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## **Age reduces resistance and tolerance in malaria-infected mice**

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## **Abstract**

Once infected, hosts can rely on two strategies to cope with parasites: fight them (resist the infection) or minimize the damage they induce (tolerate the infection). While there is evidence that aging reduces resistance, how tolerance varies as hosts become old has been barely studied. Here, we used a rodent malaria parasite (*Plasmodium yoelii*) to investigate whether 2- and 12-month old house mice differ in their capacity to resist and tolerate the infection. We found that 12-month old mice harbored higher parasitemia, showing that age reduces resistance to malaria. Infection-induced deterioration of host health was assessed using red blood cell and body mass loss. Using both traits, the rate of decline in host health, as parasitemia increased, was more pronounced in 12- than in 2-month old mice, showing that age is also associated with impaired tolerance to malaria. Overall, resistance and tolerance positively covaried; however, this was only due to the age effect, since, within age classes, the two traits were not correlated. These results show that senescing individuals might be both more susceptible to infectious diseases and less able to cope with the damage that infection induces.

*Keywords:* anemia, immunity, *Plasmodium yoelii*, senescence, virulence

*Abbreviations:* RBC = red blood cells, PBS = phosphate buffered saline, MCV = mean corpuscular volume

## 1. Introduction

Infection with parasites is usually associated with reduced host fitness. In response to this threat, hosts have evolved different strategies aiming at i) avoiding the encounter with infectious agents (i.e. avoidance), ii) limiting parasite proliferation (i.e., resistance), iii) reducing the damage induced by the infection (i.e., tolerance) (Klemme and Karvonen 2017). Since resistance is defined as the host capacity to limit parasite multiplication, it usually involves the deployment of an immune response that can, ultimately, result in the clearance of the infection. On the contrary, tolerance aims at minimizing the damage, and therefore, the fitness cost induced by the infection (Schneider and Ayres 2008; Råberg et al. 2009; Medzhitov et al. 2012). As such, mechanisms of tolerance are manifold, possibly including the regulation of the inflammatory response (Ayres and Schneider 2012; Sonnenberg and Artis 2015), the capacity to detoxify toxins produced during the infection (Seixas et al. 2009; Larsen et al. 2010), the capacity to repair and/or regenerate tissues that have been damaged during the infection (Chang et al. 2004; Soares et al. 2014), glucose and triglyceride metabolism (Wang et al. 2018; Luan et al. 2019; Wang et al. 2019), the microbiome (Palaferri Schieber et al. 2015), hormones (Vandermosten et al. 2018), or thermoregulation (Palaferri Schieber and Ayres 2016). While resistance has been the focus of much attention during the last decades, the study of tolerance in animal hosts has been boosted since the seminal paper by Råberg et al. (2007), and it is now established that tolerance is widespread across different host-parasite systems (Adelman et al. 2013; Sternberg et al. 2013; Sorci 2013; Hayward et al. 2014; Howick and Lazzaro 2014; Mazé-Guilmo et al. 2014; Athanasiadou et al. 2015; Kutzer and Armitage 2016; Decker et al. 2018; Grab et al. 2019; Stehr et al. 2019).

Age is a particularly relevant host trait that likely drives the capacity to resist or tolerate the infection. Aging is associated with profound changes in several physiological functions, including the immune system (i.e., immunosenescence) (Albright and Albright 2003).

Immunosenescence is characterized by a gradual depletion of naïve T-cells, reduced antibody production, and a low-grade activation of the inflammatory response (Desai et al. 2010; Shaw et al. 2013). Immunosenescence is not restricted to humans and animal models, as age-related variation in immunity has also been reported for free-ranging species (e.g., Nussey et al. 2012; Froy et al. 2019). Due to altered immune functioning, senescence exposes hosts to increased susceptibility to infectious diseases (Miller and Gay 1997, High 2004, Nikolich-Zugich 2018), and reduced resistance to infection is one of the many declines in function that characterize the senescent phenotype (Giefing-Kröll et al. 2015, Glynn and Moss 2020). However, while there is evidence that age is associated with reduced resistance, we still have a very partial view of whether and how age affects tolerance. To the best of our knowledge, only a handful of studies have investigated whether tolerance to infection changes with age. Using a cohort of patients infected with HIV, Regoes et al. (2014) showed that age at infection was positively associated with disease progression, independently from viral load, suggesting a decreased tolerance with age. Similarly, fruit flies (*Drosophila melanogaster*) exposed to bacterial infections suffered from increased mortality when infected at old age, although the capacity to limit bacterial proliferation was not affected by age (Ramsden et al. 2008). Again, this finding indirectly suggests that old flies were less tolerant to infection while maintaining similar resistance to young hosts. A positive effect of age on tolerance has also been reported. Jackson et al. (2014) found that tolerance to macroparasites improved in a natural population of voles (*Microtus agrestis*) between immature and mature individuals, through an increased expression of the transcription factor Gata3 (mediating the Th2 immune response).

Here, we aimed at investigating how resistance and tolerance change with age in house mice (*Mus musculus domesticus*) infected with the rodent malaria *Plasmodium yoelii*. Previous work conducted on models of rodent malaria has shown that resistance improves during the first months of life, which also mirrors the pattern found in malaria-infected humans (Shan et

al. 2012; Pinkevych et al. 2014). However, whether individuals at the age when physiological functions start to decline have altered resistance and tolerance to malaria has not been investigated, yet. To fill this gap, we infected 2- and 12-month old house mice with a non-lethal strain of the rodent malaria *Plasmodium yoelii*, and we compared resistance and tolerance to infection between the two age classes. We decided to focus on 12-month old mice (not older ones), because very old individuals suffer from a general reduction in vigor [a condition called frailty, Clegg et al. (2013)], and therefore reduced resistance or tolerance in very old individuals might reflect a general impairment of organismal functions rather than specific mechanisms related to malaria pathogenesis. Twelve-month old mice are considered to be at the onset of senescence of several (albeit not all) physiological functions. For instance, the expression of tissue-specific senescence markers, defining the senescence-associated secretory phenotype, is up-regulated from the age of 12 months onwards (Hudgins et al. 2018).

We measured resistance as the inverse of parasitemia (% infected red blood cells) during the course of the acute infection (the higher the parasitemia, the lower the resistance). To assess tolerance, we used a reaction norm approach (Simms 2000), where the negative slope of the regression of health on parasitemia provides the estimate of the magnitude of the damage suffered per unit of increase in parasitemia (the steeper the slope, the lower the tolerance). This allowed us to assess i) the change in resistance and tolerance as a function of mouse age; ii) the pattern of phenotypic covariation between resistance and tolerance both between and within age classes.

## **2. Material and Methods**

### *2.1 Animals and infections*

Female C57BL/6 mice were housed in plastic cages (18.5 x 38 x 22.5 cm - enriched with shelters), maintained under a constant temperature (24°C) and a photoperiod of 12:12

(L:D), and given ad libitum filtered water and food (standard mouse pellets). At day 0, 2-month old (n = 20) and 12-month old (n = 20) mice received (by intraperitoneal injection) 100  $\mu$ l of citrate saline diluted blood containing  $10^5$  red blood cells infected with *Plasmodium yoelii* 17XNL.

## 2.2 Parasitemia and hematology

Parasitemia was assessed as the percent of infected red blood cells (iRBCs). To this purpose, we collected a drop of blood from the tip of the tail every 2 or 3 days, from day 3 to day 33 post-infection (p.i.) (i.e., during the acute phase of the infection) to make blood smears. Smears were fixed in methanol (3 min), stained with GIEMSA (20% during 45 min) and the number of iRBCs (out of 1,000 RBCs) counted under optical microscopy (at 1,000 magnification).

Starting from day 0 and up to day 33 p.i., we also collected every 2 or 3 days, two  $\mu$ l of blood from the tail tip and diluted them in 18  $\mu$ l of Phosphate Buffered Saline (PBS). Within one hour of collection, these samples were used to count RBCs, lymphocytes and granulocytes. We also assessed mean corpuscular volume (MCV) which refers to the mean size of red blood cells, as a proxy of reticulocyte production. RBC, lymphocyte, and granulocyte counts, and measurement of MCV were done using a SCIL Vet abc Plus+ hematology analyzer.

## 2.3 Body mass and mortality

Body mass ( $\pm$  0.1 g) was measured daily (or every two days) from day 0 to day 33 p.i. Mice were also checked daily for their health status. Animals with rapidly deteriorating health condition were humanely euthanized (three mice in the 12-month old group). Since 2-month old mice are still in a stage where they gain mass, we also monitored body mass of uninfected mice (2-month old, n = 10; 12-month old, n = 10), as to infer whether any possible age-dependent variation in body mass was specifically due to the infection.

## 2.4 Statistical analyses

Parasitemia, counts of RBCs, lymphocytes, granulocytes, and values of MCV were all ln-transformed to normalize the distribution of model errors and to linearize the relationships among traits.

The temporal dynamics of traits over the course of the acute infection was modelled using mixed effects models. To take into account the non-linear relationship between traits and time p.i., we used a third-degree polynomial ( $\text{time} + \text{time}^2 + \text{time}^3$ ). All models also included age and the interactions between age and time (including squared and cubic terms) as fixed effects and a random intercept for each individual (i.e., mouse identity was included as a random effect).

Initial differences (at day 0) between age classes in counts of RBCs, lymphocytes, granulocytes, MCV, and body mass were assessed using one-way ANOVAs.

Differences in survival between age classes were assessed using a log-rank test.

To investigate the relationship between RBC counts and parasitemia, we ran a mixed effects model where RBC count was the dependent variable and parasitemia and age class (plus the interaction) were included as fixed effects. This model also included random intercepts and slopes for each individual. The same model structure was adopted to explore the relationship between variation in body mass and parasitemia.

All models were run in SAS 9.4.

### **3. Results**

#### *3.1 Parasitemia*

The dynamics of parasitemia differed between age classes, as shown by the statistically significant age x time p.i. interactions (table 1). Percent of iRBCs peaked earlier and at lower level in 2- compared to 12-month old mice (fig. 1A, table S1 in Supplementary Material). These

results, therefore, show that 12-month old mice were less resistant to *P. yoelii* infection than 2-month old mice.

### 3.2 Immune response

At day 0, 2-month old mice had similar numbers of circulating lymphocytes and lower numbers of granulocytes compared to 12-month old mice (table S2 in Supplementary Material).

Following the infection with *P. yoelii*, the number of circulating lymphocytes increased in both age classes, peaking at day 16 p.i. (table S2 in Supplementary Material). However, peak lymphocyte numbers were higher in 12- compared to 2-month old mice (table S2 in Supplementary Material). Therefore, lymphocyte dynamics differed between age classes (fig. S1A and table S3 in Supplementary Material).

Granulocyte numbers peaked at an earlier stage of the infection and at lower numbers in 2- compared to 12-month old mice (table S2 in Supplementary Material). Therefore, granulocyte dynamics differed between age classes (fig. S1B and table S4 in Supplementary Material).

### 3.3 Red blood cells

At day 0, 2- and 12-month old mice had similar RBC counts (table S5 in Supplementary Material). Infection with *P. yoelii* induced a decline in RBC counts, but the temporal dynamics of RBC counts differed between age classes (table 2). Minimum RBC count value was reached earlier on during the infection for 2- compared to 12-month old mice (fig. 1B, table S5 in Supplementary Material). Minimum RBC count was also lower for 12- compared to 2-month old mice (fig. 1B, table S5 in Supplementary Material).

RBC loss produces a physiological feedback to restore RBC number by erythropoiesis. Reticulocytes (young RBCs) are larger than mature RBCs, and therefore it is possible to estimate the dynamics of RBC renewal by assessing the changes in RBC size [mean corpuscular volume (MCV)] over time. At day 0, 2-month old mice had higher MCV compared to 12-month

old mice (one-way ANOVA,  $F_{1,38} = 245.96$ ,  $p < 0.0001$ ). However, MCV increased faster during the early phase of the infection for 2- compared to 12-month old mice, while during the late phase of the infection 12-month old mice had higher MCV than 2-month old mice (fig. 1C), resulting in statistically significant age x time interactions (table 2).

### *3.4 Body mass*

Twelve-month old mice had initially much higher body mass values (table S6 and fig. S2 in Supplementary Material). To get rid of this initial difference, we computed the percent variation of initial body mass over the course of the study. Mice in the two age classes had very different trajectories of body mass variation during the infection. Two-month old mice gained, on average, up to 17% of the initial body mass, whereas 12-month old mice lost around 11% of their initial body mass at day 21 p.i., and subsequently tended to recover during the late phase of the infection (table S7 in Supplementary Material, fig. 1D). Including initial body mass as a covariate did not change the results of this model (table S8 in Supplementary Material).

Given that 2-month old mice are still gaining body mass (fig. S2 in Supplementary Material), we also expressed changes in body mass as variation with respect to non-infected controls. To this purpose, we computed the difference between body mass at day  $i$  and the mean body mass of non-infected mice at day  $i$ , and divided it by the body mass at day 0. Therefore, this variable describes the deviation in body mass of infected mice with respect to non-infected controls at each sampling time and for both age classes. Using this variable provided similar results to the previous model (table S9, fig. S3 in Supplementary Material), indicating that the difference in body mass trajectories between 2- and 12-old mice was actually due to a more severe effect of the infection in old mice.

### *3.5 Mortality*

None of the 2-month old mice died during the course of the study vs. 20% (4/20) of 12-month old mice (one dead, three euthanized) (log-rank test,  $\chi^2 = 4.33$ ,  $p = 0.0374$ , fig. S3 in Supplementary Material).

### *3.6 Relationship between health status and parasitemia*

We investigated how age affected changes in RBC counts per unit of change in parasitemia. The results revealed that the negative slopes relating RBC counts to parasitemia were significantly steeper in 12- compared to 2-month old mice, showing that 12-month old mice were less tolerant to the infection (table 3, fig. 2A).

Changes in body mass during the course of the infection can also be used as a general indicator of host health. We therefore ran the same mixed-effect model that included percent variation of initial body mass as the dependent variable. This model showed a difference in the relationship between changes in body mass and parasitemia (table 3). Twelve-month old mice showed a negative relationship between body mass and parasitemia, suggesting that mice with high parasitemia suffered from increasingly poor health (fig. 2B). On the contrary, the slope relating changes in body mass and parasitemia was flat in 2-month old mice (fig. 2B).

### *3.7 Relationship between resistance and tolerance*

Over the whole sample, there was a negative correlation between maximum parasitemia (a measure of inverse resistance) and the slope of RBC counts on parasitemia (a measure of tolerance) (Pearson's  $r = -0.586$ ,  $p = 0.0002$ ,  $n = 36$ , fig. 3A). This finding suggests that mice that were tolerant to the infection were also the most resistant ones. However, this pattern was exclusively driven by an age effect. An ANCOVA model showed, indeed, that there was no correlation between tolerance and resistance within age classes (fig. 3B, table S10 in Supplementary Material).

## **4. Discussion**

The aim of this study was to investigate whether resistance and tolerance to infection change with age in *Plasmodium*-infected mice, and to investigate the pattern of covariation between resistance and tolerance, both within and between age classes. We found that 12-month old mice harbored more parasites and suffered higher damage per unit of parasitemia compared to 2-month old mice. We also found that the two traits positively covaried, but only across ages. These results show that age induces a reduction of both resistance and tolerance to infection with *P. yoelii*, while we did not find evidence for a trade-off between resistance and tolerance.

Age makes hosts more susceptible to infectious diseases and more likely to suffer from severe morbidities (Glynn and Moss 2020). However, increased susceptibility and/or morbidity might arise because of age-associated impairment of defense mechanisms aiming at controlling parasite proliferation (i.e., impaired resistance), age-associated impairment of mechanisms restoring homeostatic conditions during an infection (i.e., impaired tolerance), or both (Ayres 2020). Previous work on the effect of age on infectious diseases has assumed that increased susceptibility in the elderly arises from impaired immune functioning, focusing on mechanisms of parasite control (High 2004). Only a handful of studies have explicitly addressed the possible role played by the impairment of mechanisms involved in the maintenance of organismal homeostasis as a cause of infection-induced damage in aged hosts (e.g., Regoes et al. 2014). Yet, understanding if aging impairs resistance, tolerance or both is key to have treatments that effectively promote health in the elderly (Vale et al. 2014).

Although resistance and tolerance are fundamentally different host defense strategies, it has been suggested that they might be related through physiological and/or genetic trade-offs, finally producing an intertwined evolution of resistance and tolerance (Fineblum and Rausher 1995; Miller et al. 2005; Lefèvre et al. 2010; Mazé-Guilmo et al. 2014). Evidence for a genetic trade-off between resistance and tolerance has been provided for malaria-infected mice, with strains harboring the smallest number of parasite, also having the steepest slope between red

blood cell count and parasitemia (Råberg et al. 2007). Here, we could not assess the genetic correlation between resistance and tolerance because we only studied one mouse strain; however, we could assess whether the two traits were phenotypically correlated, possibly indicating a physiological trade-off. Physiological trade-offs might arise if investment into one trait diverts resources from competing traits. For instance, if investment into immune defenses diverts resources from mechanisms of tissue repair, this might produce a phenotypic trade-off between resistance and tolerance. Contrary to the hypothesis of a physiological trade-off between resistance and tolerance, we found that the two traits were positively correlated. However, this pattern was exclusively due to the heterogeneity in the age structure of our sample of mice, since 2-month old mice were both more resistant and tolerant compared to 12-month old mice. Looking at the correlation between resistance and tolerance within age classes provided no evidence for a phenotypic correlation between the two traits. This finding highlights the importance of taking into account the potential heterogeneity among individuals that, if ignored, might provide a biased view of the covariation pattern between resistance and tolerance. In agreement with this, Restif and Koella (2004) modelled the evolution of resistance and tolerance under the specific assumption that the two traits were unrelated. Nevertheless, their analysis showed that across environments, resistance and tolerance can vary in similar or opposite directions (Restif and Koella 2004). Field studies of the relationship between resistance and tolerance might, therefore, provide flawed results if potential confounding factors, such as host age or nutritional status, are not controlled for.

The lack of phenotypic correlation between resistance and tolerance within age classes suggests that i) the two strategies are based on different mechanisms; and ii) under the benign conditions experienced in the lab, the energetic requirements associated with the deployment of these mechanisms are not high enough to generate a physiological trade-off between resistance and tolerance.

There are numerous physiological mechanisms that might account for the observed age-associated decline in resistance and tolerance, including impaired immunity, a highly regulated immune response (preventing infection-induced immunopathology), reduced regenerative capacity (impaired hematopoiesis), impaired detoxification of toxins produced during the infection, or impaired metabolic functions. Identifying all the possible mechanisms accounting for age-associated changes in resistance and tolerance was well beyond the scope of the current study. Nevertheless, we suggest that age-dependent changes in immunity and hematopoiesis might play a role to explain the observed results. Asexual multiplication of *Plasmodium* parasites causes the lysis of infected RBCs and hosts harboring high parasitemia might suffer from more severe anemia because of the direct destruction of RBCs during parasite multiplication. However, it is now well established that this direct mechanism of RBCs destruction only contributes to a relatively minor fraction of the total RBC loss, whereas the majority is due to the action of the immune system, through the removal of uninfected RBCs (Evans et al. 2006; Fonseca et al. 2016). We found that granulocytes had different dynamics during the course of the infection between age classes, with 12-month old mice having higher numbers. Granulocytes mostly refer to neutrophils that have phagocytic properties and contribute to the oxidative burst. It is, therefore, possible that an exacerbated innate response, mediated by neutrophils, might account for the accentuated loss of RBCs, as shown in other systems (Evans et al. 2006). However, functional properties (i.e., phagocytosis) of neutrophils have also been shown to be compromised in senescing individuals (Butcher et al. 2000). Therefore, the causal involvement of neutrophils in the exacerbated anemic status of 12-month old *P. yoelii*-infected mice requires further investigation.

Anemia induces a physiological feedback on erythropoiesis, stimulating the renewal of RBCs that have been lost during the infection. Therefore, the capacity to rapidly and effectively restore a homeostatic RBC number should finally contribute to tolerance to malaria (Chang et

al. 2004). Age is often associated with a compromised regenerative capacity and impaired erythropoiesis is involved in the anemia of senescing humans and rodents (Price 2008; Stauder et al. 2018). Moreover, during a malaria infection, the interaction between several immune effectors and parasite-derived toxins might compromise the erythropoietic process (Vigario et al. 2001), and age might further accentuate such impaired erythropoiesis. We assessed the age-dependent capacity to renew RBCs by measuring mean corpuscular volume. Young RBCs (reticulocytes) have bigger size and therefore changes in MCV indicate a change in the age distribution of RBCs. As expected, malaria infection produced an increase in MCV in mice of both age classes; however, the dynamics of MCV was different between 2- and 12-month old mice. Two-month old mice had a faster increase in MCV during the first two weeks post-infection, whereas 12-month old mice had a slower increase in MCV but maintained higher values during the late phase of the acute infection. This finding, therefore, suggests that an impaired physiological feedback, compromising the efficacy of the erythropoietic process during the growing phase of the malaria infection, might account for the age-dependent weakened tolerance.

Aging is usually associated with a general decline in vigor (Clegg et al. 2013). One might then wonder whether the observed results reflect a general increase in frailty rather than specific mechanisms related to malaria pathogenesis. With this respect, we decided not to use very old mice (lifespan of C57BL/6 mice can exceed 30 months), and instead to compare two age classes that correspond to mature adults (2-month old) and mice (12-month old) where senescent changes are found in some but not all physiological functions. On a more general note, it should be acknowledged that the relationship between age and physiological functions is rarely linear. For logistic reasons (housing capacity), we only studied two age classes, which obviously prevents us to assess the shape of the relationship between age, resistance and tolerance to malaria infection. Indirect evidence, nevertheless suggests that resistance to malaria

increases during the first months of life, certainly reflecting the maturation of the immune system (Shan et al. 2012). Whether tolerance also follows a curvilinear age-dependent variation is not known. Further work should therefore explore how resistance and tolerance to malaria (co)-vary over the entire individual lifespan.

To conclude, we showed that age is associated with both reduced resistance and tolerance to malaria. Further work using parasites with different life cycles and pathogenicity should confirm whether compromised tolerance is a hallmark of the senescent phenotype. In such a case, treatments for the elderly might, in addition to boost the immune response (i.e., improve the responsiveness to vaccines) (Meyer et al. 2012), also target specific mechanisms underlying a compromised tolerance (Vale et al. 2014).

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**Author contributions** – GS and BF conceived the study. All authors collected the data and analyzed the samples. GS performed the statistical analyses and wrote the first draft of the manuscript. All authors revised and approved the final version of the manuscript.

**Conflicts of interest** – None to declare.

**Ethics statement** - The experiment was approved by the Comité d’Ethique de l’Expérimentation Animale Grand Campus Dijon, France (CNREEA n C2EA – 105) and by the “Ministère de la Recherche et de l’Enseignement Supérieur” (project #8675).

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Table 1. Mixed-effect model exploring the effect of time p.i. and age (2- vs. 12-month old) on the dynamics of infection with *P. yoelii* (ln-transformed % iRBCs). The model included time<sup>2</sup> and time<sup>3</sup> to take into account the non-linear relationship with parasitemia. Mouse identity was included as a random effect. For the age effect (and the interactions between age and time), the estimate for the 2-month old group is included in the intercept.

<b>Fixed effects</b>	<b>Estimate (se)</b>	<b>df</b>	<b>F</b>	<b>P</b>
<i>Intercept</i>	-2.792 (0.24)			
<i>Time p.i.</i>	1.050 (0.06)	1,450	524.06	<0.0001
<i>Time<sup>2</sup> p.i.</i>	-0.064 (0.003)	1,450	373.65	<0.0001
<i>Time<sup>3</sup> p.i.</i>	0.001 (0.0001)	1,449	230.48	<0.0001
<i>Age</i>	0.802 (0.34)	1,480	5.64	0.0180
<i>Time p.i. x Age</i>	-0.323 (0.08)	1,450	17.28	<0.0001
<i>Time<sup>2</sup> p.i. x Age</i>	0.032 (0.01)	1,450	42.81	<0.0001
<i>Time<sup>3</sup> p.i. x Age</i>	-0.0007 (0.0001)	1,449	62.30	<0.0001
<b>Random effect</b>	<b>Estimate (se)</b>		<b>Z</b>	<b>P</b>
Mouse ID	0.024 (0.01)		1.8	0.0356

Table 2. Mixed-effect model exploring the effect of time p.i. and age (2- vs. 12-month old) on the dynamics of RBC counts (ln-transformed numbers  $\times 10^6/\text{mm}^3$ ) and MCV (ln-transformed  $\mu\text{m}^3$ ). The model included time<sup>2</sup> and time<sup>3</sup> to take into account the non-linear relationship between the two traits and time. Mouse identity was included as a random effect. For the age effect (and the interactions between age and time), the estimate for the 2-month old group is included in the intercept.

Fixed effects	RBC counts				MCV			
	Estimate (se)	df	F	P	Estimate (se)	df	F	P
<i>Intercept</i>	2.642 (0.07)				3.771 (0.02)			
<i>Time p.i.</i>	-0.254 (0.02)	1,486	237.70	<0.0001	0.038 (0.01)	1,487	12.78	0.0004
<i>Time<sup>2</sup> p.i.</i>	0.015 (0.001)	1,486	68.14	<0.0001	-0.001(0.0003)	1,487	57.04	<0.0001
<i>Time<sup>3</sup> p.i.</i>	-0.0002 (0.00003)	1,486	8.11	0.0046	-6.99 $\times 10^6$ (6.44 $\times 10^6$ )	1,487	163.63	<0.0001
<i>Age</i>	-0.080 (0.10)	1,448	0.67	0.4138	0.011 (0.03)	1,449	0.19	0.6622
<i>Time p.i. x Age</i>	0.120 (0.03)	1,486	22.59	<0.0001	-0.052 (0.01)	1,487	64.40	<0.0001
<i>Time<sup>2</sup> p.i. x Age</i>	-0.015 (0.002)	1,486	68.99	<0.0001	0.005 (0.001)	1,487	121.13	<0.0001
<i>Time<sup>3</sup> p.i. x Age</i>	0.0003 (0.00004)	1,486	93.31	<0.0001	-0.0001(0)	1,487	127.77	<0.0001
Random effect	Estimate (se)		Z	P	Estimate (se)		Z	P
Mouse ID	0.008 (0.004)		1.96	0.0249	0.001 (0.003)		1.99	0.0236

Table 3. Mixed-effect model exploring the effect of parasitemia (ln-transformed % iRBCs) on changes in RBC count (ln-transformed numbers x 10<sup>6</sup>/mm<sup>3</sup>), and changes in body mass (g) in the two age classes (2- vs. 12-month old). Change in body mass is expressed as variation with respect to day 0. The model included random intercepts (mouse identity) and slopes (parasitemia) to allow for variation in the relationship between RBC count, body mass, and parasitemia at the individual level. For the age effect (and the interaction between age and parasitemia), the estimate for the 2-month old group is included in the intercept.

<b>Fixed effects</b>	<b>RBC count</b>				<b>Body mass</b>			
	<b>Estimate (se)</b>	<b>df</b>	<b>F</b>	<b>P</b>	<b>Estimate (se)</b>	<b>df</b>	<b>F</b>	<b>P</b>
<i>Intercept</i>	2.216 (0.03)				0.120 (0.01)			
<i>Parasitemia</i>	-0.369 (0.02)	1,510	1179.48	<0.0001	0.008 (0.003)	1,352	1179.48	<0.0001
<i>Age</i>	-0.019 (0.04)	1,506	0.20	0.6540	-0.148 (0.01)	1,34.9	0.20	<0.0001
<i>Parasitemia x Age</i>	-0.070 (0.02)	1,510	8.87	0.0030	-0.037 (0.004)	1,352	8.87	<0.0001
<b>Random effects</b>	<b>Estimate (se)</b>		<b>Z</b>	<b>P</b>	<b>Estimate (se)</b>		<b>Z</b>	<b>P</b>
Mouse id	0				0.0009 (0.0003)		2.60	0.0046
Covariance between intercept and slope	0.003 (0.002)		2.08	0.0375	0.001 (0.0001)		5.76	<0.0001

## Figure captions

Figure 1. Temporal dynamics of A) parasitemia (ln-transformed % iRBCs), B) RBC count (ln-transformed numbers  $\times 10^6/\text{mm}^3$ ), C) mean corpuscular value (ln-transformed  $\mu\text{m}^3$ ), D) body mass (g) in 2- and 12-month old mice infected with *P. yoelii*. Change in body mass is expressed as variation with respect to day 0. Full and dotted lines represent the model fit for the two age classes, respectively.

Figure 2. A) Changes in RBC count (ln-transformed numbers  $\times 10^6/\text{mm}^3$ ) and B) changes in body mass (variation with respect to day 0) as a function of parasitemia (ln-transformed % iRBCs) in 2- and 12-month old mice infected with *P. yoelii*. Full and dotted lines represent the slopes for 2- and 12-month old mice, respectively. Individual slopes were derived from mixed-effect models with random intercepts (mouse id) and slopes (parasitemia).

Figure 3. Covariance between tolerance and resistance. Tolerance refers to the value of the individual slope between RBC count and parasitemia, while resistance refers to maximum parasitemia. The signs + and – on the y- and x-axis indicate high and low values of tolerance and resistance. A) Relationship between tolerance and resistance over the whole sample of mice; B) Relationship between tolerance and resistance per age class (full dots = 2-month old; empty dots = 12-month old). Axes have been inverted to better visualize the positive relationship between the two traits.

Figure 1.

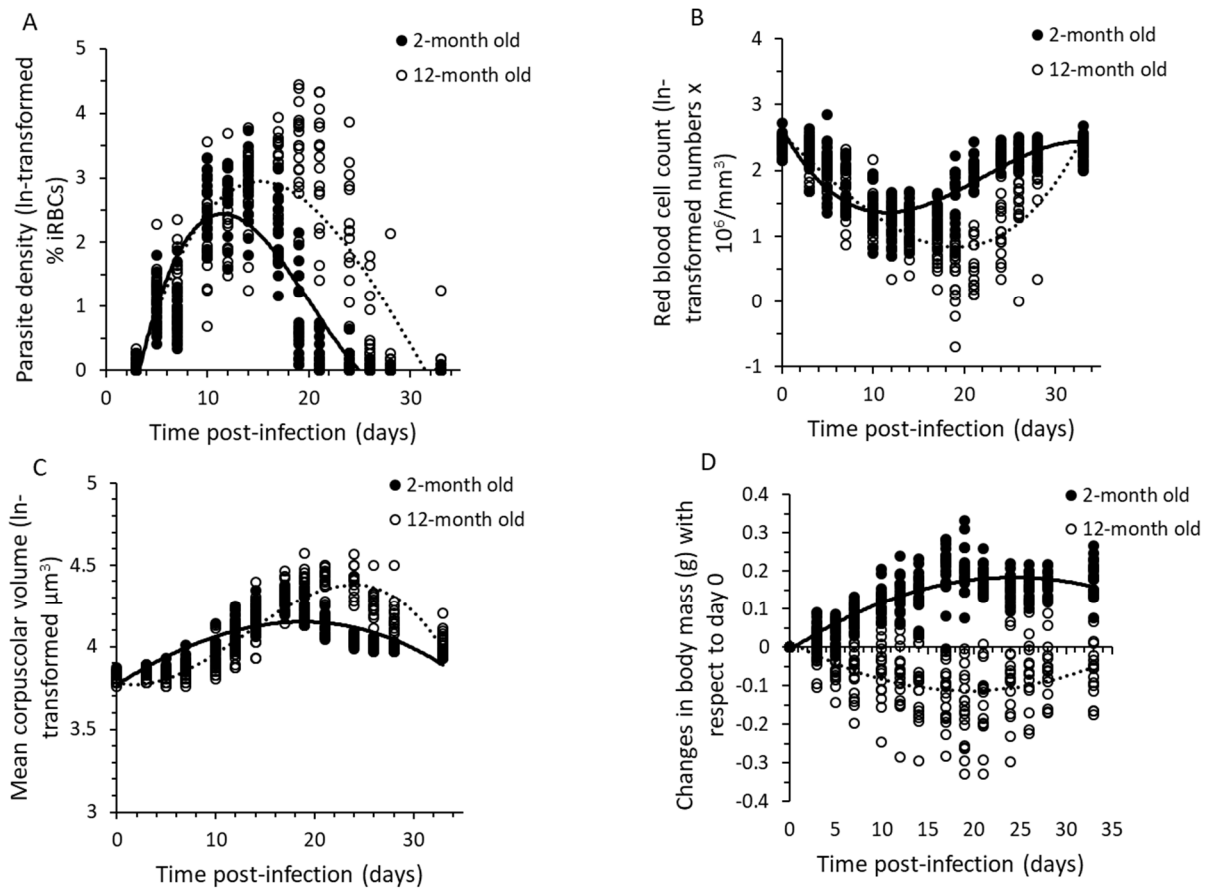


Figure 2.

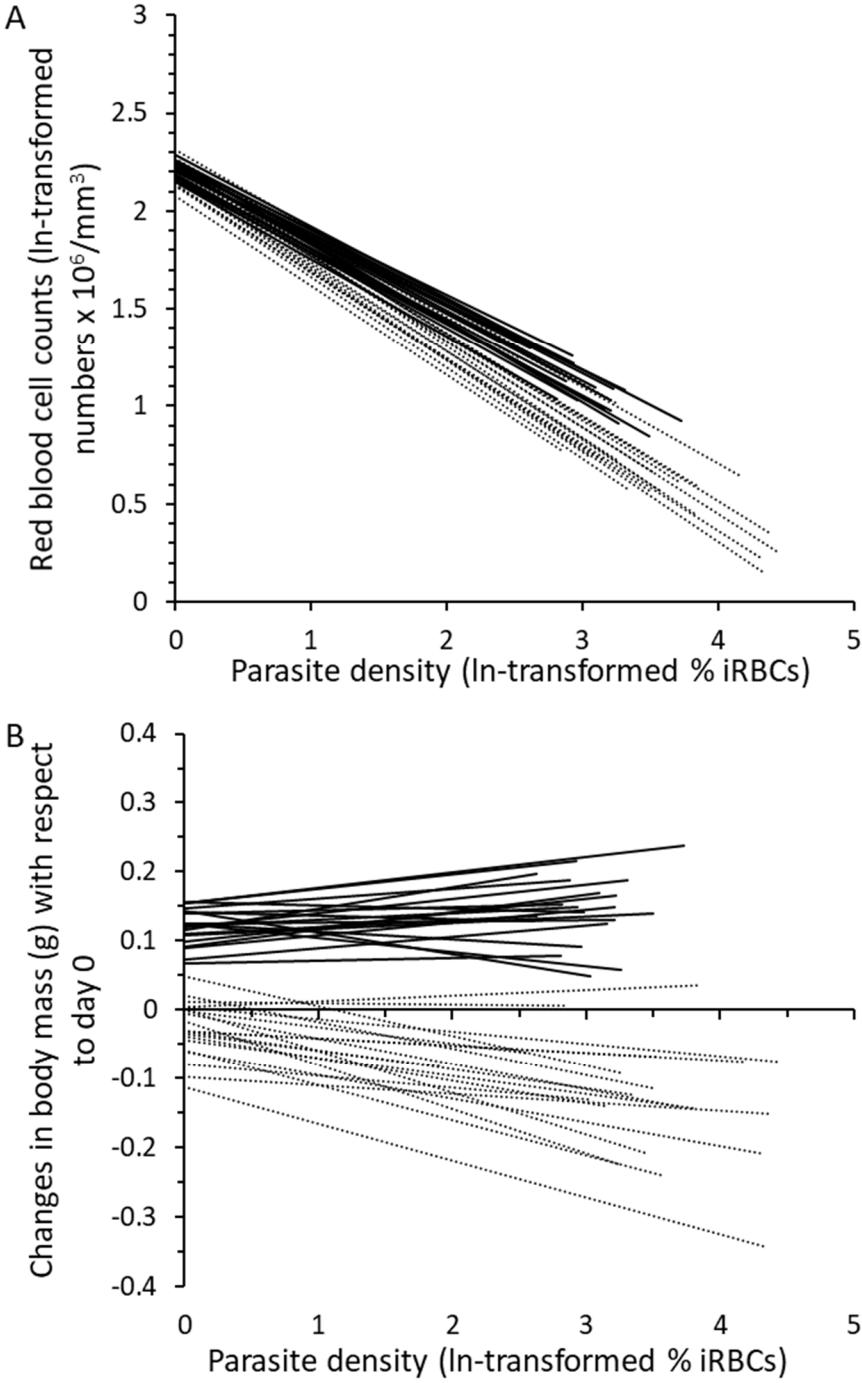


Figure 3.

