Mmm, and alpha the hybridization effect. We considered four nested hypotheses increasing in complexity, and compared them with a G-test (likelihood ratio test) to consider a more complex hypothesis only when justified by a significant increase in likelihood. Expected parasite load is fixed to be identical for both subspecies and both host sexes in hypothesis H0. The more complex H1 introduces load differences for the host sexes. H2 allows loads to be different between subspecies at the extremes of the hybrid index. H3, finally, combines differences between pure subspecies and host sexes.

Adequate distributions of values for each parasite and detection method considered were selected using log likelihood and AIC criteria and by comparing goodness-of-fits plots (density, CDF, Q-Q, P-P) (R packages MASS (Venables and Ripley 2002) and fitdistrplus (Delignette-Muller and Dutang 2015)). Negative binomial distribution should perform well for macroparasite counts (Crofton 1971, Shaw and Dobson 1995), which was confirmed for helminths. Values of (Δct Eimeria-host +5) were found be well described by a Weibull distribution.

The Negative Binomial distribution is parameterized by two arguments: its expectation (Expected Load from equation 2), and the inverse of its aggregation defined as:

Z being the deviation from the additive model, in proportion to He, which is maximal at the zone center (Baird et al. 2012). The Weibull distribution is parametrized by its shape (allowed to vary freely during maximum likelihood estimation) and its scale parameter defined as:

We fit models by maximum likelihood (using R package mle2 (Bolker and Team 2017)). The parasite load was estimated including a hybridization effect parameter or not (by setting HI = 0 in Expected Load), and we compared these two models using a G-test (maximum likelihood statistical significance test).

All graphics were produced using R package ggplot2 (Wickham 2016), R package ggmap (Kahle and Wickham 2013), and compiled using the free software inkscape.

# Results

## Host genotyping and characterisation of the HMHZ in a novel transect

A total of 660 mice were caught and genotyped during four sampling seasons (2014: N=87; 2015: N=163; 2016: N=167; 2017: N=243) in 157 farms and houses in the North-East of Germany. Overall, 4.2 mice were caught on average per locality (95% CI: [3.8, 4.6]). To estimate the amount of hybridization in each individual we estimated a hybrid index as the proportion of Mmm alleles per all (four to fourteen) genotyped alleles. A table containing hybrid indices, georeference and parasite load for each individual is available in supplementary material (table S2). To locate the centre of the HMHZ we constructed a probabilistic map of the hybrid zone centre in this transect using six autosomal markers genotyped in all mice (Es1, Gpd1, Idh1, Mpi, Np, Sod1). Describing the HMHZ centre in detail from South to North, it first makes a small bent to go in the East around the city of Berlin. Further North it turns back take a more North-Westward direction running towards the “Müritz” area through large forests with few small villages in between (figure 1).

## Parasite prevalence and intensity

To investigate *Eimera* infections we assessed 384 mice sampled in 2016 and 2017 for the presence and intensity of tissue stages. The parasite had a prevalence of 18.2% (70/384) (Sterne’s Exact method CI 95%: [14.5, 22.5]). To quantify the intensity of infection we determined the amount of *Eimeria* mitochondrial DNA per host nuclear DNA. We use ΔCt Eimeria-host, the difference in “threshold cycle” (the cycle at which quantitative PCR reaches fluorescence threshold) to express *Eimeria* intensity. “ΔCt Eimeria-host” corresponds to a log2 ratio between parasite and host DNA. The average *Eimeria* abundance was of -4.4 (95% CI: [-4.6, -4.2]) corresponding to 21.1 (95% CI: [18.4, 24.2]) times more parasite mitochondrial DNA than host nuclear DNA; The mean intensity was of -1.8 (95% CI: [-2.3, -1.1]) corresponding to 21.1 (95% CI: [18.4, 24.2]) times more parasite mitochondrial DNA than host nuclear DNA.

Between 2014 and 2017, 585 mice were investigated for helminths. Prevalence of pinworms in the transect was 52.5% (307/585) (Sterne’s Exact method CI 95%: [48.4, 56.5]) with an average abundance of 18.7 (95%CI: [15.5, 23.3]) pinworms per mouse and intensity of 35.7 (95%CI: [29.6, 44.2]) pinworms per infected mouse (maximum number of pinworms in one host: 489).

## Resistance against *Eimeria* spp. is elevated in hybrid mice compared to pure strains

*Eimeria* spp. tissue stage intensity was modelled with a Weibull distribution of errors, along the hybrid index. Our most basic model (H0) containing no load differences between Mmd and Mmm pure subspecies and between host sexes was found to fit our observations significantly better than more complex models including those factors (supplementary table S1). The fit integrating a hybridization effect showed significantly higher likelihood than a model without hybridization effect (G-test; p-value < 0.001). Hybrids have significantly lower load of *Eimeria* spp. tissue stages (a hybridization effect parameter of 0.86) than expected if load was linear along the hybrid index (Fig. 2a, values of parameters of the fitted model given in table 1).

A reduced abundance (strength of infection in both infected and uninfected hosts) of *Eimeria* spp. (and of any parasite) could be explained by either ecological and epidemiological factors or by host-intrinsic factors: resistance. To test more specifically the resistance of hybrids compared to pure mice, we considered only individuals infected by *Eimeria* spp. tissue stages (N = 70). Again the model containing no differences between Mmd and Mmm taxa and between host sexes was also found to fit significantly better our observations than more complex models (supplementary table S1). The fit integrating a hybridization effect showed significantly higher likelihood than the model without it (G-test; p-value = 0.026). Infected hybrids have significantly lower load of *Eimeria* spp. tissue stages than expected if the load was linear along hybrid index, with a hybridization effect parameter alpha of 0.88 (Fig. 2b, values of parameters of the fitted model given in table 1).

## Hybrid vigour against pinworms and comparisons of it’s strength with previous reports

We modelled pinworm abundance with a negative binomial distribution of errors along the hybrid index. The most complex model tested (H3) including a load difference between Mmd and Mmm pure subspecies and between host sexes was found to fit our observations significantly better than the less complex models (table 1) in this case. For both sexes, the model including a hybridization effect showed significantly higher likelihood than the model without (G-test; p-value < 0.01 for both males and females). Hybrids have significantly lower pinworms load than expected if the load was linear along the hybrid index, with a hybridization effect parameter alpha of 1.11 (females) and 1.33 (males) (Fig. 3a, values of parameters of the fitted model given in supplementary table S1).

To compare the strength of hybridization effect between our Brandenburg transect and the Czech-Bavarian transect investigated by Baird et al., we considered the selected hypothesis for pinworms in this former study, H1 (taxon differences but no sex differences). We compared the model fitted to our data (table 1) to a model fitted on the same data, optimized with a fixed hybridization parameter of 1.39 (corresponding to the alpha parameter of Baird et al. 2012 for pinworm abundance). Comparison between the models with freely varying alpha and restricted alpha did not show any significant differences in likelihood (G-test; p-value = 0.25). Therefore we conclude that the strength and direction of hybrid vigour are coherent with results in the previously studied Czech–Bavarian transect of the HMHZ.

## Resistance against pinworms is elevated in hybrid mice compared to pure strains

To exclude ecological and epidemiological explanation also for pinworms we again tested the resistance of hybrids compared to pure mice in our Brandenburg transect, we considered only individuals infected by pinworms (N = 307). Model H3 integrating a difference between taxons and sexes was found to fit our observations significantly better than lower models of lower (supplementary table S1). For both sexes, the fit including a hybridization effect showed significantly higher likelihood than the model without it (G-test; p-value = 0.04 for females, p-value < 0.01 for males). Infected hybrids have significantly lower pinworm load than expected if the load was linear along hybrid index, with a hybridization effect parameter alpha of 0.89 (females) and 1.43 (males) (Fig. 3b, values of parameters of the fitted model given in table 1).

# Discussion

We tested hybrid vigour vs. susceptibility analysing infections of house mice with the intracellular parasites *Eimeria* spp. in a novel transect of the HMHZ. We found a positive effect of hybridization (that is, hybrid vigour) both when considering all host (parasite abundance) or infected hosts only (parasite intensity).

While the HMHZ is one of the best studied animal hybrid zone, conflicting results on the impact of parasitism on hybridization emerging from several studies emphasize the importance of studying multiple parasites, multiple time points and multiple areas (Theodosopoulos et al. 2018).

To our knowledge no previous study tested an effect of hybridization in mice on other than helminth infections using field sampling in the HMHZ. To quantify the intensity of *Eimeria* infections, we used as a quantitative PCR as proxy for parasite load. Such qPCR data of intestinal parasites reflects the intensity of infection more accurately than traditional coprological (flotation) techniques (Nolan et al. 2015, Jarquin et al (under preparation), Al-Khlifeh et al. (under preparation)), we additionally argue that the intensity of tissue stages is a better proxy for host health than the intensity of reproductive stages of the parasite detected in faeces. Moreover, we chose mitochondrial primers for *Eimeria* detection: the high copy number of mitochondria per cell in *Eimeria* (180 predicted in *Eimeria falciformis* (Heitlinger et al. 2014)) guarantee a high sensitivity of this technique.  
House mouse hybrids are thoroughly admixed (“late-generation”) (Macholán et al. 2007) and therefore should not be considered in categories, but rather on a continuous scale. We adapted the statistical analysis of Baird et al. (Baird et al. 2012) and explicitly model the effect of hybridization on parasite load by approximating the amount of recombined alleles using expected heterozygosity. We use this to derive non-linear predictions for hybridization effect based on the observed hybrid index. To increase reproducibility we make our analysis available in an R package (Balard and Heitlinger 2019).

Different measures of response to parasites can be chosen (Fritz et al. 1999), including two different measures of load: in this work we consider both parasite abundance (parasite load amongst all potential hosts) and parasite intensity (parasite load amongst infected hosts). Practically this means in- or excluding non-infected hosts from the analysis. The latter might not only reduce problems in statistical inference caused by false negative measurements (so called zero-inflation) but we also address two different hypotheses: (i) are hybrid hosts differentially likely to be infected strongly by parasites than more pure subspecies host? and (ii): do infected host present a different parasite intensity when they are hybrids? We argue here that ii) isolates hybrid resistance from potential ecological and epidemiological determinants. Host density and *Eimeria* spp. prevalence are, for example, negatively correlated in bank voles (Winternitz et al. 2012). Considering that hybrid populations might be less dense (e.g. because of selection against hybrids or the HMHZ falling into areas of low population density due ecological factors (Dureje et al. 2012)) it could be problematic that abundance is logically correlated with prevalence (Morand and Guégan 2000). Therefore we propose to consider only infected individuals for load estimations, arguing that parasite intensity relates more closely to intrinsic resistance of individual hosts.

We replicated the result of Baird et al 2012 (Baird et al. 2012), confirming hybrid vigour in response to pinworm infection. We additionally show that not only pinworm abundance in our novel transect suggests hybrid vigour but also resistance of infected hosts (pinworm intensity excluding uninfected hosts). Following the same logic we apply to *Eimeria* infections this argues for hybrid vigour to be an effect intrinsic to host individuals.

We found that our own results were very similar to the results of Baird et al., both in the direction and strength of the hybridization effect. In this context it is noteworthy that prevalence and abundance were both lower in our Brandenburg transect (respectively 52.5% and 18.7) than in the previously studied Czech-Bavarian transect (70.9% and 39.19) (Baird et al. 2012). Other differences compared to the Baird et al. 2012 study include a significant sex difference for pinworm load in our transect. Ecological differences may apply in different parts of the hybrid zone, as well as over time. The similar strength of hybridization effect in the presence of these epidemiological differences in replicate transects reinforces our confidence hybrid resistance being a host-intrinsic effect instead of by-product of altered epidemiology in the HMHZ.

When results contradictory to the hypothesis of hybrid susceptibility were first found in laboratory experiments (Derothe et al. 2004), Derothe formulated the parasite constraint hypothesis. It states that parasites need to exert high enough selective pressure on their hosts to lead to the evolution of co-adapted gene complexes. Hybridization would then lead to the erosion of these complexes and to Bateson-Dobzhansky-Muller incompatibilities (Orr 1996) in recombinants. In contrast to pinworms, *Eimeria* spp. are both pathogenic and prevalent and can thus be expected to exert a high selective pressure. Hybrid vigour observed in response to this infections with this parasite hereby is evidence against the parasite constraint hypothesis.

Hybrid mice of the HMHZ likely have higher fitness when confronted with parasite infections than hosts of pure subspecies faced with the same infections. Parasitism can therefore be expected to have direct consequences for the strength of the species barrier and the dispersal of hybrids. In the HMHZ male hybrid mice have reduced fertility compared to more pure individuals (Albrechtová et al. 2012) and it is therefore important to stress that hybridization can have different effects on different components of Darwinian fitness: while reproductive fitness can lower in hybrids, e.g. health upon infection and (potentially resulting) survival to adulthood could be higher. While some physiological systems (e.g. reproductive) are more dependent on “co-adapted complexes”, others benefit from diversity. In the HMHZ context, parasitism is likely to increase the “net-fitness” of hybrids and thereby reduce the “net-strength” of isolating barriers. In particular the immune system could fall in this category leading to increased resistance of hybrids to parasites.  
A reduction in the strength of isolating barriers will first and foremost allow introgression of alleles that confer resistance to parasites through the HMHZ. Indeed, cline analyses have found enrichment of immune functions (MHCI-mediated immmunity) in genes at loci introgressing particularly far across the HMHZ (Teeter et al. 2008).

A prime candidate locus for mediating a positive effect of hybridization on the immune system is the major histocompatibility complex (MHC). In mice two genes of the MHC have been shown to present different levels of polymorphism as well as population structure with many alleles inferred to be shared between subspecies by maintenance of ancestral polymorphism (Čížková et al. 2011). Additionally, the small demes of house mice function as reservoir for MHC alleles, contributing to the diversity of this system across demes and populations (Linnenbrink et al. 2018). Increased MHC diversity does, however, not necessarily correlates with parasite resistance, as studies on hybrid mouse lemur revealed (Sommer et al. 2014). The genetic structure of the MHC and especially polymorphism shared across subspecies should make this locus one among a number of other loci (including Toll-like receptors (Skevaki et al. 2015), Imminity related GTPases (Lilue et al. 2013)) investigated to uncover the mechanism behind hybrid vigour.

Recently, concepts have emerged that provide an alternative view on the susceptibility vs. vigour argument. Instead of resistance against parasites assumed to decrease host fitness (almost by definition), the holobiont concept focusses on incompatibilities between genomes of different organisms. While this concept is widely accepted concerning “interactions” of the bacterial microbiome and the host (Wang et al. 2015) it’s slowly gaining attention in eukaryotes including parasites. The genetic structure of the pinworm *Syphacia obvelata*, for example, matches the HMHZ and hybrid parasites are found associated geographically with the host zone centre (Bellocq et al. 2018). “Co-adapted complexes” could then span the genomes of host and parasite. In a classical parasitological view the parasite would then “fail to target common host genotypes” (Baird et al. 2012) at its own detriment and resulting in hybrid vigour of the host. In a symbiont view “holobiont failure” would be detrimental for both organisms unified in the holobiont. It is not straight forward to derive testable hypotheses and predictions to assess which of these concepts (holobiont or ressistance/susceptibilty) has more explanatory value. One such prediction might be that non-specialist parasites without a hybrid zone population structure (e.g. Trichuris muris, Wasimuddin et al. 2016) should be less likely to be a target of hybrid vigour. Parasites being able to infect hosts other than house mice and showing no population structure in the HMHZ have usually a low prevalence in house mice. This is a motivation for the further investigation of host-specificity and population structure of *Eimeria* spp. (Jarquin et al (under preparation)).

Hybridization with introduced species can threaten autochtonal endangered animals, by diminishing gene pool diversity, producing less adapted offsprings or preventing gene flow (Simberloff 1996). Parasites being omnipresent in natural systems, it is of major concern for conservation biologists to comprehend to which extend the strength of parasitism influences the level of hybridization. Closer to us, Eurasian human immune system seems to have been shaped by interactions and hybridization with other human species, as a result of adaptive introgression in response to pathogens pressure (Abi-Rached et al. 2011). The house mouse is the most studied animal system worldwide. Sub-species gene flow has molded this biomedical model (Yang et al. 2011). We argue that improving our understanding of how hybridisation in the HMHZ is impacted by parasites provides valuable information on the house mouse as the model species with the most thoroughly understood immune system. A transfer of knowledge from this model might help to understand the impact of parasites on the hybridisation of our own species and species relevant for conservation.

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# Ethics statement

Trapping and handling of mice was approved by local authorities (“Landesamt fuer Gesundheit, Umwelt und Verbrauchersschutz Brandenburg”) under permit number 35 –101 2014– 2347.

# Tables

**Table 1. Parametrisation of the selected fitted models**   
Parameters estimated by maximum likelihood for each dataset.

**Supplementary table S1. Full parametrisation of the fitted models and comparison by G-test of the four nested hypotheses**   
H0: same expected load for the subspecies and between sexes; H1: same expected load across sexes, but can differ across subspecies; H2: same expected load across subspecies, but can differ between the sexes; H3: expected load can differ both across subspecies and between sexes.

**Table S2. Hybrid indexes, georeference and parasite load for each individual**

# Figures

**Figure 1. Map of the spatial range of both house mouse subspecies in the European House Mouse Hybrid Zone**. Spatial organisation was infered using six autosomal markers (Es1, Gpd1, Idh1, Mpi, Np, Sod1). Mmd is found in the West of the hybrid zone (blue), Mmm on the East (red). Numbers in the map indicate posterior probabilities of population membership for each mouse sub-species.

**Figure 2. Abundance and intensity of *Eimeria* spp. tissue stage infection** Hybrid index is represented as a gradient ranging from 0 (pure Mmd, in blue) to 1 (pure Mmm, in red). Map of all individuals tested for detection and quantification of *Eimeria* spp. tissue stages and corresponding fitted model (a), and map of positive individuals tested for detection and quantification of *Eimeria* spp. tissue stages and corresponding fitted model (b). The optimised fit is represented by a solid line; 95%CI of the fit is plotted as a grey ribbon, where all parameters are allowed to vary in their 95%CI and likelihood is maximized (upper bound) or minimized (lower bound); 95%CI of the hybridization parameter alpha is plotted as dashed lines, where all parameters are fixed to their fitted value while alpha is allowed to vary in its 95%CI and likelihood is maximized (upper bound) or minimized (lower bound).

**Figure 3. Abundance and intensity of pinworms** Hybrid index is represented as a gradient ranging from 0 (pure Mmd, in blue) to 1 (pure Mmm, in red). Map of all individuals tested for detection and quantification of pinworms count and corresponding fitted model (a), and map of positive individuals tested for detection and quantification of fecal pinworms count and corresponding fitted model (b). Pinworm load given on a log transformed y-axis. Females individual loads and fit are represented in orange, males ones in green. The optimised fit is represented by a solid line; 95%CI of the fit is plotted as a grey ribbon, where all parameters are allowed to vary in their 95%CI and likelihood is maximized (upper bound) or minimized (lower bound); 95%CI of the hybridization parameter alpha is plotted as dashed lines, where all parameters are fixed to their fitted value while alpha is allowed to vary in its 95%CI and likelihood is maximized (upper bound) or minimized (lower bound).

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