International Journal for Parasitology 48 (2018) 445–455

Contents lists available at ScienceDirect

International Journal for Parasitology

journal homepage: www.elsevier.com/locate/ijpara

Contrasting consequences of different defence strategies in a natural multihost–parasite system

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ARTICLE INFO

Article history:
Received 8 August 2017
Received in revised form 1 November 2017
Accepted 7 November 2017
Available online 31 January 2018

Keywords:
Species jump
Reservoir host
Disease ecology
Arms race
Host selection: Host switch

ABSTRACT

Hosts counteract infections using two distinct defence strategies, resistance (reduction in pathogen fitness) and tolerance (limitation of infection damage). These strategies have been minimally investigated in multi-host systems, where they may vary across host species, entailing consequences both for hosts (virulence) and parasites (transmission). Comprehending the interplay among resistance, tolerance, virulence and parasite success is highly relevant for our understanding of the ecology and evolution of infectious and parasitic diseases. Our work investigated the interaction between an insect parasite and its most common bird host species, focusing on two relevant questions: (i) are defence strategies different between main and alternative hosts and, (ii) what are the consequences (virulence and parasite success) of different defence strategies? We conducted a matched field experiment and longitudinal studies at the host and the parasite levels under natural conditions, using a system comprising *Philornis torquans* flies and three bird hosts – the main host and two of the most frequently used alternative hosts. We found that main and alternative hosts have contrasting defence strategies, which gave rise in turn to contrasting virulence and parasite success. In the main bird host, minor loss of fitness, no detectable immune response, and high parasite success suggest a strategy of high tolerance and negligible resistance. Alternative hosts, on the contrary, resisted by mounting inflammatory responses, although with very different efficiency, which resulted in highly dissimilar parasite success and virulence. These results show clearly distinct defence strategies between main and alternative hosts in a natural multi-host system. They also highlight the importance of defence strategies in determining virulence and infection dynamics, and hint that defence efficiency is a crucial intervening element in these processes.

1. Introduction

As a response to infection, hosts engage in two distinct defence strategies, although many hosts combine the two. They may resist infection, by investing in processes that reduce the fitness of parasites, and they may also tolerate an infection, through mechanisms that reduce the cost and damage of parasitism (Raberg et al., 2007, 2009). The relationship between levels of tolerance and resistance is predicted to be negative, and this has been confirmed in several systems (Fineblum and Rausher, 1995; Kover and Schaal, 2002; Raberg et al., 2007; Vincent and Sharp, 2014; Sears et al., 2015) (but see Maze-Guilmo et al., 2014). These trade-offs may arise because resistance and tolerance are redundant and costly traits (Raberg et al., 2009), and because some resistance mechanisms affect tolerance by inflicting autoimmune or inflammatory damage (Graham et al., 2005; Sears et al., 2011; Best et al., 2012). Previous studies have focused on the resistance/tolerance responses of individual host species (but see Rohr et al., 2010; Sears et al., 2015; Knutie et al., 2016). Here, by contrast, we examine the responses of several alternative, coexisting hosts to a shared parasite species.

Another relevant component of host–parasite interactions is virulence: the cost paid by the host as a result of an infection (Leggett et al., 2013). Virulence is not simply the inverse of tolerance, as the latter measures the damage per parasite (the slope of the relationship between host fitness and parasite burdens) (Raberg, 2014), whereas virulence is usually measured as morbidity or mortality, reflecting the damage caused by a typical infection (at mean infection intensity). Thus, virulence depends on factors

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https://doi.org/10.1016/j.ijpara.2017.11.001
0020-7519/

inherent to the host (tolerance and resistance) and to the parasite (exposure and pathogenicity) (Raberg et al., 2009, Raberg, 2014; Johnson et al., 2012). We note, too, that the efficiency of defence may be of particular significance for the outcomes of host–parasite interactions. For example, large efforts to reduce parasite fitness or damage, achieving little control of the infection and its consequences (low defence efficiency) will result in high virulence due to poorly constrained infection intensity and pathogenicity, plus the cost of resistance and the associated immunopathology.

The effect of availability of multiple host species on the ecology and evolution of tolerance and resistance has been little explored by theoretical and empirical studies, even though in nature most parasites use more than one host species (Woolhouse et al., 2001; Schmid-Hempel, 2011). This is of special relevance, as many emerging infectious diseases (e.g. HIV in humans, rinderpest in ruminants, canine distemper in wild carnivores), are the result of switches from one host species to another (Woolhouse et al., 2005). It is known that infections are often less virulent in 'reservoir' hosts than they are in alternative ones (Mandl et al., 2015), but the mechanisms underlying this have not been studied in the context of resistance and tolerance. Establishing how switching from main to alternative hosts affects the outcome of host–parasite interactions is critical to our understanding of the dynamics of parasites and their effects on different hosts, which in turn is essential to comprehend and attempt to control zoonotic and emerging infectious diseases.

A few studies in natural multi-host systems have shown that defence strategies and their outcomes may vary across host species, using either laboratory experiments (Rohr et al., 2010; Johnson et al., 2012; Sears et al., 2015) or field studies under natural conditions (Knutie et al., 2016), but to the best of our knowledge, there have been no studies investigating the interplay among tolerance, resistance, virulence and parasite productivity under natural conditions and in a naturally co-evolving multi-host system, nor has there been research comparing defence strategies (resistance and tolerance) and their outcomes between main and alternative hosts. This is the focus of the work described here.

Some neotropical parasitic flies of the genus *Philornis* Meinert, 1890 (Diptera: Muscidae) have unique life history traits that provide the rare opportunity to collect detailed empirical data on the interaction between host and parasite under natural conditions, which can shed light on the consequences of using different hosts on the fitness of both parasite and host. (For a description of the characteristics of this system, see Supplementary Data S1). Many *Philornis* spp. have larvae that are permanent s.c. parasites of bird nestlings (Texeira, 1999). Because nestlings can be monitored daily for the whole period they are susceptible to infection by *Philornis* spp., collection of sequential infection data from the whole bird community is feasible (Manzoli et al., 2013). Moreover, larvae of s.c. *Philornis* are relatively large and stay at the site where they penetrated the skin, so they can also be individually followed throughout this parasitic stage, enabling assessment of parasite success.

The predominant *Philornis* sp. in central Argentina is *Philornis torquans* (Nielsen, 1913; Monje et al., 2013; Quiroga et al., 2016). Although *P. torquans* is considered a generalist (Löwenberg-Neto, 2008), it is closely associated with Great Kiskadees, *Pitangus sulphuratus* (Linnaeus, 1766) (Passeriformes: Tyrannidae) (de la Peña et al., 2004; Antoniazzi et al., 2011; Manzoli et al., 2013). Given that prevalences are highest in Great Kiskadees, that the abundance of their broods determines the occurrence of *P. torquans* in the whole bird community, and that other host species are generally ignored if sufficient Kiskadee nestlings are available (Antoniazzi et al., 2011; Manzoli et al., 2013), Great Kiskadees are arguably the main host of *P. torquans* in central Argentina. Many other bird species can be alternative hosts of *P. torquans*, notably Thornbirds (*Phacellodomus ruber* and *Phacellodomus sibilatrix*) (de la Peña et al., 2004; Antoniazzi et al., 2011; Manzoli et al., 2013).

Here we offer an integrative analysis of the interplay of host resistance, tolerance, virulence and parasite success in this multi-host–parasite system, and offer quantitative data from experimental and longitudinal studies carried out in natural populations under natural conditions, to address two relevant questions: (i) are defence strategies different between main and alternative hosts and, (ii) what are the consequences of different defence strategies for hosts (virulence) and parasites (parasite success)?

2. Materials and methods

2.1. Community studied

The data were collected from two similar native forest patches located in the centre of Santa Fe Province (Argentina). One is a reserve belonging to Universidad Nacional del Litoral, Argentina (centre at 60°55′W, 31°23′S), the other one, a private field known as "Mihura" (centre at 60°47′W, 31°30′S). The area sampled within each site was 40 ha. Both sites are located alongside the Salado River and represent relics of the biogeographic province 'El Espinal'. The forest is dominated by medium sized tree species such as *Aspidosperma quebracho blanco*, *Geoffroea decorticans*, *Acacia caven* and *Prosopis* spp. (Arturi, 2006), which allow access to most nests present in the area. The climate in the region is Pampean Temperate, with an average annual temperature of 18 °C (mean minimum = 12 °C; mean maximum = 23 °C) (from www.climayagua.inta.gov.ar). Approximately 100 bird species have been reported to breed in this region, mostly of the Order Passeriformes (de la Peña, 2005).

At the study area, more than 20 species have been found to be parasitised by *P. torquans* larvae, but the Great Kiskadee is by far the preferred host, with prevalences over three times higher than those of the second most used host species (Manzoli et al., 2013). Among the most frequently parasitised alternative hosts are the Greater Thornbird, *Phacellodomus ruber* (Vieillot, 1817) and the Little Thornbird, *Phacellodomus sibilatrix* (Scalter, 1879) (Antoniazzi et al., 2011). For a description of the hosts studied see Supplementary Data S2. Molecular studies demonstrated that *P. torquans* is the only species of the genus present in the area, and to date the only lineage documented in central Argentina (Monje et al., 2013).

2.2. General approach

Studying host–parasite interactions in the wild is indispensable to be able to understand the ecological patterns underlying the dynamics and risks of infection. However, generating sound data from natural systems is challenging. Field (observational) studies are confounded by a myriad of interacting factors that make it difficult to separate pertinent patterns from background noise. Manipulative experimental approaches, on the other hand, can overcome this issue, allowing the dissection of the variables of interest, but some limitations arise when it comes to studying host–parasite interactions in natural systems. For example, either the manipulation (e.g. applying insecticides to reduce ectoparasites, see Knutie et al., 2016) or the controlled conditions employed (e.g. hosts maintained in containers, see Sears et al., 2015) may affect some host species more than others, causing distortion in the effects being measured and jeopardizing comparisons among host species.

To overcome these difficulties, we combined the virtues of matched experimental designs and the realism of observational longitudinal studies. First, a larval removal field experiment was

conducted on Kiskadees and Thornbirds to assess the effect of *Philornis* infection on nestling survival, growth, red blood cells (RBCs) and white blood cells (WBCs). This enabled us to establish cause-effect relationships between *Philornis* and most of the dependent variables selected to assess tolerance, resistance and virulence. Then we conducted a longitudinal study following all nestlings of the three host species of interest present in two similar bird communities during two breeding seasons, which produced a large sample size that allowed us to establish temporally coherent associations between *Philornis* burdens and the variables related to nestling fitness (e.g. whether RBC counts decline soon after infection), while controlling for potential confounders and effect modifiers (see Supplementary Table S1). Finally, a third study based on following individual larvae on each host species allowed assessment of parasite success (survival) and an additional measure of resistance.

2.3. Haematological procedures

Once a week, a blood sample was obtained from every nestling by clipping the tip of a nail and collecting the emerging drops in heparinised capillary tubes. This allowed extraction of approximately 10 μl of blood each time. The tubes containing the blood samples were kept refrigerated in a cooler until taken to the laboratory (within a 4 h period). The direct Rees Ecker Method was used for absolute counts of RBCs and WBCs. The blood was extracted from the capillary tubes and homogenized on a glass slide, and a 2 μl aliquot was mixed with 98 μl of Ress Ecker solution (Sodium citrate: 3.8 g; 40% formaldehyde: 0.2 ml; cresyl blue: 0.3 g; distilled water: 100 ml), thus achieving a 1:50 dilution (modified from Lucas and Jamroz, 1961). Counts were made using a haemocytometer (Neubauer Improved, Brand, Germany). This solution leaves WBCs tainted in blue and refringent, facilitating the count.

2.4. Measures of resistance, tolerance, virulence and parasite success

Operationally, resistance has been defined as the inverse of the number of parasites per host given constant parasite exposure (Raberg et al., 2007, 2009; Rohr et al., 2010). This has two major problems; first, parasite exposures are rarely constant and are difficult to quantify in nature, and second, it neglects that resistance efforts may vary in efficiency (as it only measures the outcome, not the investment). Different investments in resistance may yield similar parasite reductions, and vice versa, and this needs to be acknowledged for a better understanding of the phenomenon. To overcome these issues, we provide two measures of resistance that are independent of parasite burden, one of which focuses on investment in resistance (inflammatory response).

The host immune response to myiasis-causing larvae combines non-specific and specific elements (Otranto, 2001). In our system, the non-specific response predominates, as specific immune responses against botflies do not appear until a few weeks post-infection (Otranto, 2001), when nestlings have already left the nest or are fully fledged and about to leave it. The non-specific immune response to burrowing larvae consists primarily of an inflammatory reaction, which is reflected locally by a cellular infiltrate and edema, and systemically by an elevated WBC count (Owen et al., 2010). Hence, we used WBC counts as a proxy for the investment in resistance. With a field experiment (see Section 2.6), we provided evidence to confirm that *Philornis* caused increases in WBCs. The investment in resistance was expressed as regression coefficients representing the change in host WBCs per parasite, obtained from Generalized Linear Mixed Models (GLMM, see Section 2.9) with WBCs per microlitre as the response (with a Poisson distribution) and, as independent variables, the burdens of larvae at their first (L1) or last instar (L3) stage present during the preceding visit (2–3 days previously) (plus several potential confounders). These data were obtained from longitudinal study 1 (see Section 2.7). L1 burdens were used to evaluate early responses, whereas burdens of L3s (which had been on the host for 3–4 days) were used to assess whether the inflammatory response was sustained.

The outcome of resistance was approximated by conducting a survival analysis with individual larvae as the study unit (see Section 2.8). Survival estimates were used as a measure of parasite success, whereas their inverse (probability of failure) reflected the outcome of resistance. In summary, we measured resistance in two novel ways: one that focuses on the efforts aimed at controlling the parasite (host immune response per parasite), and another that shows the actual success achieved (parasite failure). The combination of both measures can give an idea of the efficiency of resistance efforts, by relating control success to resistance investment.

Tolerance was operationally defined as the regression slope between parasite burden and host fitness (Raberg et al., 2009). These slopes were calculated with models constructed with data from longitudinal study 1 (see Section 2.7). The proxies of fitness used were the presence/absence of nestlings during the following 2–3 days, daily growth in tarsus length, and RBC levels.

Virulence is not merely the inverse of tolerance, but an outcome of the host–parasite interaction determined by the interplay between infection and host defences, as it depends on the detrimental effect per parasite (reflected by tolerance) and on the intensity of parasitism (affected by resistance, and dependent on parasite exposure) (Read et al., 1999, 2009). The inextricable relationships among tolerance, resistance, virulence and parasite success are depicted in Fig. 1. Virulence is therefore the damage caused by the parasite burden, which is determined by parasite exposure and host resistance. Such damage not only depends on the infection intensity, but also on host tolerance. Here we attempt to assess the virulence of a typical infection, and hence we estimate it by multiplying the mean parasite intensity of each host species (mean number of parasites found in the infected hosts) by the per-larva damage documented in longitudinal study 1 (see Section 2.7). It is noteworthy that measures of virulence were inevitably obtained using the same fitness parameters used for tolerance (nestling survival, growth and RBCs), but while tolerance reflects the (inverse of) damage per parasite, virulence is measured as the damage caused by a typical infection, having as a reference fitness in the absence of parasites (vigour, see Fig. 1). That is, the impact on each fitness parameter caused by a burden equal to the mean larval intensity for each host species.

Besides the estimate of parasite fitness described above (parasite survival as estimated by longitudinal study 2), we also calculated an estimate of parasite productivity that indicates the number of larvae that achieve development per available host at a given time, using this simple formula:

$$\text{Parasite productivity in host}_i = \text{prevalence in host}_i * \text{mean intensity in host}_i * \text{larval survival in host}_i$$

2.5. Data collection

During the breeding seasons of 2008–2009 (September–April) and 2009–2010 (September–May), both 40 Ha areas were examined exhaustively each week, looking for signs of active nests. Once nests under construction or with activity were found, those were marked with a flagging tape and geo-located. Nestling manipulation was conducted following guidelines by Ralph et al. (1993) and de Beer et al. (2001). All procedures conducted in this study comply with the current National and Provincial laws, and were

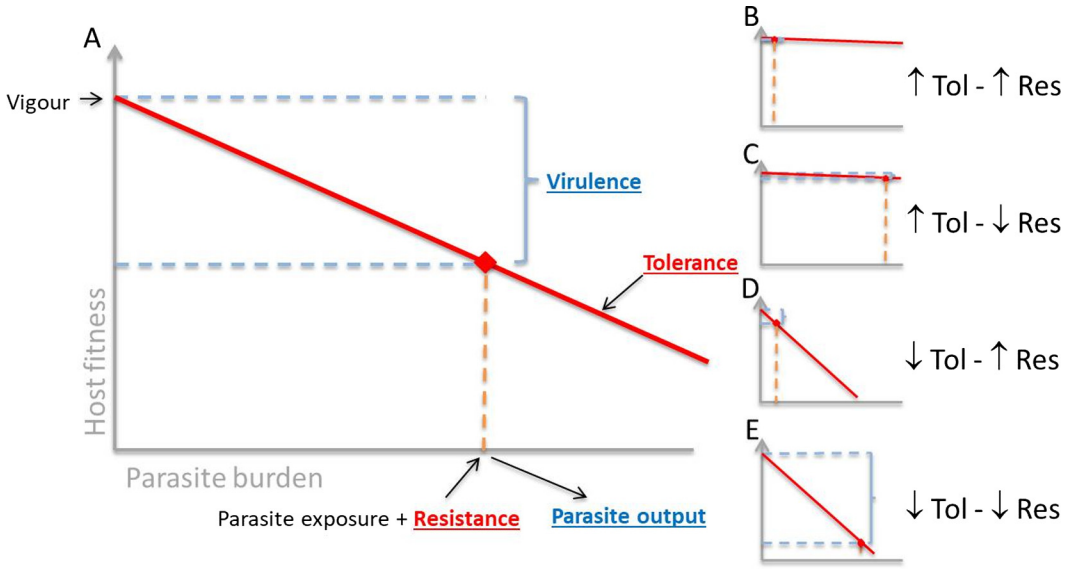

Fig. 1. The relationships among the components of the host–parasite interaction and its outcomes (virulence and parasite output). (A) The parasite burden results from parasite exposure and the ability of the host to control the parasite: its resistance. The host tolerance is the ability to reduce the damage caused by each parasite, and here it is represented by the slope of the red line. The steeper the slope, the less tolerant the host. The virulence is the loss of host fitness at a given parasite burden, having as a reference the vigour (fitness when parasite burden equals zero). For a given parasite, both virulence and parasite output depend on parasite exposure, resistance and tolerance. Using mean infection intensities (diamond), the virulence of a typical infection may be estimated. (B-E) Different scenarios of resistance (Res) and tolerance (Tol). Assuming constant parasite exposure, a highly tolerant and resistant host (B) yields the lowest levels of virulence and parasite output. The highest parasite output occurs in hosts with high tolerance and low resistance (C), and the greatest virulence in hosts that are low in both tolerance and resistance (E). In a host that is highly resistant but with low tolerance (D), the virulence is not high because burdens are low.

approved by the Bioethics Committee of Universidad Nacional del Litoral, Argentina.

For the three studies, all broods of the three species of interest were periodically visited and each nestling was thoroughly examined for parasites, from hatching until they left the nest or died. Larvae were individually identified and their stage and location recorded every time the host was examined, as L1 (<4 mm), L2 (4–7 mm) or L3 (>7 mm). In addition, morphometric measures were taken, including the length of the tarsus (in mm). Once a week, a blood sample of approximately 10 μl was taken with a heparinized capillary tube, by cutting the tip of a nail. Additional information about the host, the brood, the nest and the environment was recorded to account for potential confounding and effect modification in the statistical analysis (Supplementary Table S1).

2.6. Matched field experiment

Broods of Great Kiskadee and Greater Thornbird identified in the surroundings of the area surveyed for the longitudinal studies (below) were recruited for a larval removal experiment. The Little Thornbird was excluded from this study because our early observations suggested that larvae often failed to progress to stages beyond L1, so removal would not result in the desired contrast between treated and control hosts. Recruited broods were followed daily from egg to fledging, and only the subset that was parasitized was considered for the analysis. The final number of broods used was 36 (23 Great Kiskadees and 13 Greater Thornbirds). Within each brood, nestlings were marked and randomly assigned to either of two treatments: complete daily removal of L1s; and mock removal (hereafter, controls; so both treatments were exposed to similar manipulation stress). These procedures were repeated every day until the nestlings left the nest or died. If in a given brood, all nestlings of one of the treatment groups died, the remaining siblings were followed for 2 more days.

2.7. Longitudinal study 1: Repeated measures of nestlings

Nests of Kiskadees or Thornbirds that were identified with activity (nest building or lining) were recruited for longitudinal study 1. Each brood was systematically followed and sampled three times each week (at intervals of 2–3 days) as described in Section 2.6.

2.8. Longitudinal study 2: Larval survival

The subset of data used for this study came from records of longitudinal study 1, and consisted of all larvae that were initially recorded at their first stage (L1, <4 mm) and on young nestlings (≤5 days old, to reduce the variability in larval success that might arise with age). The aim of this study was to compare larval success on each of the three studied hosts (116 larvae of 13 Great Kiskadee nesltings, 51 larvae of five Greater Thornbirds, and 46 larvae of 15 Little Thornbirds). Larval success (presence and growth) was assessed 2–3 days later (when the larva was supposed to be at its final instar, but before it was ready to leave the host), allowing the probability of survival to be estimated.

2.9. Statistical analysis

Analyses were carried out using Linear Mixed Models (LMM; daily tarsus growth and RBC levels in the field experiment and longitudinal study 1), Generalised Linear Mixed Models (GLMM; WBC concentration with a Poisson response and survival with a binomial response for the field experiment and longitudinal study 2), and Cox Mixed effects Models (survival analysis in longitudinal study 1). Inference was performed using Information theory, and model selection was conducted by comparing all possible models using the Akaike Information Criterion (AIC) (Burnham and Anderson, 2002). The software used was R 3.2.3 (the R-Project for Statistical Computing; //http:www.R-project.org).

3. Results

3.1. Quantifying Philornis parasitism

In the two 40 Ha forest patches that were examined exhaustively every week during two breeding seasons, we recorded 2291 observations based on 43 broods of Greater Thornbird, 79 broods of Little Thornbird and 70 broods of Great Kiskadee.

Parasite exposure (presence of at least one larva) was documented in 59% of 69 broods of Great Kiskadee, 38% of 78 broods of Little Thornbird and 26% of 42 broods of Greater Thornbird. The prevalence (proportion of nestlings parasitised at a given time) was 41.2% in Great Kiskadees, 12.6% in Greater Thornbirds and 10.6% in Little Thornbirds. Mean intensities (mean burden in infected hosts) were 11.1, 13.4 and 3.6 larvae per nestling, respectively.

3.2. Establishing cause-effect relationships: Larval removal experiment

Daily larval removal resulted in a 36-fold increase in the odds of survival of Greater Thornbird nestlings to a level similar as Great Kiskadees, which showed no detectable effect of larval infestation on survival (Table 1, Supplementary Fig. S1). In both species, RBC counts were lower in parasitized nestlings compared with treated ones, depending on the number of larvae (L3) that were parasitizing the brood. Each L3 on a control host accounted for a difference of approximately 54,000 RBCs per microliter between treated and control nestlings (Table 2). No effect of larval removal was observed on growth (Supplementary Table S2).

An effect of larval removal on WBC counts was also only observed in Greater Thornbirds (Fig. 2; Table 3). Parasitized Greater Thornbird nestlings had 50% greater WBC levels than those that had their larvae removed on a daily basis.

Table 1
Generalized Linear Mixed Model with a binomial response, showing the effect of daily larval removal on the probability of survival, by nestling species (Great Kiskadee and Greater Thornbird). Brood ID was included as a random intercept.

Model: Survival ~ Host species + Larval removal + Host sp. × Larval removal; Random intercept: brood ID				
Parameter	Coefficient (log odds)	S.E.	*z* value	95% CI
Intercept	−1.5183	0.9217	−1.647	−3.325, 0.288
Larval removal	3.5726	1.1848	3.015	1.250, 5.895
Host sp. (Grt. Kiskadee)*	3.1620	1.2899	2.451	0.634, 5.690
Larval removal × Host sp. (Grt. Kiskadee)	−2.9357	1.3463	−2.181	−5.574, −0.297

CI, confidence interval; Grt., Great.
*Reference host sp. = Greater Thornbird.

Table 2
Linear Mixed Model showing the effect of daily larval removal on red blood cell levels (cells/microlitre), adjusting by age and by the mean larval burden that were left on control siblings. Host species and its interaction with the treatment were removed from the model because those were not important for the model, indicating no differences between host species. The random intercept included was brood ID/nestling ID.

Model: RBC ~ Mean L3 burden on controls + Removal + Age + L3 burden × Larval removal; random intercept: brood ID/nestling ID				
Parameter	Coefficient	S.E.	*t* value	95% CI
Intercept	1,039,924	101,403	10.255	839,980, 1,242,032
Mean L3 burden on controls	−30,933	12,630	−2.449	−55,843, −6035
Larval removal	−40,576	86,965	−0.467	−212,037, 131,154
Larval removal × L3 burden	54,288	15,520	−3.498	23,670, 84,911
Age	71,021	8917	7.965	53,387, 88,687

CI, confidence interval.

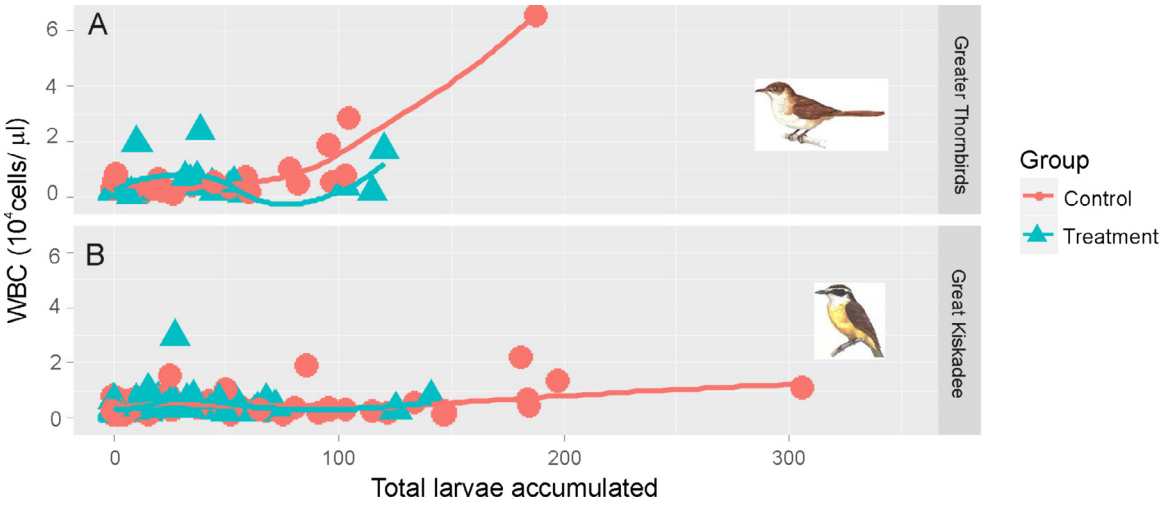

Fig. 2. Inflammatory response by treatment and species. White blood cell (WBC) counts in nestlings of Greater Thornbird (A) and Great Kiskadee (B) by cumulative burden of *Philornis* larvae (number of larvae that have fed on a nestling at the time of observation). Circles represent records from control nestlings, and triangles treated ones. In the latter, the x-axis represents the number of larvae that have been removed, whereas in the former the total number that was left parasitizing the host. Bird illustrations were extracted from: de la Peña, M.R. Aves Argentinas (Tomo 2). Eudeba – Ediciones UNL. Buenos Aires, Argentina. 496 pp. ISBN 9789876579865..

3.3. Defence strategies and their outcomes across hosts: Longitudinal studies

3.3.1. Tolerance

Consistent with the experimental findings, *Philornis* infection had no effect on nestling survival in Great Kiskadees, but it affected both Thornbird species considerably (Table 4; Fig. 3). Every larva reduced the probability of survival over the following 2–3 days by 6% for Greater Thornbirds and by 16% for Little Thornbirds (Table 4; Fig. 3C). Also in agreement with the field experiment, *Philornis* infection affected RBC counts and this effect was not significantly different between host species (Supplementary Table S3). Unlike results from the experimental study, there was a significant negative effect of *Philornis* infection on growth (increases in tarsus length) that did not differ between host species. However, an interaction with age shows that the negative effect decreases as the nestling grows older (Supplementary Table S4). For example, for a 5 days old nestling every larva reduces the daily growth by 0.02 mm, whereas the effect drops to 0.01 mm for a 10 days old nestling.

3.3.2. Resistance investment

Great Kiskadees showed no increase in WBC counts in response to *Philornis* infection (Table 5, Fig. 4), as also observed in the experiment, but both Thornbirds showed strong positive effects of L1 burdens on WBC counts (of greater magnitude in Greater Thornbirds, Fig. 4A). However, for L3s (representing prolonged exposure) the correlation becomes highly positive in Little Thornbirds and negative in Greater Thornbirds (Fig. 4B).

3.3.3. Resistance outcome

The results of longitudinal study 2 showed that larval success was remarkably different among host species (Table 6; Supplementary Fig. S2). The larval failure rate in Great Kiskadees was very low, reaching just 8% (95% Confidence Interval (CI): 2%; 23%). In Little Thornbirds, on the other hand, larval failure was highest at 82% (59%; 94%), while Greater Thornbirds achieved relatively low larval failure at 22% (5%; 51%).

3.4. Virulence, parasite success and summary of results

3.4.1. Virulence

With the effect sizes from the models presented in Section 3.3 (Table 4, Supplementary Tables S3, S4) and the mean intensity values described in Section 3.1, virulence estimates were as follows: i) at mean burdens, there is no impact on survival in Great Kiskadees, whereas its probability decreases 42% for Little Thornbirds and 46% for Greater Thornbirds; ii) typical infections cause a substantial reduction in daily tarsus growth of Greater Thornbirds and Great Kiskadees (0.47 mm and 0.46 mm less growth than a non-infected nestling, respectively), but only a small effect in Little Thornbirds (0.19 mm); iii) similarly, RBC counts decreased on average by 205,000 and 173,000 cells per microlitre in infected Greater Thornbirds and Great Kiskadees, respectively, and only 50,500 in Little Thornbirds.

3.4.2. Parasite fitness

Larval survival (inverse of parasite failure, above) was very high in Great Kiskadees (91.8%; Table 6), relatively high in Greater Thornbirds (78.4%), and low in Little Thornbirds (16.4%). Taking into account the prevalence and mean burden present in each host species (see Section 3.1) and the estimates of larval survival, parasite productivity was 4.2 larvae per available host at a given time in Great Kiskadees, 1.3 in Greater Thornbirds, and only 0.07 in Little Thornbirds.

Fig. 5 summarises the results, showing the different strategies of each host with their resulting contrasting outcomes for both host and parasite.

4. Discussion

Only a few studies have explored whether defence strategies and their outcomes may vary across host species (Rohr et al., 2010; Johnson et al., 2012; Sears et al., 2015; Knutie et al., 2016). Experimental studies comparing trematode infections in amphibian hosts found that the pace of life (life history style in terms of timing, comprising a slow-fast continuum) is associated with resis-

Table 3
Generalized Linear Mixed Model with a Poisson response, showing effect of larval removal on white blood cells, by nestling species (Great Kiskadee and Greater Thornbird). Brood ID was included as a random intercept.

Model: WBC ~ Host species + larval removal + Host sp. × larval removal; random intercept: brood ID				
Parameter	Coefficient	S.E.	*t* value	95% CI
Intercept	8.6961	0.1661	52.37	8.362; 9.026
Larval removal	−0.4089	0.1799	−2.27	−0.767; −0.051
Host sp. (Grt. Kiskadee)*	−0.3874	0.2023	−1.91	−0.789; 0.022
Larval removal × Host sp (Grt. Kiskadee)	0.4419	0.2168	2.04	0.011; 0.874

CI, confidence interval; Grt., Great.
*Reference host sp. = Greater Thornbird.

Table 4
Mixed Cox Proportional Hazard model showing the effect of *Philornis* infection on survival for each of the three nestling species studied, while adjusting for brood size. Random intercepts included were brood ID/nestling ID.

Model: Survival ~ Larval burden + Host species + Brood size + Larval burden × Host species; random intercept: brood ID/nestling ID				
Parameter	Coefficient	S.E.	z value	95% CI
Larval burden	0.003	0.012	0.23	−0.021, 0.027
Host sp. (Grt. Thornbird)*	−0.023	0.606	−0.04	−1.210, 1.165
Host sp. (Ltl. Thornbird)*	0.562	0.491	1.15	−0.400, 1.524
Larval burden × Host sp. (Grt. Thornbird)	−0.059	0.028	−2.12	−0.114, −0.004
Larval burden × Host sp. (Ltl. Thornbird)	−0.155	0.056	−2.80	−0.268, −0.045
Brood size	−0.812	0.143	−5.68	−1.092, −0.531

CI, confidence interval; Grt., Greater; Ltl., Little.
*Reference host sp. = Great Kiskadee.

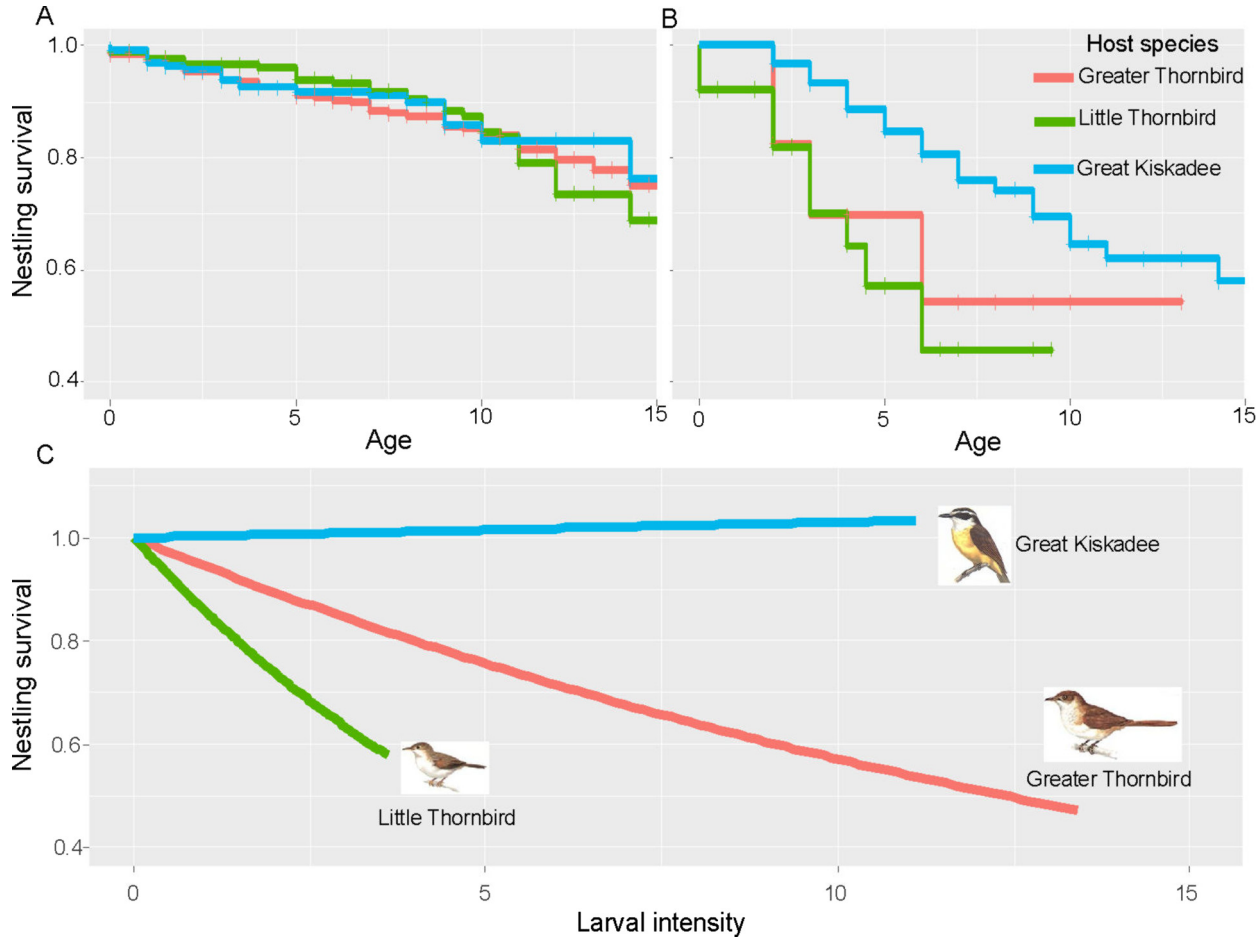

Fig. 3. Impact on survival by species. The probability of survival as nestlings age are shown for individuals not parasitized by *Philornis* (A) and for nestlings harbouring ≥ 5 larvae (B). (C) The probabilities of survival (relative to uninfected hosts) as a function of *Philornis* burdens, as estimated by the Mixed Cox Proportional Hazard model (see Table 4) are shown for each host species. The length of each line represents the third quartile of the burden recorded for each species. Bird illustrations were extracted from: de la Peña, M.R. Aves Argentinas (Tomo 2). Eudeba – Ediciones UNL. Buenos Aires, Argentina. 496 pp. ISBN 9789876579865.

Table 5
Generalized Linear Mixed Model with a Poisson response, evaluating the effect of *Philornis* infection (at different larval instars, L1 and L3) on white blood cell levels. Additional terms and interactions were included to account for potential confounding or effect modification (see Supplementary Table S1 for details). The random intercept included was brood ID/nestling ID. Bold terms are statistically significant.

Parameter	Coefficients	S.E.	95% CI	
Host sp. (Grt. Thornbird)*	−0.106	0.165	−0.430	0.218
Host sp. (Grt. Kiskadee)*	−0.078	0.145	−0.361	0.206
$L1_{t-1}$	**0.128**	**0.043**	**0.043**	**0.213**
$L3_{t-1}$	**0.615**	**0.188**	**0.247**	**0.982**
Host sp. (Grt. Thornbird) × $L1_{t-1}$	0.070	0.215	−0.351	0.490
Host sp. (Grt. Kiskadee) × $L1_{t-1}$	**−0.129**	**0.049**	**−0.225**	**−0.034**
Host sp. (Grt. Thornbird) × $L3_{t-1}$	**−0.702**	**0.246**	**−1.184**	**−0.219**
Host sp. (Grt. Kiskadee) × $L3_{t-1}$	**−0.612**	**0.188**	**−0.981**	**−0.244**
Age	0.069	0.036	−0.002	0.141
Age^2	**−0.006**	**0.002**	**−0.010**	**−0.002**
Age^2 × Host sp. (Grt. Thornbird)	0.001	0.001	−0.001	0.003
Age^2 × Host sp. (Grt. Kiskadee)	**0.004**	**0.001**	**0.003**	**0.006**
$Rain_{t-1}$	**−0.005**	**0.001**	**−0.007**	**−0.003**
Year (II)	−0.092	0.169	−0.423	0.240

CI, confidence interval, Grt. Thornbird, Greater Thornbird; Grt. Kiskadee, Great Kiskadee.
*Reference host sp. = Little Thornbird.

tance and tolerance (Johnson et al., 2012; Sears et al., 2015). One of these studies found that fast-lived species exhibited lower resistance and tolerance (i.e. had the highest levels of parasite load and pathology) compared with hosts with a slow pace of life (Johnson et al., 2012). A second experiment showed a different pattern, as tadpole pace-of-life was again a negative predictor of tolerance, but a positive predictor of behavioural resistance (Sears et al., 2015). Recently, Knutie et al. (2016) found that Galapagos mocking birds tolerate infections by an introduced parasite, *Philornis downsi*, whereas Darwin's finches are heavily impacted. Here we offer data

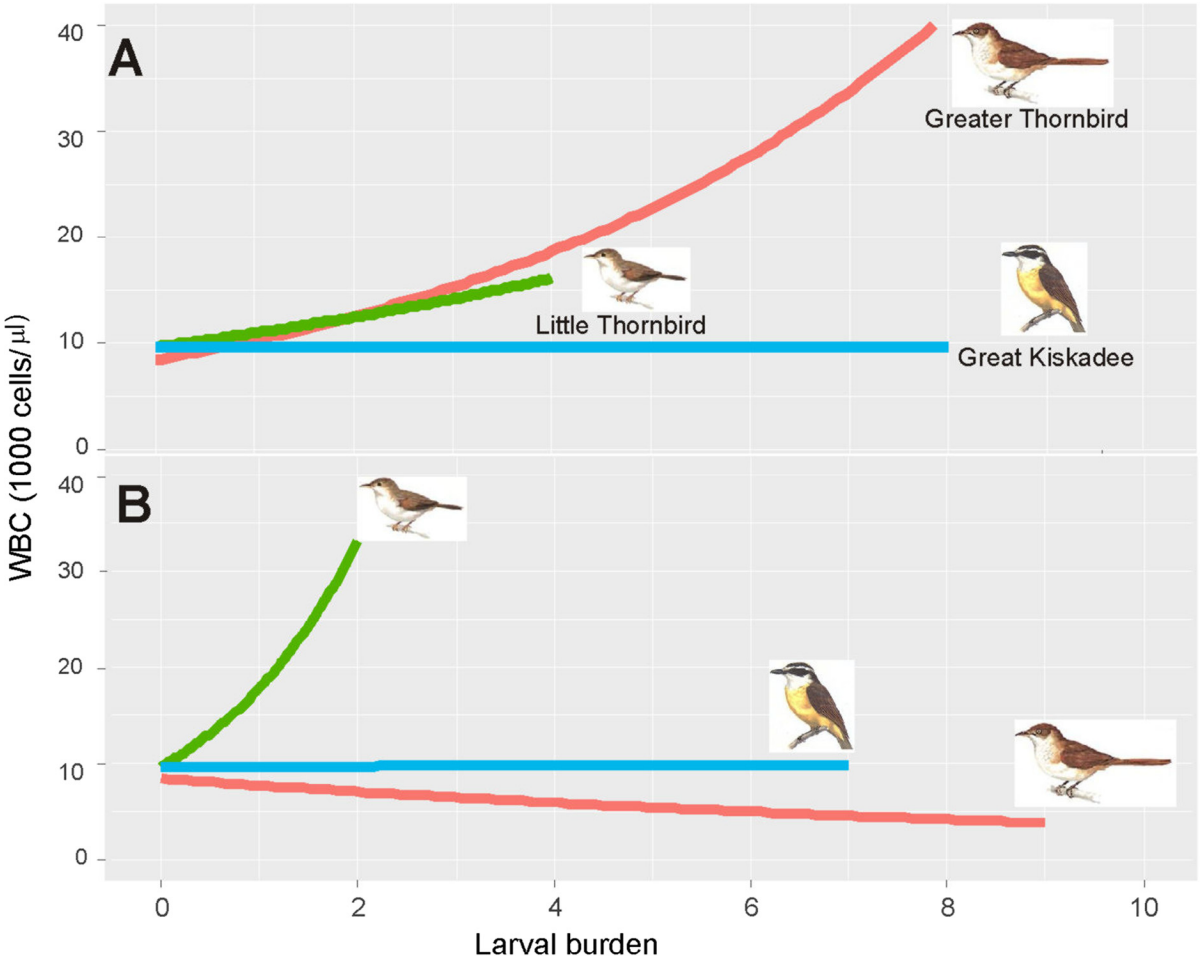

Fig. 4. Inflammatory response as a proxy of resistance efforts. Predicted host white blood cell (WBC) levels as a function of *Philornis* larval burden, (A) L1, (B) L3, by host species, as predicted by a Generalized Linear Mixed Model with a Poisson response (see Table 5). The length of each line represents the third quartile of the burden recorded for each species. Bird illustrations were extracted from: de la Peña, M.R. Aves Argentinas (Tomo 2). Eudeba – Ediciones UNL. Buenos Aires, Argentina. 496 pp. ISBN 9789876579865.

Table 6
Generalized Linear Mixed Model with a binomial response, showing the effect of host species on larval survival. Nestling ID was included as a random intercept.

Model: Larval survival ~ Host species + Age; random inercept: nestling ID				
Parameter	Coefficient	SE	95% CI	
Intercept	−1.487	0.602	−2.740	−0.357
Host species (Grt. Thornbird)*	2.778	0.706	1.457	4.418
Host species (Grt. Kiskadee)*	3.907	0.649	2.682	5.506
Age	−0.439	0.131	−0.763	−0.169

CI, confidence interval; Grt. Thornbird, Greater Thornbird; Grt. Kiskadee, Great Kiskadee.
*Reference host sp. = Little Thornbird.

under natural conditions comparing defence strategies (resistance and tolerance) and their outcomes between main and alternative hosts in a naturally co-evolving system.

Defence strategies and infection outcomes were dissimilar across the three studied hosts. Such results may be explained by two plausible mechanisms: parasite-derived and host-derived traits. A parasite-derived explanation may be that *Philornis* larvae show phenotypic plasticity and are able to inhibit the inflammatory response successfully in Great Kiskadees, but fail to do so in Thornbirds. A second explanation is that these hosts have evolved different defence strategies against the same parasite. Although establishing this important distinction warrants further research, in this case we prefer to favour the host-derived explanation due to the following reasons. Firstly, both the field experiment and the longitudinal study showed no sign of inflammatory response in Great Kiskadees. If this was due to perfect inhibition achieved by the larvae, it makes little biological sense that the same inhibiting mechanisms would perform so poorly in other birds of the Order Passeriformes (Otranto, 2001), especially in the presence of co-evolutionary history. Second, while the existing literature supports the view that the same parasite may have varying pathological effects across a range of hosts species due to differences in host defences (e.g. Graham et al., 2005; De Roode and Altizer, 2010; Zhao et al., 2015), evidence of changes in pathogenicity attributable to parasite phenotypic plasticity related to using different hosts is still lacking.

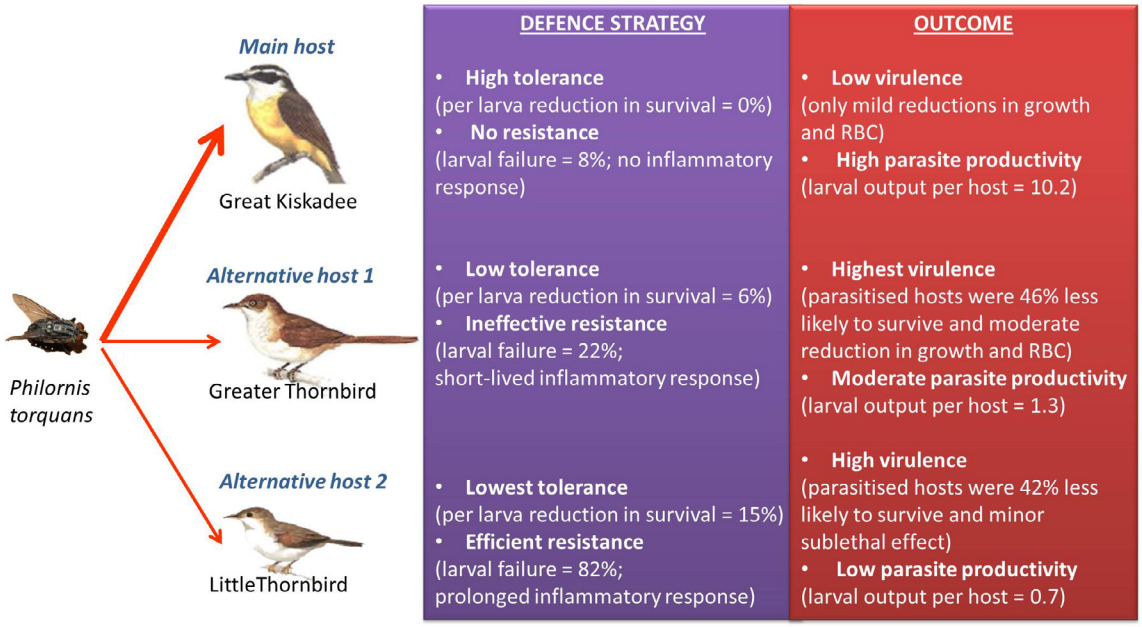

Fig. 5. Representation of the interaction between *Philornis torquans* and its main hosts. Adult *P. torquans* females largely prefer Great Kiskadees over other potential host species (represented by the thicker arrow, the equal narrow width of arrows going to Thornbirds denote similar lower exposures in these). Great Kiskadees have a strategy of tolerance without resistance, whereas both Thornbirds invest in efforts to reduce parasite fitness. The larger the resistance efforts, the lower the tolerance, but the greater the parasite reduction achieved. These diverse strategies resulted in contrasting outcomes: virulence was greatest in Greater Thornbirds and lowest in Great Kiskadees. Parasite productivity (the number of larvae that successfully develop per available host at a given time) was highest in Great Kiskadees, very low in Little Thornbirds, and intermediate in Greater Thornbirds. Bird illustrations were extracted from: de la Peña, M.R. Aves Argentinas (Tomo 2). Eudeba – Ediciones UNL. Buenos Aires, Argentina. 496 pp. ISBN 9789876579865.

In the main host, the strategy was to tolerate without resisting, which appeared to benefit both parasite and host. On the other hand, both Thornbirds fought the infection and showed little tolerance, but the outcomes differed between them, depending on the magnitude and efficiency of the resistance efforts elicited. The Little Thornbird was the least tolerant of the host species studied, but as its parasite burdens were generally low, the population-level impact of *Philornis* infection on survival, growth and RBCs (i.e. the mean virulence) was lower than that suffered by Greater Thornbirds, which despite being more tolerant, had higher larval intensities.

The importance of the defence strategy employed for the outcome of host–parasite interactions is poorly understood. A revealing experiment recently conducted with laboratory rodents infected with *Listeria monocytogenes* showed that the strategy adopted by hosts was genetically determined and resulted in two different infection outcomes, survival or death (Lough et al., 2015). Here, our empirical data provide further evidence for host resistance and tolerance playing a major role in determining infection outcomes – in this case in a naturally co-evolving system. As parasites evolve quickly and hosts have to focus on many selection pressures besides their multiple parasites, it has been argued that hosts are unlikely to win the 'arms race' against parasites (Roy and Kirchner, 2000; Boots 2008). This, in turn, has generated some parasite-centric views on the ecology and evolution of host–parasite interactions. For example, virulence has been considered a trait of that interaction mainly determined by parasite evolution, which results from trade-offs between host damage and transmission (the so called trade-off theory for virulence) (Alizon et al., 2009; Leggett et al., 2013). In the *Philornis*-nestling system studied here, however, the main determinant of differential virulence between host species appears to be the host defence strategy, leaving little room for the trade-off theory and strongly suggesting an important role for hosts in the evolution of virulence. Being highly tolerant and showing negligible resistance, Great Kiskadees are able to keep virulence at minimum levels, which also benefits the parasite (i.e. there is optimal host exploitation and 'transmission' without virulence). Further, our results emphasise the clear distinction between virulence and tolerance, despite both being calculated using per parasite damage data. Although the damage per parasite was greatest in Little Thornbirds (lowest tolerance), infections in Greater Thornbirds were typically more virulent (greater impact on survival, growth and RBCs).

In this system, our results suggest that the inflammatory response may be a mechanism contributing substantially to the pathogenicity observed in Thornbirds. Conversely, preventing this response seems to be one of the main mechanisms of tolerance in Kiskadees. A systemic inflammatory response (as observed in Thornbirds here) is among the costlier host defence efforts (Lee et al., 2005), and is also known to exert profound immunopathological effects (Sears et al., 2011). The regulation of the inflammatory response has been implicated as a mechanism of tolerance, whereas inflammation promotes resistance but reduces tolerance to parasites (Sears et al., 2011). The fact that the inflammatory response causes resistance at the expense of tolerance suggests that these distinct defence strategies are not as independent as previously argued (Ayres and Schneider, 2008; Maze-Guilmo et al., 2014), at least in systems where resistance is highly costly and/or self-damaging. This, in turn, confirms that resistance, tolerance, virulence and parasite success are deeply inter-related. In our system, the most tolerant host was also the least resistant and the one with greatest parasite outputs, whereas the most resistant host was the least tolerant, and the host lying between the other two in terms of tolerance and resistance showed the highest virulence levels. The inflammatory response to control the infection and, crucially, its efficiency, appear to have a central mechanistic role underlying those associations.

The efficiency of defence efforts has been neglected by previous studies, as resistance has been traditionally measured as the outcome of these efforts (Raberg et al., 2009), often not considering

their magnitude (i.e. investment for parasite control). Here we show that in phylogenetically related hosts that choose to resist, virulence and parasite productivity depend on the efficiency of resistance. Following *Philornis* infection, both Thornbird species elicited an inflammatory response, initially higher in the Greater Thornbird but sustained only in the Little Thornbird. These responses proved to be efficient in reducing the parasite burden in Little Thornbirds, whereas they achieved very limited parasite control in Greater Thornbirds.

Undoubtedly, tolerance cannot be as passive as cancelling the immune response. The host exploitation by the parasite and the efforts elicited to reduce the damage must carry a cost to the physiological economy of the host, which in Great Kiskadees was observed as moderate reduction in RBC counts and growth, but no effect on survival. Mechanisms of tolerance include tissue repair, immunity against parasite toxins, and regulation of the immune response to limit immunopathology (Raberg et al., 2009; Sears et al., 2011; Medzhitov et al., 2012; Gause et al., 2013). Parts of the tolerance mechanisms may be extrinsic to the hosts. One plausible external mechanism of tolerance in our system might be that food provision by parents may be greater in parasitized than in non-parasitized nestlings, as has been reported for other bird species (Bouslama et al., 2002; Hund et al., 2015). Indeed, evidence indicates that Galapagos mocking birds tolerate the introduced parasite *P. downsi* through this mechanism (Knutie et al., 2016).

In a multi-host system, tolerance has the potential to create positive feedback loops that reinforce selection of tolerant hosts, leading to the establishment of specific associations. As this strategy does not imply a negative selection pressure for parasites, the 'arms race' co-evolutionary dynamics of hosts and parasites does not apply, and tolerance tends to become fixed (Roy and Kirchner, 2000). In this way, host tolerance strategies can establish evolutionarily stable host-pathogen associations which neither host nor pathogen have an incentive to depart from. It has been suggested that stable host–parasite associations based on tolerance could give rise to mutualism (Roy and Kirchner, 2000). The fact that there is an overlap in the trophic niche of Kiskadees and other birds that are greatly affected by *Philornis* (Alessio et al., 2005), hints that breeding *Philornis* may aid Great Kiskadees in their competition with other bird species (including Thornbirds). Nonetheless, it should be considered that high tolerance in Great Kiskadees might result in the evolution of greater within–host parasite growth, thus gradually tending to higher virulence (Miller et al., 2006), which creates room for the trade-off theory for virulence in this system.

As tolerance appears to be such a successful strategy against *Philornis*, the fact that in this naturally co-evolving system some hosts choose to resist requires an explanation. We hypothesize that, in a multi-host system, one major benefit of resistance is avoiding host selection. Host selection is a trait that has drawn much attention for parasitoids and vectors (e.g. Henry et al., 2009; Campbell et al., 2013), but which has been often neglected for other parasites. Parasites would tend to select hosts in which their fitness is maximised, i.e. low resistance and high tolerance. Switching to alternative hosts represents a cost to the parasite (Leggett et al., 2013), and thus the evolutionary trend would be avoiding highly resistant hosts. Data from trematode-amphibian systems support this: cercariae discriminated among host species and chose the tadpoles that least limited the infection (Sears et al., 2012). The data provided here further supports this notion, and may explain why *P. torquans* selects Thornbirds only when the availability of Kiskadee nestlings has been low (Manzoli et al., 2013). However, despite *P. torquans* larvae being much more successful in Greater than in Little Thornbirds, the parasite does not appear to prefer one over the other (see Section 3.1). This is perhaps because both *Phacellodomus* spp. are too phylogenetically close to be distinguished by the fly, but the current selection pressure exerted by Little Thornbirds might eventually drive the fly to evolve a way to tell one apart from the other.

The body of literature on zoonotic pathogens and emerging infectious diseases often focuses on reservoir species, which maintain and are the main source of a given pathogen. It is known from descriptive research that species deemed reservoir hosts are generally not significantly affected by infection; but quantitative studies comparing defence strategies and parasite outputs in reservoir versus alternative hosts have not been available (Mandl et al., 2015). Here we provide data that suggest that defence strategies in main and alternative hosts are profoundly different. The common observation that reservoir hosts show little evidence of disease can be explained by high tolerance, whereas its low resistance maximises parasite fitness and transmission, reinforcing selection of reservoir hosts by the parasite. Nonetheless, being able to use alternative hosts is beneficial for the parasite to survive through times when the main host has low availability. Transfer of a parasite to a novel host is a common mechanism of disease emergence (Woolhouse et al., 2005; Allison et al., 2012). Infecting a new host species imposes ecologically and evolutionary costs on the parasite, one of which is loss of infectivity, which is referred to as the 'species barrier' (Woolhouse et al., 2005). In this study we showed that this reduction in parasite fitness may also occur when host switching involves usual hosts and in the presence of co-evolutionary history.

The findings reported here support an expanded knowledge about the consequences of differential defence strategies across hosts and involve a number of implications that warrant further investigation. They highlight the importance of defence strategies in determining virulence and infection dynamics, suggest that there are clearly distinct defence strategies between main and alternative hosts, and hint that defence efficiency is a crucial intervening element in these processes. They also open avenues for theoretical studies on the ecology and evolution of host–parasite interactions, exploring the inclusion of heterogeneous defence strategies and host selections in multihost–parasite systems.

Acknowledgements

This study was funded by Wildlife Conservation Society, USA (Wildlife Health Fund, www.wcs.org), Morris Animal Foundation, USA, (www.morrisanimalfoundation.org, Grant No. D08ZO-304), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina (www.conicet.gob.ar, Grant No. PIP11220100100261). We would like to thank Dr. Martín de la Peña for ornithological advice and Prof. Mike Begon for his critical review of this manuscript and the insightful comments provided. The authors declare no competing interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ijpara.2017.11.001.

References

Alessio, V., Beltzer, A., Lajmanovich, R., Quiroga, M., 2005. Ecología alimentaria de algunas especies de Passeriformes (Furnariidae, Tyrannidae, Icteridae y Emberizidae): consideraciones sobre algunos aspectos del nicho ecológico. Insugeo Miscelánea 14, 441–482.

Alizon, S., Hurford, A., Mideo, N., Van, B.M., 2009. Virulence evolution and the trade-off hypothesis: history, current state of affairs and the future. J. Evol. Biol. 22, 245–259.

Allison, A., Harbison, C., Pagan, I., Stucker, K., Kaelbe, J., Brown, J., Ruder, M., Keel, M., Dubovi, E., Holmes, E., Parrish, C., 2012. The Role of Multiple Hosts in the Cross-Species Transmission and Emergence of a Pandemic Parvovirus. J. Virol. 86, 865–872.

Antoniazzi, L., Manzoli, D., Rohrmann, D., Saravia, M., Silvestri, L., Beldomenico, P., 2011. Climate variability affects the impact of parasitic flies on Argentinean forest birds. J. Zool. 283, 126–134.
Arturi, M., 2006. Situación ambiental de la ecorregión Espinal. In: Brown, A., Martinez, Ortiz U., Acerbi, M., Corcuera, J. (Eds.), La situación ambiental argentina 2005. Fundación Vida Silvestre Argentina, Buenos Aires, pp. 240–260.
Ayres, J.S., Schneider, D.S., 2008. A signaling protease required for melanization in *Drosophila* affects resistance and tolerance of infections. PLoS Biol. 6, 2764–2773.
Best, A., Long, G., White, A., Boots, M., 2012. The implications of immunopathology for parasite evolution. Proc. Biol. Sci. 279, 3234–3240.
Boots, M., 2008. Fight or learn to live with the consequences? Trends Ecol. Evol. 23, 248–250.
Bouslama, Z., Lambrechts, M., Ziane, N., Djenidi, R., Chabi, Y., 2002. The effect of nest ectoparasites on parental provisioning in north-African population of the blue tit *Parus caeruleus*. Ibis 144, E73–E78.
Burnham, K., Anderson, D., 2002. Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer, New York, USA.
Campbell, R., Thiemann, T.C., Lemenager, D., Reisen, W.K., 2013. Host-selection patterns of *Culex tarsalis* (Diptera: Culicidae) determine the spatial heterogeneity of West Nile virus enzootic activity in northern California. J. Med. Entomol. 50, 1303–1309.
de Beer, S., Lockwood, G., Raijmakers, J., Raijmakers, J., Scott, W., Oschadleus, H., Underhill, L., 2001. SAFRING Bird Ringing Manual. University of Cape Town, Cape Town, South Africa, Avian Demography Unit.
de la Peña, M., 2005. Reproducción de las aves Argentinas. LOLA, Buenos Aires, Argentina.
de la Peña, M., Beldomenico, P., Antoniazzi, L., 2004. Pichones de aves parasitados por larvas de *Philornis* sp. (Diptera: Muscidae) en un sector de la provincia biogeográfica del Espinal de Santa Fe, Argentina. Rev. FAVE Cs. Vet. 2, 141–146.
De Roode, J.C., Altizer, S., 2010. Host–parasite genetic interactions and virulence-transmission relationships in natural populations of monarch butterflies. Evolution 64, 502–514.
Fineblum, W., Rausher, M., 1995. Tradeoff between resistance and tolerance to herbivore damage in a morning glory. Nature 377, 517–520.
Gause, W.C., Wynn, T.A., Allen, J.E., 2013. Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nat. Rev. Immunol. 13, 607–614.
Graham, A.L., Allen, J., Read, A., 2005. Evolutionary causes and consequences of immunopathology. Ann. Rev. Ecol. Evol. System. 36, 337–397.
Henry, L.M., Ma, B.O., Roitberg, B.D., 2009. Size-mediated adaptive foraging: a host-selection strategy for insect parasitoids. Oecologia 161, 433–445.
Hund, A., Aberle, M., Safran, R., 2015. Parents respond in sex-specific and dynamic ways to nestling ectoparasites. Anim. Behav. 110, 187–196.
Johnson, P.T., Rohr, J.R., Hoverman, J.T., Kellermanns, E., Bowerman, J., Lunde, K.B., 2012. Living fast and dying of infection: host life history drives interspecific variation in infection and disease risk. Ecol. Lett. 15, 235–242.
Knutie, S., Owen, J., McNew, S., Bartlow, A., Arriero, E., Herman, J., DiBlasi, E., Thompson, M., Koop, J., Clayton, D.H., 2016. Galápagos mockingbirds tolerate introduced parasites that affect Darwin's finches. Ecology 97, 940–950.
Kover, P.X., Schaal, B.A., 2002. Genetic variation for disease resistance and tolerance among *Arabidopsis thaliana* accessions. Proc. Natl. Acad. Sci. 99, 11270–11274.
Lee, K.A., Martin, L.B., Wikelski, M.C., 2005. Responding to inflammatory challenges is less costly for a successful avian invader, the house sparrow (*Passer domesticus*), than its less-invasive congener. Oecologia 145, 244–251.
Leggett, H.C., Buckling, A., Long, G.H., Boots, M., 2013. Generalism and the evolution of parasite virulence. Trends Ecol. Evol. 28, 592–596.
Lough, G., Kyriazakis, I., Bergmann, S., Lengeling, A., Doeschl-Wilson, A.B., 2015. Health trajectories reveal the dynamic contributions of host genetic resistance and tolerance to infection outcome. Proc. R. Soc. B 282.
Löwenberg-Neto, P., 2008. The structure of the parasite–host interactions between *Philornis* (Diptera: Muscidae) and Neotropical birds. J. Trop. Ecol. 24, 575–580.
Lucas, A.M., Jamroz, C., 1961. Atlas of Avian Hematology. United State Department of Agriculture, Washington, USA.
Mandl, J.N., Ahmed, R., Barreiro, L.B., Daszak, P., Epstein, J.H., Virgin, H.W., Feinberg, M.B., 2015. Reservoir host immune responses to emerging zoonotic viruses. Cell 160, 20–35.
Manzoli, D.E., Antoniazzi, L.R., Saravia, M.J., Silvestri, L., Rorhmann, D., Beldomenico, P.M., 2013. Multi-level determinants of parasitic fly infection in forest passerines. PLoS One 8, e67104.
Maze-Guilmo, E., Loot, G., Paez, D.J., Lefevre, T., Blanchet, S., 2014. Heritable variation in host tolerance and resistance inferred from a wild host–parasite system. Proc. R. Soc. B 281, 20132567.
Medzhitov, R., Schneider, D.S., Soares, M.P., 2012. Disease tolerance as a defense strategy. Science 335, 936–941.
Miller, M.R., White, A., Boots, M., 2006. The evolution of parasites in response to tolerance in their hosts: the good, the bad, and apparent commensalism. Evolution 60, 945–956.
Monje, L.D., Quiroga, M., Manzoli, D., Couri, M.S., Silvestri, L., Venzal, J.M., Cuervo, P., Beldomenico, P.M., 2013. Sequence analysis of the internal transcribed spacer 2 (ITS2) from *Philornis seguyi* (Garcia, 1952) and *Philornis torquans* (Nielsen, 1913) (Diptera: Muscidae). Syst. Parasitol. 86, 43–51.
Otranto, D., 2001. The immunology of myiasis: parasite survival and host defense strategies. Trends Parasitol. 17, 176–182.
Owen, J.P., Nelson, A.C., Clayton, D.H., 2010. Ecological immunology of bird-ectoparasite systems. Trends Parasitol. 26, 530–539.
Quiroga, M., Monje, L.D., Arrabal, J., Beldomenico, P.M., 2016. New molecular data on subcutaneous *Philornis* (Diptera: Muscidae) from southern South America suggests the existence of a species complex. Rev. Mex. Biodivers. 87, 1383–1386.
Raberg, L., 2014. How to live with the enemy: understanding tolerance to parasites. PLoS Biol. 12, e1001989.
Raberg, L., Graham, A.L., Read, A.F., 2009. Decomposing health: tolerance and resistance to parasites in animals. Philos. Trans. R. Soc. Lond B 364, 37–49.
Raberg, L., Sim, D., Read, A.F., 2007. Disentangling genetic variation for resistance and tolerance to infectious diseases in animals. Science 318, 812–814.
Ralph, C., Geupel, G., Pyle, P., Martin, T., DeSante, D., 1993. Handbook of field methods for monitoring landbirds. Forest Service, United States Department of Agriculture, Albany.
Read, A.F., Aaby, P., Antia, R., Ebert, D., Ewald, P.W., Gupta, S., Moxon, R., 1999. What can evolutionary biology contribute to understanding virulence? In: Stearns, S. (Ed.), Evolution in Health and Disease. Oxford University Press, Oxford, UK, pp. 205–215.
Rohr, J.R., Raffel, T., Hall, C., 2010. Developmental variation in resistance and tolerance in a multi-host–parasite system. Funct. Ecol. 24, 1110–1121.
Roy, B.A., Kirchner, J.W., 2000. Evolutionary dynamics of pathogen resistance and tolerance. Evolution 54, 51–63.
Schmid-Hempel, P., 2011. Evolutionary Parasitology. Oxford University Press, Oxford, UK.
Sears, B.F., Rohr, J.R., Allen, J.E., Martin, L.B., 2011. The economy of inflammation: when is less more? Trends Parasitol. 27, 382–387.
Sears, B.F., Schlunk, A.D., Rohr, J.R., 2012. Do parasitic trematode cercariae demonstrate a preference for susceptible host species? PLoS One 7, e51012.
Sears, B.F., Snyder, P.W., Rohr, J.R., 2015. Host life history and host-parasite syntopy predict behavioural resistance and tolerance of parasites. J. Anim. Ecol. 84, 625–636.
Texeira, D., 1999. Myasis caused by obligatory parasites. Ib. general observations on the biology of species of the Genus *Philornis* Meinert, 1890 (Diptera, Muscidae). In: Guimaraes, J., Papavero, N. (Eds.), Myiasis in man and animals in the Neotropical region. Editora Plêiade, São Paulo, pp. 71–96.
Vincent, C.M., Sharp, N.P., 2014. Sexual antagonism for resistance and tolerance to infection in *Drosophila melanogaster*. Proc. R. Soc. B 281, 20140987.
Woolhouse, M.E., Haydon, D.T., Antia, R., 2005. Emerging pathogens: the epidemiology and evolution of species jumps. Trends Ecol. Evol. 20, 238–244.
Woolhouse, M.E., Taylor, L.H., Haydon, D.T., 2001. Population biology of multihost pathogens. Science 292, 1109–1112.
Zhao, J., Shi, N., Sun, Y., Martella, V., Nikolin, V., Zhu, C., Zhang, H., Hu, B., Bai, X., Yan, X., 2015. Pathogenesis of canine distemper virus in experimentally infected raccoon dogs, foxes, and minks. Antiviral Res. 122, 1–11.