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**Article** *in* Behavioural Processes · October 2016

DOI: 10.1016/j.beproc.2016.10.012

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# Effect of an anti-malaria drug on behavioural performance on a problem-solving task: An experiment in wild great tits

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ARTICLE INFO

*Article history:*
Received 17 March 2016
Received in revised form 18 October 2016
Accepted 27 October 2016
Available online 29 October 2016

*Keywords:*
Activity
Cognition
Learning
Parasite
Problem-solving

ABSTRACT

Malaria parasites have been shown to decrease host fitness in several species in the wild and their detrimental effects on host cognitive ability are well established in humans. However, experimental demonstrations of detrimental effects on non-human host behaviour are currently limited. In this study, we experimentally tested whether injections of an anti-malaria drug affected short-term behavioural responses to a problem-solving task during breeding in a wild population of great tits (*Parus major*) naturally infected with malaria. Adult females treated against malaria were more active than control females, even though they were not more likely to solve the task or learn how to do so, suggesting that energetic constraints could shape differences in some behaviours while changes in cognitive performances might require more time for the neural system to recover or may depend mainly on infection at the developmental stage. Alternatively, parasite load might be a consequence, rather than a cause, of inter-individual variation in cognitive performance. These results also suggest that inter-individual as well as inter-population differences in some behavioural traits may be linked to blood parasite load.


## 1. Introduction

Parasites are well known to affect many of their hosts' traits, in particular behaviour (Kershaw et al., 1959; Kavaliers et al., 1995; Barber et al., 2000; Gegear et al., 2006; Barber and Dingemanse, 2010; Poulin, 2013). Alterations of behaviour in infected hosts may result from side-effects of the host reaction to infection (e.g. host energy reallocation to the immune system; Moret and Schmid-Hampel, 2000) or pathogenic effects (e.g. Hermes et al., 2008), i.e. they may be non-adaptive. Alternatively, these alterations may represent an adaptive process either for the host, e.g. by facilitating the attempt to face infection (i.e. the so-called 'sickness behaviour'; reviews in Hart, 1988; Larson and Dunn, 2001) or for the parasite, in order to facilitate transmission (i.e. the behavioural manipulation hypothesis; Lefèvre and Thomas, 2008; Poulin, 2010). Although we do not know much about the mechanisms underlying such behavioural changes in the wild yet, there is no doubt that infection-associated behavioural changes can impact the fitness of infected individuals (Barber et al., 2000), because their behavioural changes are themselves exposed to selective pressures. Understanding how, why and which behavioural changes arise following parasite infection in natural populations could help us to quantify their evolutionary and ecological significance.

Cognition is at the heart of decision-making by individuals facing environmental variation in the wild and cognitive abilities may be a key target of parasite effects on host behaviour, either (i) because of the diversion of energy from brain functions into the immune system, (ii) because of an adaptive change as part of the general host response to face the infection, mediated by the action of the immune system on the brain (e.g. via the action of cytokines; review in Larson and Dunn, 2001), or (iii) via direct manipulation by the parasite. Because individual innovation and learning performance can play a central role in shaping the response to environmental changes (Sol et al., 2002; Mery and Kawecki, 2004; Sol, 2009; Cantalapiedra et al., 2014; Aplin et al., 2015; Kozlovsky et al., 2015), the effects of parasites on these cognitive abilities need specific attention when assessing the evolutionary consequences of host-parasite interactions.

Malaria is a widespread and harmful blood parasitic infection in many animal populations (Levine, 1988). In birds for

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http://dx.doi.org/10.1016/j.beproc.2016.10.012
0376-6357/

example, malaria parasites (genera *Haemoproteus* and *Plasmodium, sensu* Perez-Tris et al., 2005) have been experimentally shown to decrease host reproductive success, body condition and survival (Knowles et al., 2010; Marzal et al., 2005; Merino et al., 2000 but see Podmokła et al., 2014). However, whether malaria parasites negatively affect individual's fitness because of their direct impact on health and/or because of a side-effect on fitness-related behaviours has been overlooked. Yet, assessing the relative role of both mechanisms could yield a better understanding of host-parasite interactions and the selective pressures acting on them in the wild via host behavioural changes. Interestingly, malaria parasites are well known to impair cognitive functions in terms of attention, memory and visuospatial performance in humans (Bangirana et al., 2006; Kihara et al., 2006). However, only a few studies have examined their effect on behavioural and cognitive traits in other animal species, in particular in birds where malaria is common (Garamszegi et al., 2015). In great tits (*Parus major*), a correlative study showed that malaria-infected females performed worse on a problem-solving task than non-infected females (Dunn et al., 2011). In another songbird species, the canary (*Serinus canaria*), early exposure to malaria parasites negatively affected the development of the high vocal centre song nucleus in the brain and, as a consequence, song complexity (Spencer et al., 2005). Because problem-solving performance has been linked to reproductive success in great tits (Cole et al., 2012; Cauchard et al., 2013) and song complexity is a sexual secondary trait favoured by females during mate choice in canaries (Drăgănoiu et al., 2002), the effect of malaria on problem-solving and singing behaviours may impact natural and sexual selection processes. Moreover, if malaria parasites can influence behavioural traits, within-species differences in malaria prevalence between populations, even on a small geographical scale (e.g. Wood et al., 2007; Szoellosi et al., 2011), may partly explain behavioural differences between these populations.

Because previous studies on the links between behavioural variation and parasitaemia in natural populations were correlative (e.g. Dunn et al., 2011; Garamszegi et al., 2015), the question whether parasites cause behavioural changes or differential parasitaemia derives from between-individual variation in behavioural traits remains unanswered so far. Problem-solving and learning performances, by incorporating new behaviours, may affect the chance of encountering parasites and get infected in natural populations, e.g. via differential habitat use (Cole et al., 2012). To explore the causal links between malaria infection and changes in behavioural and cognitive traits and thereby shed light on the evolutionary consequences of malaria, an experimental manipulation of malaria infection level in the wild is needed. In this study, we experimentally tested whether Primaquine, an anti-malaria drug, could affect short-term behavioural responses to a novel problem-solving task in a natural population of great tits (*Parus major)* strongly infected with malaria. Primaquine has been successfully used to reduce avian malaria infection during breeding, as well as protect uninfected individuals against acquiring novel infection, in a phylogenetically close passerine species, the blue tit (*Cyanistes caeruleus*) (Merino et al., 2000; Marzal et al., 2005; Martínez-de la Puente et al., 2010). Indeed, infection intensity and/or the risk of acquiring novel infections increases over the course of the nesting cycle (Atkinson and van Riper III, 1991). We injected adult great tit females at an early breeding stage with physiological salt either alone (control) or with Primaquine (Merino et al., 2000; Marzal et al., 2005; Martínez-de la Puente et al., 2010). We subsequently measured females' behavioural responses to a problem-solving task in terms of problem-solving performance and its improvement over successive attempts to solve the task, as well as activity on the nest box. If Primaquine reduces malaria parasite infection, even temporarily, and parasites affect host behavioural responses, we predicted that females treated with Primaquine could invest more in the behavioural traits measured, thus be more active and show enhanced problem-solving performance and improvement over successive attempts than control females.

## 2. Material and methods

### 2.1. Study system, monitoring of breeding and anti-malaria drug injections

We carried out the study in a population of great tits breeding on the island of Gotland (Sweden) in spring 2011 and 2012. Nest boxes were visited regularly from the start of the breeding season as part of the long-term monitoring of the population to record breeding data (laying and hatching dates, clutch size, fledgling number and condition) (see Cauchard et al., 2013 for details). In our population, nest building can take up to two weeks, laying lasts 8–9 days on average (one egg laid per day), and incubation lasts 12 to 14 days on average.

During early nest building, 70 females were caught within nest boxes, ringed, aged (i.e. yearling *vs.* 2 years or more) and weighed. Only females were used for this study because catching males early in the breeding season can prove difficult since the nest is almost solely built by the female in this species. A blood sample (~30–50 μl) was taken from the brachial vein and stored to allow the determination of female malaria infection status (i.e. infected *vs* uninfected) prior to the experimental treatment, using molecular determination of the malaria species present (genera *Haemoproteus* and *Plasmodium*; for more details, see Dubiec et al. in prep). Females were then randomly assigned to one of the two treatment groups and injected intraperitoneally with 0.1 ml of physiological salt (PBS) either alone (control) or with 0.01 mg of Primaquine (anti-malarial drug; Aldrich). The effects of Primaquine are expected to be limited in time and observations suggest that the effects wear off around 40 days after injection (A. Marzal, personal communication). Behavioural tests were conducted during the nestling period, thus on average (± SE) 35.5 (±2.9) days after PBS/Primaquine-injection. Therefore, we could not catch females a second time early enough to directly test for the effect of Primaquine injection on malaria infection status after the experimental treatment. Blood samples were analysed in the laboratory after the breeding season.

### 2.2. Behavioural measures on a novel problem-solving task

Problem-solving performance and its improvement over successive attempts to solve the task were measured using a string-pulling task that has been previously successfully used in this population and for which the solving motivation stems from parents' drive to feed their young during the nestling rearing period (Cauchard et al., 2013). The task consisted of a door blocking the nest box entrance that could be opened by parents by pulling a string placed below the door using their legs (see Cauchard et al., 2013). Once the bird had entered, the door then closed automatically behind it, but could be simply pushed open from inside the nest box by parents to get out. Tests lasted only 1 h to prevent nestling starvation if parents were unable to enter and were conducted only if chicks were satiated (i.e. not loudly begging) upon the arrival of the experimenter to the nest box. When begging behaviour of the nestlings was very intense, reflecting that nestlings were hungry, the test was delayed to the day after to avoid nestling starvation if the parents were not able to solve the task. The test was abandoned only when chicks were in very poor condition on this second visit (overall, it happened only twice). To give parents more time to possibly solve the task, we presented this task twice, once a day on two consecutive days, during the peak of chick provisioning, i.e. on 7 to 9 days old chicks. We observed no deser-

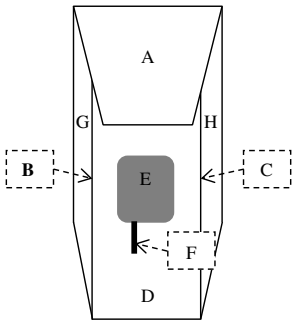


**Fig. 1.** Zones of the nest box used to quantify activity behaviour. A: roof, B and C: left and right corners, D: front, E: task door, F: task string, G and H: left and right sides.

tion or total reproductive failure after the tests, thus suggesting no major detrimental effect of the perturbation in this population (see Cole et al., 2012). All tests were video recorded and recordings were scored blindly with respect to the treatment (i.e. control *vs.* Primaquine) received by the female.

From video recordings, we determined whether experimental females succeeded in opening the door and entering ('solvers') or contacted the nest box but failed to enter ('non-solvers'). Among solvers, we quantified the first solving latency as the time elapsed between the first contact with the string that caused a movement of the door and the bird's entrance into the nest box, excluding the time spent away from the nest box. This measure thus discarded the time before the bird was able to see that the door could be opened. We then determined whether females solved the task more efficiently over successive entrances by examining the change in solving latency over the first three entrances. We chose to use only the first three entrances to standardize our measure of improvement between females because the number of entrances can vary greatly between birds tested in the wild (i.e. from 2 to 34 entrances during the two hours of test, with most of the solving females entering 3 times). For the second and third entrances, we quantified solving latency as the time elapsed between the first contact with the nest box following the previous entrance and the bird's next entrance, excluding again the time spent away from the nest box. These successive solving latencies slightly differed from the first latency because upon successive entrances, we assumed that the birds had already been able to see the door opening. Individuals that showed a decrease in solving latency across solving events (i.e. a negative slope on the regression line of the three latencies) were named 'learners'. In contrast, birds that failed to solve the task again after their initial entrance even though they returned to the nest box or birds that showed no decrease in solving latency across successive entrances were named 'non-learners'. Finally, we measured activity during the problem-solving test as the total number of movements between zones defined on the nest box (i.e. front, lid, sides; see Fig. 1) until the first entrance (for solvers) or the end of the test (for non-solvers).

Neophobia, an animal's aversion to novelty, could blur our measures of problem-solving performance and improvement because the problem-solving task itself was new to the bird. We recorded the time elapsed between first landing on the nest box and first contact with the task as a measure of neophobia and controlled for its effect on problem-solving performance and improvement over successive attempts to solve the task by including it as a covariate in our models (Cauchard et al., 2013).

Birds were caught, handled and ringed under a licence from the Stockholm Museum Ringing Center. Behavioural tests and blood sampling were conducted under a general licence from the Swedish Committee for Experiments on Animals for all experiments on the site (licence number C 108/7).

### 2.3. Statistical analyses

The Primaquine injection could only reduce malaria infection in females that were infected prior to the treatment. Nevertheless, to test whether the medication per se, rather than its effect on malaria load, could be responsible for behavioural changes, we also analysed responses of females that were uninfected prior to the treatment when a behavioural change was observed among infected females. If behavioural changes are observed following Primaquine injection in previously infected females only, they are likely to result from the reduction of malaria load. If behavioural changes are observed following Primaquine injection in all females regardless of their prior infection status, they are likely to be due to medication per se. Unfortunately, the low number of uninfected females at the start of the experiment (i.e. revealed by the analyses of blood samples: N = 8 out of 57 experimental females; control: N = 5; Primaquine: N = 3) prevented us to reach sufficient statistical power to directly test for an interaction between experimental treatment and infection status prior to the experiment. Therefore, we analysed infected and uninfected females separately using appropriate tests given sample sizes.

We used generalized linear models (GLM) to test the effect of treatment (control vs Primaquine injection) on infected females' probability to solve the task (i.e. solvers *vs.* non-solvers) and to improve in solving the task (i.e. learners *vs.* non-learners), with binomial error and logit link function. We used linear models (LM) to analyze the effect of treatment on infected females' problem-solving latency, neophobia and activity. All initial models included treatment, year, age and the pairwise interactions between these factors as explanatory variables, as well as the measure of neophobia (except when it was the response variable) and date and time of the day when the test was performed as covariates. Finally, because activity is expected to strongly depend on the total time a bird spent on the nest box during the test, we also added it as a covariate in the initial model for activity.

Among uninfected females, we tested the effect of treatment on female's probability to solve the task and improvement over successive attempts (i.e. binary variables) using chi-square tests (i.e. Fisher's exact test; Graham, 1992) and on problem-solving latency and activity (i.e. continuous variables) using *t*-tests corrected for the heterogeneity of variance. Because we could not add the total time spent on the nest box during the test as a covariate in the *t*-test when analysing activity, we used the residuals of activity on time spent on the nest box as the dependant variable for activity.

We excluded from our analyses two females that did not sufficiently participate in the test, i.e. were present during less than 50% of the fastest observed solving latency over the sample of tested females (19 s). We also excluded from the 2012 data the females that had already been tested in 2011 (N = 13) to avoid pseudoreplication. Normality was checked and data were transformed to reach normality when needed. Homogeneity of variance was also checked. Non-significant effects were backward eliminated and all tests were two-tailed. Sample sizes varied between analyses because some behavioural data were missing (e.g. we could not assess neophobia for five females and solving latency for one female). All analyses were conducted using in SPSS 18.0 (Chicago, SPSS Inc. 2009).

## 3. Results

We measured the behavioural response to the problem-solving task for a total of 57 experimental females (control: N = 27; Primaquine: N = 30). Of the 49 infected experimental females (control: N = 22; Primaquine: N = 27), 31 solved the task (control: N = 13; Primaquine: N = 18). 22 out of these 31 solving females attempted to

enter again (control: N = 10; Primaquine: N = 12) and 16 succeeded to solve it again twice or more (control: N = 8; Primaquine: N = 8).

Among infected females at the start of the experiment, the treatment affected neither the probability to be a solver ($F_{1,48} = 0.08$, P = 0.77; Fig. 2a), the latency to solve the task ($F_{1,28} = 0.84$, P = 0.37; Fig. 2b) nor, among solvers, the probability to be a learner ($F_{1,21} = 0.27$, P = 0.60; Fig. 2c). These results were not confounded by an effect of neophobia on behavioural performance, because neophobia affected neither the probability to be a solver ($F_{1,43} = 1.08$, P = 0.30), the latency to solve the task ($F_{1,26} = 2.13$, P = 0.16) nor, among solvers, the probability to be a learner ($F_{1,21} = 1.32$, P = 0.25). Moreover, the level of neophobia did not differ between treatments ($F_{1,43} = 0.72$, P = 0.40). However, the level of activity differed between experimental groups: females injected with Primaquine were more active than control females ($F_{1,42} = 6.22$, P = 0.017; Fig. 2d), accounting for the total time a bird spent on the nest box during the test ($F_{1,42} = 17.46$, P < 0.001; activity increased with increasing time spent on the box), year ($F_{1,42} = 4.40$, P = 0.042; females were more active in 2012 than in 2011) and date of the test ($F_{1,42} = 4.20$, P = 0.047; activity increased with increasing date). The level of activity was not related to the probability to solve or to be a learner (problem-solving: $F_{1,51} = 1.05$, P = 0.30; learning: $F_{1,24} = 1.28$, P = 0.26) but the latency to solver the task increased with the level of activity ($F_{1,31} = 86.30$, P < 0.001).

Among uninfected females at the start of the experiment, the level of activity taking the time spent on the nest box during the test into account did not vary between treatments (mean ± SE = −0.07 ± 1.26 for uninfected females injected with PBS and 0.10 ± 0.26 for uninfected females injected with Primaquine; $t(3.3) = -0.27$, P = 0.80). Moreover, the level of activity taking the time spent on the nest box into account did not differ between uninfected females and infected females treated with Primaquine ($t(31) = -0.77$, P = 0.44).

## 4. Discussion

In this study, we experimentally tested whether injection of an anti-malaria drug affected behavioural and cognitive traits measured on a problem-solving task in a wild population of a passerine bird. Our results showed that females injected with Primaquine were not more likely to solve the task or solve it more rapidly over successive attempts, but they were significantly more active (i.e. made more movements per unit of time) when interacting with the task as compared to control females. Moreover, although the sample size was limited because uninfected females were rare, there was no difference in activity level among uninfected females injected with Primaquine or PBS alone (control females). This suggests that the observed difference in activity level between females injected with Primaquine and control females was due to the reduction of malaria load, rather than to the drug itself.

Infected great tit females injected with Primaquine showed a higher activity level than control ones. Activity is generally a costly behaviour, in particular in small animals because of high costs of locomotion and thermoregulation (Taylor et al., 1982; Karasov, 1992). The costs of activity could not be directly assessed here, but flight activity and parental provisioning behaviour have been shown to entail energetic costs to small passerine birds (Drent and Daan, 1980; Nudds and Bryant, 2000). This suggests that females relieved from parasite load were not constrained by energy drain from costly parental care behaviour (Drent and Daan, 1980) towards immune response, and were therefore able to invest more energy to maintain a high activity level during breeding, which could ultimately allow parents to achieve higher reproductive success. Alternatively, females relieved from parasite load did not need to reduce activity to facilitate the body response to the infection, if reducing activity is part of a 'malaria sickness syndrome' (Hart, 1988). Decreasing activity could decrease the encounter rate with parasite vectors and therefore reduce the risk of novel infections. Interestingly, a decrease in behaviours that could *a priori* enhance parasite transmission (e.g. novelty-seeking behaviour) in infected hosts has also been described in other host-parasite systems (e.g. Skallová et al., 2005). Importantly, both hypotheses (energy constrain and adaptive reduction of activity) are not mutually exclusive. On the contrary, if reduced activity increases encounter rate with parasite vectors (i.e. for example mosquitoes on an immobile prey), this result may be in line with the hypothesis that parasites can manipulate host behaviour to enhance transmission (Vyas et al., 2007; Lefèvre and Thomas, 2008; Poulin, 2010). Such examples of parasite manipulation remain however rare, and usually involve a trophic transmission between the intermediary and final host, which is not the case here.

The injection of the anti-malaria drug did not enhance female problem-solving performance or its improvement over successive attempts to solve the task. This suggests either that malaria infection does not affect problem-solving and learning performances in adult great tits or that the effects of malaria infection are long-lasting and thus require longer time for recovery than the period during which the Primaquine is effective. The high mortality associated with malaria infection during the early stages of infection (Nordling et al., 1998; Knowles et al., 2009) implies that only individuals with high quality immune system can resist infection and survive in the wild with chronic infections (Atkinson and van Riper III, 1991; Bensch et al., 2007). Chronic infections may affect only some behavioural traits, such as activity in our study population, but not others. Such selectivity of behaviours affected by chronic infection has been described previously in other host-parasite systems, for instance *Toxoplasma* infection in rats, where chronic infection converted the aversion of rats to feline odors into attraction in order to enhance the transmission of the parasite to his final host, the domestic cat (Vyas et al., 2007). However, chronic *Toxoplasma* infection did not influence learned fear, anxiety-like behaviour, olfaction, or non-aversive learning in rats (Vyas et al., 2007). Alternatively, behaviours such as activity that may vary rapidly could be impacted more by a short-term reduction of the parasite load induced by the anti-malaria treatment than problem-solving and learning performances, for which changes might require more time for the neural system to recover (Bangirana et al., 2006). Thus, the absence of effect of the treatment on problem-solving performance and improvement over successive attempts to solve the task may have been due to the short delay between injection and the problem-solving test (i.e. 5 weeks) and/or the short duration of the effect of the anti-malaria drug here. To identify which behavioural traits may be affected by malaria infection, future studies may experimentally infect hosts and measure a set of individual behavioural responses including cognitive traits on a longer term.

Importantly, the effect of parasitism on host behaviour can be expected to strongly depend on the timing of infection, and more particularly whether individuals were infected during ontogeny or at the adult stage (Kihara et al., 2006). Parasites often have developmental, long-term detrimental effects on cognitive abilities of hosts infected during early growth (e.g. John et al., 2008) while the effects may only be transient for adults (e.g. Dugbartey et al., 1998). Therefore, experimental infections by malaria at different life stages (nestling, adults) would be required to fully understand the short- and long-term effects of malaria infections on behavioural and cognitive traits in the wild.

Another explanation for the lack of effect of the anti-malaria drug on problem-solving performance and its improvement may be that these performances influence malaria load rather than the

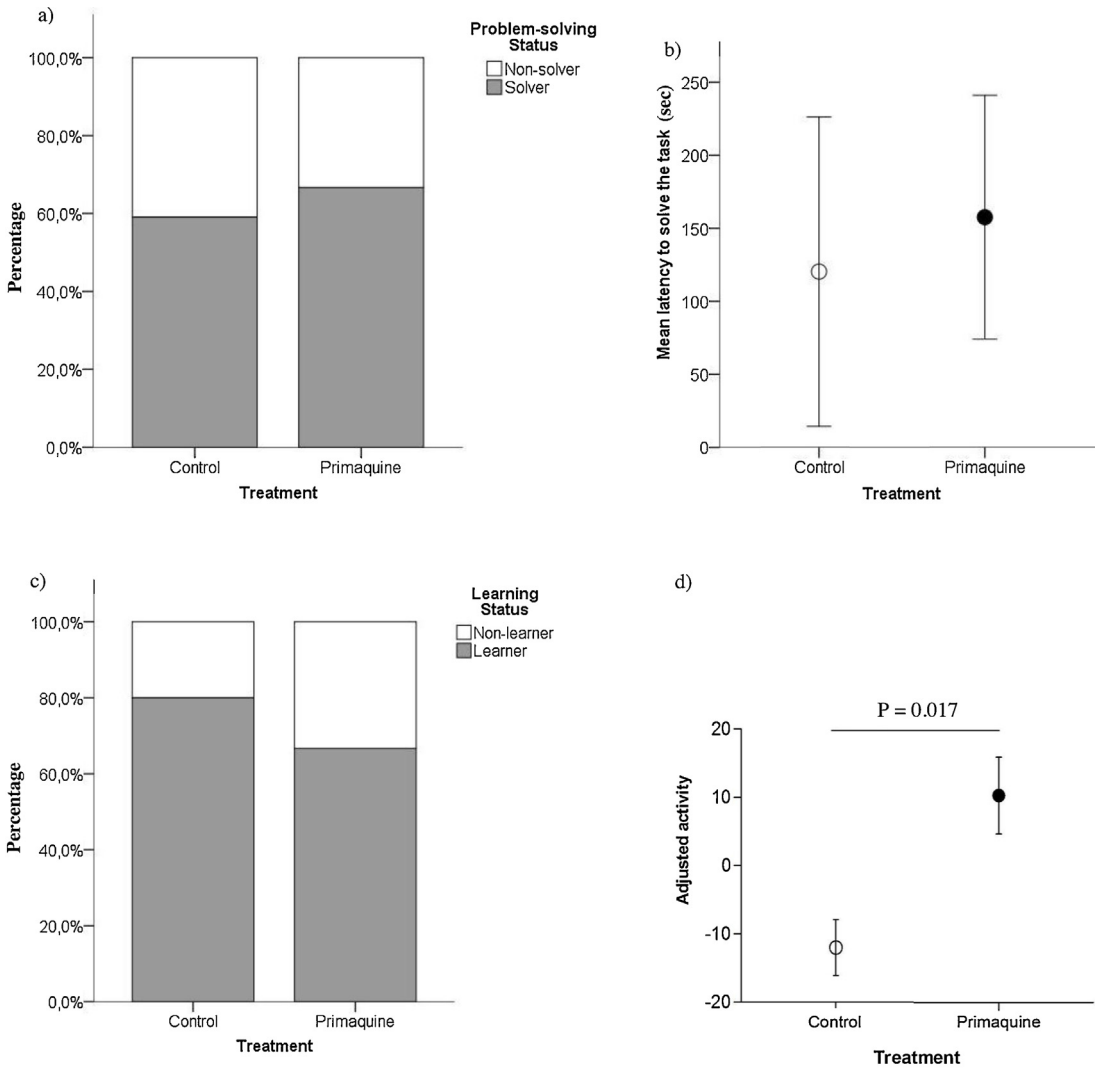


**Fig. 2.** Behavioural traits measured in infected great tits females injected with 0.1 ml of physiological salt either alone (Control) or with 0.01 mg of Primaquine (Primaquine). (a) Probability to solve the task (solvers *vs.* non-solvers); (b) mean latency to solve the task among solver females; (c) probability to improve the problem-solving performance among solver females that attempted to enter again (learners vs. non-learners); and (d) mean adjusted activity level on the nest box. Values were adjusted for the time spent on the nest box during testing, year and date of the test (see text). Whiskers indicate standard errors.

reverse. For example, novelty-seeking behaviour such as innovation may result in higher encounter and/or infection probability for hosts showing a higher propensity to explore new habitats (Barber and Dingemanse, 2010). Previous studies, conducted both at the inter- and intra-specific levels, found positive associations between operationalized measures of innovation and a proxy of infection level (Garamszegi et al., 2007; Audet et al., 2015). However, this contrasts with previous results reported for another great tit population, in which problem-solving performance measured using a different problem-solving task (rewarded by food and performed in captivity) was negatively correlated with malaria parasite load in females (but positively in males) (Dunn et al., 2011). Yet, contrasting results between studies could result from the reciprocity of effects between hosts and parasites (Blanchet et al., 2009; Poulin, 2013). In our study for example, more innovative individuals could be more prone to explore new habitats, thus more prone to encounter parasites and get infected (i.e. a positive relationship), but these infections could then lead host to reallocate energy from these behaviours to immune defenses or adaptively reduce cognitive performance as part of a sickness behaviour (i.e. a negative relationship). The effects of malaria parasites on cognitive traits may then be blurred and difficult to detect depending on the stage of this dynamic process at which the study is performed. Assessing the immunocompetence of individuals with different cognitive abilities at various stages of their life may help testing this hypothesis, for example using the immune response to phytohemagglutinin (PHA) injection, which has been shown to relate to exploration and problem-solving performance (Audet et al., 2015).

Importantly, malaria, as other parasitic infections, can be caused by different parasite species and lineages, and parasite prevalence and community composition can vary between locations, even at a small geographical scale, resulting in different populations being subjected to different parasite pressures (Wood et al., 2007; Marzal et al., 2011; Szoellosi et al., 2011). Behavioural alterations due to parasite infections can be caused by different molecular mechanisms that can be parasitemia-dependent (Larson and Dunn, 2001; Thomas et al., 2005; Poulin, 2010). Thus, the number and nature of behavioural traits affected by infection could depend on the number and type of parasites that infect an individual host. Further studies should examine in more details the impact of these different malaria parasites and the timing of infection (i.e. during or after development) on host behavioural traits to better understand the role of malaria parasite in shaping inter-individual and inter-population variation in behaviours.

## 5. Conclusions

Our study showed that anti-malaria drug injections increased previously infected adult female great tit activity level without affecting problem-solving and learning performances. These results suggest that some behaviours can respond to a short-term reduction in parasite load, probably due to the ability to reallocate energy from the immune system or a release from the need to adjust behaviour to face infection, while changes in cognitive performances might require more time if the neural system has to recover. Alternatively, parasite load might be a consequence, rather than a cause, of inter-individual variation in cognitive performance. Distinguishing these alternative explanations require further experimental work to investigate the effects of infection at larger time scales. Our study provides experimental support for the hypothesis that, besides ecological factors such as food availability and predation, malaria parasites constitute another ecological factor causing variation in host behavioural traits in wild animals and, as such, need to be taken into account when comparing behavioural responses between individuals and populations.

## Acknowledgements

We thank A. Dubiec and E. Podmokła for sharing their data regarding the treatment used per females and their initial malaria status, E. Camprasse and E. Pośliński and J. Lázaro Tapia for video recordings, D. Quent, U. Courcoux-Caro and S. Harrison for video analysis. We also thank L. Gustafsson for providing access to the site. We are grateful to J-N Audet and A. Marzal for helpful comments on the MS. This work was supported by a PICS grant from the CNRS (INEE, no 31520) to B.D., a NSERC grant to B.A. and a scholarship from the Biological Sciences Department of the Université de Montréal to L.C.

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