Making the right choice: testing the drivers of asymmetric infections within hosts and their consequences for pathology

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Despite the ubiquity of bilateral symmetry among animals, a long-standing mystery centers on why parasites that infect paired organs often do so non-randomly. Examples from diverse host and parasite taxa continue to accumulate, yet little is known about their causes or implications for host–parasite fitness. We combined field surveys, experimental infections, and parasite choice assays to evaluate both competing explanations for – and consequences of – asymmetric infections of amphibian kidneys by echinostome trematodes, which are widespread and potentially pathogenic infections of larval amphibians. Samples from 6001 hosts representing 26 species indicated that echinostome infections exhibit a consistent, right-kidney bias, with ~ 62% of parasites in the right kidney. This pattern could not be explained by variation in kidney size or total infection. Experimental infections of three anuran species reproduced this pattern, with 64% of infections in the right kidney, and indicated it was not the result of differential host or parasite mortality. Based on sequential infection experiments and parasite choice assays, we further showed that earlier infections did not affect the distribution of subsequently colonizing parasites and that echinostome cercariae followed host-derived cues rather than exhibiting congenital 'sidedness'. We advance the hypothesis that variation in the position of the right kidney along the anterior–posterior axis controls cue strength in the right nephric duct and thus determines parasite encystment. Correspondingly, anatomical measurements from a subset of larval amphibian hosts revealed that the relative position of the right kidney explained 83% of the variation in infection bias, with no additional contributions associated with kidney volume or host size. We also show that the degree of right-kidney bias associated positively with host growth in experiments. Morphological asymmetries could therefore function as a unique form of tolerance to mitigate the consequences of infection, despite the oft-cited costs of asymmetry for mate selection and enemy vulnerability.

A key innovation in the evolution of animals was the development of bilateral symmetry, which facilitated the arrangement of body axes and subsequent structural specialization (Finnerty et al. 2004). Bilaterally symmetric organisms, which include 99% of modern-day animals, typically exhibit matched pairing of internal and external structures, including limbs, antennae, eyes, ears, lungs, kidneys, testes/ovaries and brain hemispheres. Given the pervasiveness of bilateral symmetry and the potentially deleterious consequences of deviations from it, exceptions to the 'rule' of symmetry can be especially revealing (Møller and Thornhill 1998, Perrett et al. 1999, Møller 1990, 1996, 2006, Galeotti et al. 2005). For instance, owls exhibit asymmetry in left-right ear positioning that helps them localize high-frequency sound and identify prey position (Payne 1971, Norberg 1977), while squid (*Histioteuthis* sp.) exhibit a greatly enlarged left eye and optic lobe, likely to help detect predators above them (Wentworth and Muntz 1989). More enigmatic, however, is why parasites that infect paired structures within bilaterally symmetric hosts will often do so non-randomly. Parasites that preferentially invade a particular eye, lung, gill, ear, wing, kidney or other paired feature of their host have been reported across a wide range of host and parasite taxa (Table 1, Supplementary material Appendix 1 Table A1). Giant kidney worms *Dioctophyma renale*, for instance, which can grow to 100 cm in length and often cause significant renal impairment, almost always (99%) occur in the right kidneys of their mammalian hosts (Mech and Tracy 2001, Loukmas et al. 2010). Despite a growing list of such examples (Table 1), neither the mechanisms underlying such observations nor their implications for host tolerance and parasite fitness have been well explored. In some cases, non-random parasite distribution on/within hosts may be adaptive, increasing mating opportunities for parasites while limiting host and parasite mortality (Thiemann and Wassersug 2000, McLachlan et al. 2008).

Four primary patterns of infections within paired host structures have been reported, each of which can have multiple, competing mechanistic explanations. The first (and perhaps least interesting) is randomness, in which parasites are equally likely to be recovered from either structure

Table 1. Patterns of parasite infection from paired host structures reported from the literature. Citations given by numbered footnotes.

Parasite type	Parasite name	Host type/species	Paired structure	Bias type	Proposed mechanism
Trematode	*Echinostoma* sp.	larval amphibians	kidneys	right side[1–3]	host morphology[1] host mortality[1]
Trematode	*Diplostomum* sp.	fish	eyes	inconsistent[4–8]	cue-following[4–5] host mortality[6–7]
Nematode	*Dioctophyma renale*	mink	kidneys	right side[9–11]	parasite preference[12]
Dipteran	*Protocalliphora* sp.	hawk *Buteo lineatus*	ears	inconsistent[13]	
Mite	*Unionicola* sp.	midge *Para-trichocladius* sp.	wings	uniform[14]	host fitness/mortality[14]
Mite	*Dicrocheles phalaenodectes*	noctuid moths (mostly *Leucania* sp.)	ears	inconsistent[15–16]	cue-following[15] host mortality[15–16]
Mite	*Ensliniella trisetosa*	wasp *Ancistrocerus antelope*	Acarinaria ('mite chambers')	uniform[17]	
Isopod	Epicaridea	decapods (*Munida* sp.)	branchial region	right side[18–19]	parasite mortality[20]
Monogenea	*Bothrithrema bothi*	eels *Scophtalmus aquosus*	external surface	inconsistent[21]	host morphology[22]
Monogenea	*Pseudodactylogyrus anguillae*	eels *Anguilla anguilla*	gills	left side[23]	parasite fitness/mortality[23]
Monogenea	*P. bini*	eels *A. anguilla*	gills	right side[23]	parasite mortality[23]
Monogenea	*Entobdella soleae*	flatfish *Solea solea*	body	nadiral (bottom)[24]	parasite mortality[24]
Copepod	*Leposphilus labrei*	fish *Crenilabrus melops*	lateral line	left side[25]	host behavior/morphology[25]
Copepod	*Lepeophtheirus thompsoni*	flatfish *Psetta maxima*	gill cavity	nadiral (bottom) (biological right)[26]	host morphology/physiology[26]
Copepod	*L. europaensis*	flatfish *Scophthalmus rhombus*	gill cavity	nadiral (bottom) (biological right)[26]	host morphology/physiology[26]
Copepod	*L. europaensis*	flatfish *Platichthys flesus*	gill cavity	zenithal (top) (biological right)[26]	host morphology/physiology[26]

[1]Thiemann and Wassersug 2000, [2]Holland 2009, [3]Holland et al. 2007, [4]Rau et al. 1979, [5]Graczyk 1991, [6]Karvonen and Seppala 2008, [7]Rintamaki-Kinnunen et al. 2004, [8]Dwyer and Smith 1989, [9]Mace 1976, [10]Mech and Tracy 2001, [11]Loukmas et al. 2010, [12]Measures 2001, [13]King et al. 2010, [14]McLachlan et al. 2008, [15]Treat 1957, [16]Payne et al. 1966, [17]Cooper 1954, [18]Rayner 1935, [19]Rasmussen et al. 2008, [20]Housa 1963, [21]Hendrix 1990, [22]Bilong-Bilong 1995, [23]Buchmann 1988, [24]Kearn 1984, [25] Donnelly and Reynolds 1994, [26]de Meeus et al. 1995.

(e.g. frog lung flukes; Whitehouse 2002). Second, infection may be consistently more common in a left or right organ or structure (consistent bias), as described for giant kidney worms. This pattern is often hypothesized to result from structural or behavioral asymmetries on the part of the host or from congenital preferences by the parasite. Third, parasites can exhibit an infection bias that is not consistent to one side or another (inconsistent bias). Colonies of mites that infect the tympanic organs of noctuid moths are equally likely to occur in the left or right ear, but never occur in both (Treat 1957, Payne et al. 1966). Because heavy infestations tend to destroy ear function and may make moths more vulnerable to predators, patterns such as these can stem from differential mortality, such that hosts with infection in both structures are lost from the population, or as a result of parasites following physical/chemical cues established during earlier infections (Treat 1957, Rau et al. 1979, Leung et al. 2010). Finally, parasite distributions can be more even than expected by chance (uniform). For instance, unionicolid mites exhibit a non-randomly even distribution on the left and right side of their midge hosts. Because both mites and midges depend on the host's flight for dispersal and reproduction, a uniform distribution by these relatively large parasites is hypothesized to minimize interference with the host's aerobatic performance, which is supported by experimental studies using wind tunnels (Cooper 1954, McLachlan et al. 2008).

Interactions between amphibians and echinostome trematodes offer a particularly useful system in which to study the puzzle of asymmetric infections. These widespread parasites colonize the kidneys of their hosts and have been reported to exhibit a consistent, right-side bias in some species (Table 1). Echinostomes have complex life cycles often involving freshwater snails, amphibians, and mammalian or avian definitive hosts (Johnson and McKenzie 2008). Free-swimming parasites (cercariae) emerge from infected snails and infect larval amphibians, entering through the cloaca and traveling along the nephric ducts to either the left or right kidney (mesonephros) where they establish a cyst (metacercaria) (Fig. 1A). After a bird or mammal predator consumes the infected amphibian, the metacercaria develops into an adult worm, reproduces sexually, and releases eggs that hatch and infect snails. Tadpoles exposed to echinostome cercariae either early in development or at high dosages can develop severe pathology associated with impairment of osmoregulation, including whole-body edema and mortality (Beaver 1937, Fried et al. 1997, Schotthoefer et al. 2003, Holland et al. 2007). Because these infections are easy to quantify within amphibian hosts and amenable to manipulation, they offer an excellent system in which to evaluate the potential mechanisms for biased infections and test their implications for both host and parasite.

Here, we combined large-scale survey data, experimental infection studies, and a competing-model approach to address the following questions: 1) do echinostome infections in amphibian kidneys exhibit a consistently biased distribution? 2) to what extent are observed patterns
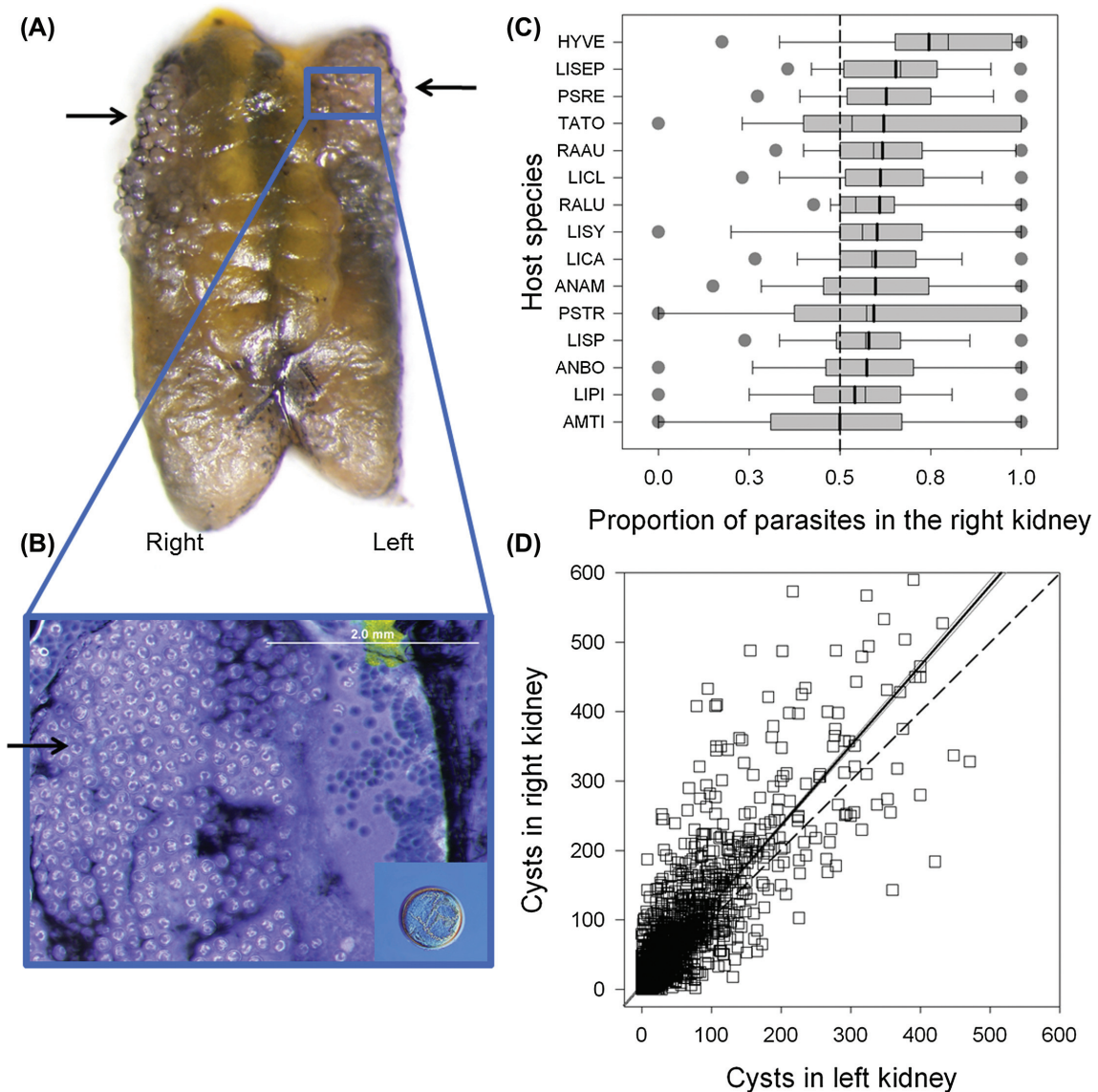
Figure 1. (A) Amphibian kidneys infected with echinostome metacercariae. Arrows indicate clusters of metacercariae (B) Phase-contrast image of amphibian kidney tissue with encysted infections (40 × magnification). Inset: isolated metacercaria (200×). (C) Box plots of the average proportion of echinostome metacercariae in the right kidney for field-collected host species with a sample size > 20. Thick black lines depict the mean proportion while the thin gray lines are the median; outliers are represented as points outside the box plots. The vertical dashed line is the expected value of 0.5. See Supplementary material Appendix 1 Table A2 for species codes. (D) Relationship between the number of echinostome parasites in the left and right kidneys of naturally occurring amphibian hosts. Outlier hosts with > 1000 total parasites were excluded. Dashed line indicates the 1:1 expectation of equal parasite numbers in each kidney whereas the thin solid line is the fitted relationship (with 95% CI, $R^2 = 0.75$, n = 5965).

explained by variation in kidney sizes, kidney position, total infection intensity, parasite preference, or differential mortality by hosts or parasites? And finally, 3) what are the consequences of asymmetric infection for host and parasites? Using infection data from 6001 infected hosts representing 26 amphibian species and 424 wetlands across the USA, we assessed the generality of right-biased infection in amphibians and developed a statistical modeling framework to differentiate among alternative hypotheses for this consistent bias. We paired field analyses with detailed anatomical observations, parasite choice assays and experimental infections of three amphibian host species to evaluate whether field-observed biases persisted under controlled conditions, examine whether parasites exhibit inherent 'sidedness', and test the consequences of intra-host parasite aggregation for host and parasite responses, including infection success, host survival and host growth.

Material and methods

Field sampling

Between 2003 and 2012, metamorphosing amphibians (Gosner 1960, stages 42–46) representing 26 species (Supplementary material Appendix 1 Table A2) were

collected from wetlands and examined fresh for macro-parasite infections. Sites represented a broad range of lentic water bodies and geographic regions from the USA, including 424 sites distributed across 29 states. From each host, the kidneys were carefully removed and the number of encysted echinostome metacercariae quantified under a compound microscope (100–200×) while applying gentle pressure on the coverslip (Fig. 1B). Beginning in 2009, the length and width of each kidney was measured using digital calipers to allow incorporation of kidney volume and the disparity in volume between the left and right kidneys into statistical models of parasite distribution. Given the broad geographic range included in this study, quantified parasites almost certainly represent a mixture of species in the echinostome group, including *Echinostoma trivolvis*, *Echinostoma revolutum* and *Echinoparyphium* spp., all of which have relatively similar life cycles as described above.

Experimental infections
To determine whether asymmetric infections could be reproduced in experimentally infected hosts and to examine potential causes and consequences of the right-kidney bias, we collected egg masses of Pacific chorus frogs *Pseudacris regilla* and wood frogs *Lithobates sylvaticus* from field sites in California and Manitoba, respectively, allowed them to hatch in the laboratory, and exposed individuals to cercariae of *Echinostoma trivolvis* as they reached (Gosner 1960) stage 26–27 (Supplementary material Appendix 1 Table A1). To obtain cercariae, we collected naturally infected *Helisoma trivolvis* snails, isolated them into 50 ml centrifuge tubes exposed to overhead light, and collected emerging cercariae within 4 h of release. Cercariae were pooled among ~10 snails and we used a stereodissecting microscope to isolate the correct number of parasites for each replicate. A subset of cercariae was vouchered for molecular identification (Supplementary material Appendix 1). For *P. regilla*, 30 tadpoles were exposed individually to 35 *E. trivolvis* cercariae in 100 ml of water on day 1 and 4 of the experiment (total of 70 cercariae per host). Twenty additional animals, which were not exposed to cercariae, were maintained as controls (total of 50 tadpoles). For *L. sylvaticus*, 70 tadpoles were exposed individually to a single dosage of 25 cercariae following a similar protocol. Control tadpoles (*P. regilla* only) were sham-exposed by adding a similar volume of water (~2 ml) from uninfected snails. Either 14 (*P. regilla*) or 21 days (*L. sylvaticus*) post exposure, all tadpoles were measured (snout–vent length, mm), massed (blotted dry, mg), and necropsied to quantify infection and measure kidney size. Hosts that died prior to this period were excluded. We quantified the number and proportion of echinostome metacercariae in the kidneys (mesonephroi) and in the head kidneys (pronephroi), which are larval excretory structures present in immature amphibians that atrophy as hosts approach metamorphosis (Schotthoefer et al. 2003).

Fluorescent dye experiment
To explicitly explore how the sequence of parasite infection affected aggregation between the kidneys and whether early infection events affected distribution of subsequent infections, we exposed southern leopard frog *Lithobates sphenocephalus* tadpoles to either 30 or 60 *E. trivolvis*

cercariae over two exposure periods separated by three days. This included five treatments: 0 then 30 cercariae, 0 then 60, 30 then 0, 30 then 30, and 60 then 0 (10 tadpoles per treatment, each maintained individually within 1-l containers). An additional five tadpoles were maintained as controls with no exposure to parasites on either date. Three egg masses were collected near Tampa, Florida, hatched in the laboratory, and randomly assigned to treatments as larvae reached Gosner (1960) stages 26–27. We followed the same methods described above with the exception that cercariae were labeled with fluorescent dye prior to the exposures. By labeling cercariae with a fluorescent dye, we were able to differentiate infections from the first exposure (green dye) and the second exposure (red dye) (Supplementary material Appendix 1). Two days after the second exposure, we euthanized tadpoles, measured their kidneys, and quantified metacercariae using a fluorescent stereomicroscope.

Parasite choice assays
We used cercarial choice assays involving different cues (water or one of two concentrations of kidney extract) to assess whether parasites had a congenital directional preference or instead responded to host cues. We created mazes from high-density plastic blocks and carved a Y-shape into each (Supplementary material Appendix 1). We placed either de-chlorinated water or kidney extract derived from the macerated and centrifuged kidneys of 10 bullfrog tadpoles into the right and left arms following a 2 × 2 factorial design (Supplementary material Appendix 1). We also compared the effects of high and low kidney extract concentration by placing either a low (the supernatant resulting from centrifuging diluted 50% with Ringer's solution) or high (undiluted supernatant) concentration in the right and left arms. We added 100 μl of de-chlorinated water into each maze followed by 20 μl of the particular cue to each of the short arms. After 10 min to allow time for cue diffusion and gradient formation, we pipetted 15 echinostome cercariae into the long arm of each maze and quantified the number in each segment (left or right) after 20 min (Supplementary material Appendix 1). By rotating the cues between arms and the orientation of the mazes themselves, any gradients in the room, such as temperature and light, would have been consistent across the mazes. Although experimental mazes differ from host anatomy in several important ways (e.g. mazes were larger than amphibian nephric ducts) and should not be construed as an accurate representation of parasite migration through the host nephric system, our primary goal was to assess the responses of parasite cercariae to different cues.

Kidney position
Finally, to more thoroughly assess positional differences between the amphibian kidneys and how they influenced patterns of infection, we obtained fine-scale anatomical measurements from a subset of 20 wild-caught American bullfrog *Lithobates catesbeianus* tadpoles (Gosner 1960, stage mean ± 1 SE: 33.3 ± 0.98 [range: 28–41]; mean snout–vent length (mm) ± 1 SE: 43.3 ± 1.2 [range: 33.4–51.2]) collected from a wetland known to support echinostome infections (BNPND024; latitude: 37°92′71.01″; longitude: −122°16′12.95″). Given that

amphibians acquire echinostome infections as tadpoles, our goal here was to conduct a detailed anatomical examination of the size, volume, and position of larval amphibian kidneys to test their competing influence on infection asymmetries. We used *L. catesbeianus* because this species generally requires ~ 2 years to reach metamorphosis and can therefore offer variation in both body size and infection load useful to differentiate among alternative explanatory factors. To this end, we first removed all of the organs except for the kidneys (mesonephroi) in each host. We then captured a coronal image of the ventral surface of the kidneys using a stereomicroscope and used ImageJ software to measure any difference in position between the bottom of the left and right kidneys along the antero-posterior axis. We then measured each kidney, carefully dissected them out of the host, and recorded their masses using a digital microbalance. As an additional estimate of kidney volume, we measured the water displacement of each kidney in a 10 ml graduated cylinder. Finally, we counted the number of echinostome metacercariae in each kidney using the methods described above.

Analysis of field data

We initially used parametric statistics to determine whether 1) amphibian hosts exhibited a random distribution of infection between the two kidneys, 2) one kidney consistently supported more parasites than the other, and 3) observed patterns correlated with variation in total echinostome infection and/or the proportional difference in kidney sizes. Using all hosts in which echinostome infections were detected (n = 6001), we tested whether the observed distribution between kidneys corresponded with the expected value (0.5) using one-sample t-tests followed by paired t-tests to assess whether one supported more infection. We then used generalized linear mixed models with a negative binomial distribution and a log-link function to evaluate how the number of echinostomes per kidney was affected by kidney identity (left vs right) and kidney volume (calculated as $4/3 \times \pi \times L/2 \times W/2 \times 1$, where L and W are the length and width and the depth is held constant, which we also validated using measures of kidney mass and water displacement, Supplementary material Appendix 1). This analysis used only animals with complete kidney measurements (n = 4670). Random effects included host individual (with kidneys nested within individual), site-visit (a unique combination of site and year sampled), and amphibian species. If the right-side bias was explained by kidney volume, we expected there to be no effect of kidney identity (right vs left) after accounting for volume and the hierarchically nested random effects. We also compared left and right kidney volume in a subset of amphibian hosts without echinostome infection (Supplementary material Appendix 1). Analyses were performed using the R package glmmADMB.

To evaluate how the degree of right-kidney bias changed with infection intensity, we tested whether the relationship between parasites in the left vs. the right kidney was linear or nonlinear (polynomial or exponential) using least-squares regression. In a linear model, a non-zero intercept indicates that right kidney infections are more likely even when the left kidney is uninfected while a slope > 1 would

reinforce a consistent bias in favor of the right kidney. A nonlinear model suggests that the degree of bias is not constant, as might be expected if earlier infections positively or negatively affect subsequent parasite kidney settlement. For the *L. catesbeianus* larvae with additional measurements of the relative position of each kidney, we used a binomial GLM with a logit link function to determine how disparity in kidney volume and the difference in anterior–posterior position between the two kidneys affected the proportion of parasites in the right kidney.

Analysis of experimental data

We compared the proportion of cysts in the right kidney and the right pronephros to both an expected value of 0.5 and the number of cysts in left versus right structures using paired t-tests. Among exposed animals, we tested whether the number of cysts (in the kidneys and overall, both $\log_{10}$-transformed) or the proportion of metacercariae in the right kidney (arcsin-square root transformed) correlated with host growth over the course of the experiment (Supplementary material Appendix 1). In the fluorescent dye experiment, we tested how the degree of right-side bias was affected by the number of parasites administered in each time period and their interaction. Analyses were performed using generalized linear models with a binomial distribution and a logit link function for survival and a Normal distribution and identity link for remaining responses. Individuals without kidney infections were excluded. For the cercarial choice assay, we used a generalized linear model with a binomial distribution to predict the number of cercariae detected in the right arm of the maze relative to the total. Treatment was included as a fixed effect with four categories as a function of the cue placed in the left and right chambers, respectively: kidney–kidney, water–water, kidney–water, and water–kidney. We used an identical approach to compare the effects of kidney extract concentration (high vs low) on cercariae choice.

Results

Field data

The distribution of parasites between the left and right kidneys differed from the expected 0.5 value (one-sample t = 34.90, p < 0.0001), with consistently greater infections in the right kidney (paired t-test = 20.139, p < 0.0001; n = 6001). The average proportion of parasites in the right kidney was 0.62, although this value varied among host species (Fig. 1C). Among hosts with kidney measurements (n = 4670), the proportional volume occupied by the right kidney was also slightly greater (mean proportional volume of the right kidney = 0.522; paired t-test = 16.06, DF = 4669, p < 0.0001). However, the degree of difference was considerably smaller than that observed for infection. Correspondingly, in the mixed model analysis, kidney volume explained little variation in echinostome infection relative to kidney position (left vs right) (full model: coefficient of kidney identity [right] = 0.462 ± 0.011, z = 39.21, p < 0.00001; coefficient of kidney volume = 0.0026 ± 0.00096, z = 1.70, p = 0.089). Based on AIC

values, the full model with both fixed effects and all three random effects was the best supported; however, removal of kidney volume increased the AIC value by ~1, suggesting a minor explanatory role for this variable. Removal of any other variable caused a ΔAIC increase of >1000. Indeed, even after accounting for the effects of total infection, volume disparity, site-visit and host species in a binomial GLM, the probability of right kidney infections was estimated as 0.60. Infection in the left kidney correlated allometrically rather than isometrically with infection in the right kidney ($R^2 = 0.752$, n = 5965); the linear predictor has an intercept of 5.74 ± 0.4002 ($t = 14.34$, $p < 0.0001$) and a slope of 1.158 ± 0.0086 ($t = 134.33$, $p < 0.0001$) (Fig. 1D).

Experimental infections

Experimentally exposed amphibian hosts exhibited a consistent, right-kidney infection bias similar to naturally occurring hosts (*P. regilla*: one sample $t = 2.47$, DF = 22, $p = 0.022$; *L. sylvaticus*: $t = 5.29$, DF = 65, $p < 0.0001$) (Fig. 2A). Among *P. regilla*, 66% of metacercariae in the kidneys and 60% of those in the pronephros were recovered from the right side (paired t-tests: kidneys $t = 2.12$, DF = 25, $p = 0.044$; pronephros $t = 2.55$, DF = 25, $p = 0.017$). An average (± 1 SE) of 40.46 ± 2.202 metacercariae were recovered from each *P. regilla*, and this number was unrelated to the proportion of parasites recovered on the right side ($p > 0.05$). Among *L. sylvaticus*,
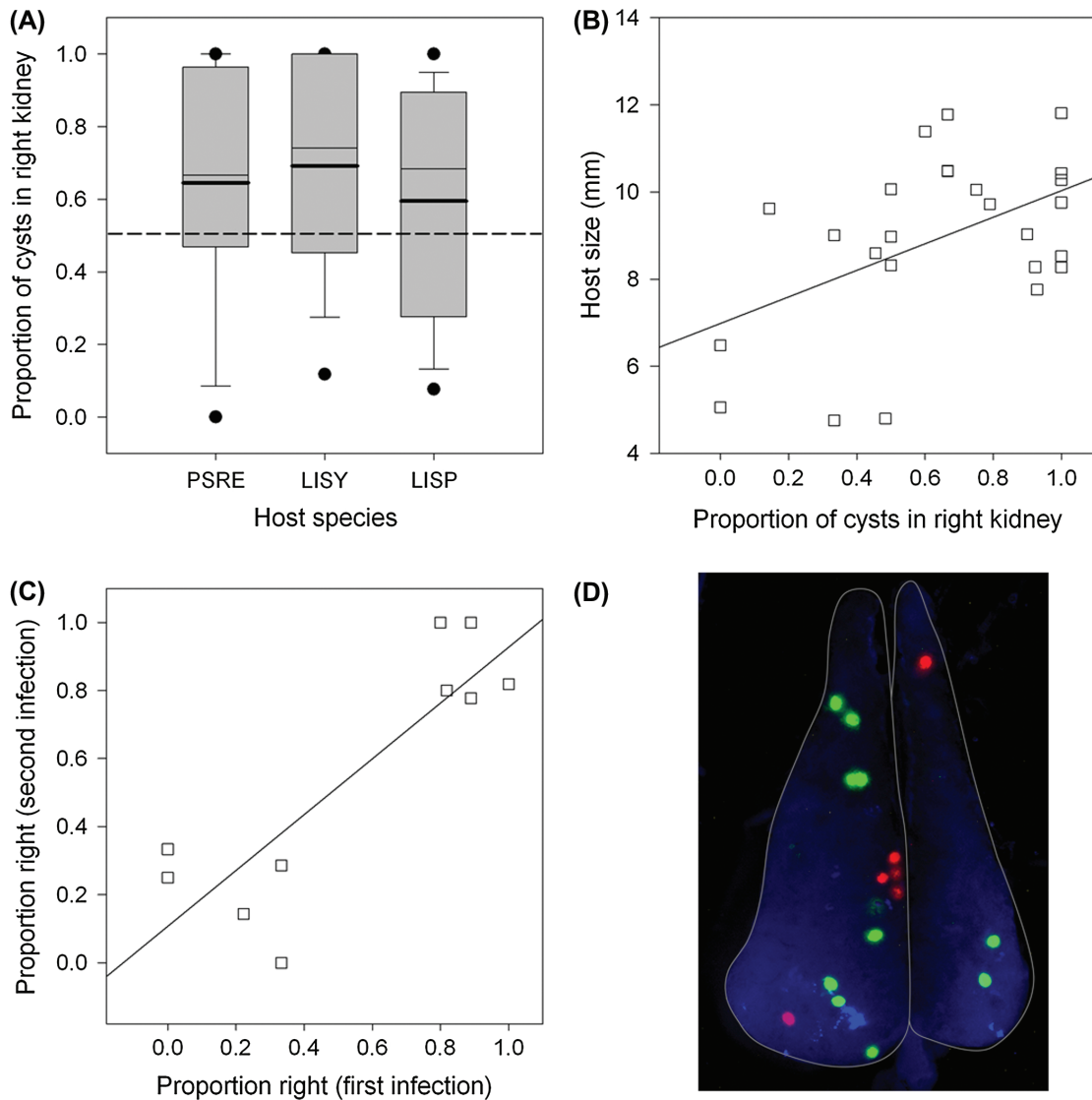

Figure 2. (A) Box plots of the proportion of echinostome metacercariae in the right kidneys of experimentally exposed *Pseudacris regilla* (PSRE), *Lithobates sylvaticus* (LISY), and *L. sphenocephalus* (LISP). The vertical dashed line is the expected value of 0.5. Boxplot parameters are described in Fig 1. (B) Relationship between the degree of right-kidney bias and host size among *P. regilla* ($R^2 = 0.22$, n = 23). (C) Isometric relationship between the degree of right-kidney bias across two infection events for *L. sphenocephalus* exposed to fluorescently labeled parasites ($R^2 = 0.73$, n = 10). (D) Composite image of amphibian kidneys (in blue) illustrating echinostome parasites dyed green (first infection) or red (second infection event). See Supplementary material Appendix 2 for details.

69% of metacercariae in the kidneys and 44% of those in the pronephros occurred on the right (paired t-tests: kidneys $t = 5.29$, DF $= 65$, $p < 0.0001$; pronephros $t = -1.36$, DF $= 62$, $p = 0.177$). Total echinostome infection (mesonephroi and pronephroi) negatively affected *P. regilla* host stage ($R^2 = 0.152$; $p = 0.04$) and had a marginally negative effect on host size ($R^2 = 0.139$; $p = 0.06$), while for *L. sylvaticus* total kidney echinostomes had a negative effect on host growth ($R^2 = 0.575$; $p = 0.016$). Interestingly, however, the proportion of metacercariae recovered from the right kidney [arcsin-square root transformed] associated positively with chorus frog size ($R^2 = 0.221$; $p = 0.022$) and stage ($R^2 = 0.349$; $p = 0.003$) (Fig. 2B), while for wood frogs, right-side aggregation was positively related to growth only at higher infection intensities (interaction $p = 0.046$; full model $R^2 = 0.398$). No such relationships were observed between the proportion of parasites recovered from the right pronephros and any fitness measures. Few hosts died during the experiment (two controls and four exposed for *P. regilla*, one exposed for *L. sylvaticus*), and there were no significant effects of echinostome exposure on mortality.

Fluorescent dye experiment

Lithobates sphenocephalus larvae exhibited a right side bias similar in magnitude to the previous experiments (one sample $t = 2.03$, DF $= 47$, $p = 0.024$), with 59.5% of kidney metacercariae recovered from the right side (paired t-test: $t = 2.44$, DF $= 52$, $p = 0.018$). Degree of bias was unrelated to the number of parasites administered at time 1, time 2, or their interaction (GLM, all $p > 0.05$). Separating out the parasites by exposure event (for the 30–30 treatment), we found that the proportion of parasites in the right kidney at time 1 linearly predicted the proportion that infected this kidney at time 2 ($R^2 = 0.728$, $F_{1,8} = 21.3$, $p = 0.0017$), with a slope approaching unity (intercept constrained to zero, slope ± 1 SE $= 0.95 \pm 0.101$, n $= 10$) (Fig. 2C–D). Correspondingly, the proportion did not differ between the two infection events (paired-t $= 0.187$, $p = 0.85$), indicating that, while some hosts had a greater fraction of parasites in the right kidney than others, this fraction was unaffected by prior infection.

Parasite choice assay

Echinostome cercariae exhibited no directional preference when both arms of the experimental maze contained identical concentrations of kidney extract ($47.29 \pm 4.7\%$ in the right arm; paired $t = 0.83$, DF $= 31$, $p = 0.41$) or water (paired $t = 1.39$, DF $= 47$, $p = 0.17$) (Fig. 3). However, this percentage changed directly in relation to which pair of cues was included in the treatment: $36.0 \pm 4\%$ of parasites selected the right arm when it contained water and the left contained kidney extract, whereas $50.6 \pm 4.0\%$ did when these treatments were reversed. Based on the binomial model, kidney extract in the right well functioned to increase the proportion of cercariae detected in the right chamber at the end of the trial while kidney extract in the left well decreased it (right-kidney $z = 1.993$, $p = 0.046$; left-kidney $z = -2.523$, $p = 0.0116$) relative to
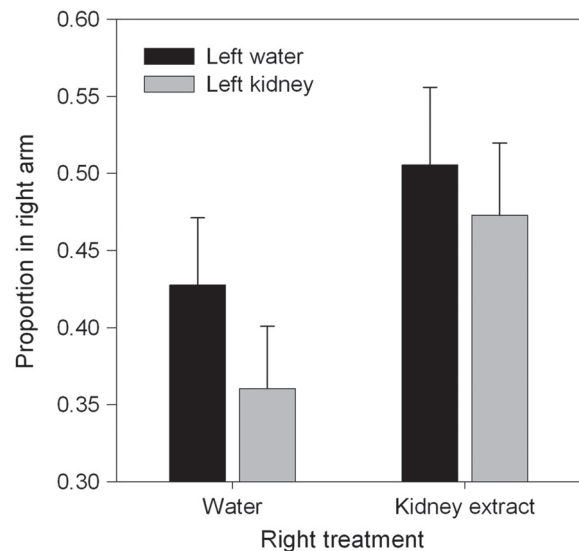

Figure 3. Results of the cercarial choice assays. Depicted is the proportion of echinostome cercariae recovered from the right arm of experimental mazes as a function of whether kidney extract or water was added to the right arm (see x-axis) or left arm (see differently shaded bars).

the reference condition of kidney extract in both wells. Adding water to both wells had no effect on parasite distribution relative to the reference condition ($z = 0.184$, $p = 0.854$). Parasite behavior also depended on the concentration of kidney extract. Cercariae moved preferentially toward the arm with a higher concentration of kidney extract regardless of whether it was in the right or left side ($z = -2.156$, $p = 0.031$). In each case, 53.8% of cercariae were recovered from the chamber with concentrated kidney extract, such that the overall distribution of parasites when the treatments were combined did not differ from the expected value 0.5 (two-tailed $t = 0.99$, $p = 0.89$; DF $= 61$).

Kidney position

Among the *L. catesbeianus* examined for kidney position, individuals were infected with 16–281 echinostome metacercariae (mean $= 67.85 \pm 13.15$; infection prevalence $= 100\%$). Between 30 and 91% of infections occurred in the right kidney (mean $= 56.2\%$, one-tailed t-test $p = 0.026$). On average, the right and left kidneys weighed 0.0955 ± 0.0071 and 0.0606 ± 0.0071 g, with the right kidney larger than the left (two-tailed paired $t = 8.13$, $p < 0.0001$). Importantly, however, the right kidney also averaged 1.88 ± 0.102 mm lower (i.e. more posterior) than the left kidney (Fig. 4B). In a binomial GLM, the difference in kidney position – but not volume – was a strong, positive predictor of proportion of parasites in the right kidney, accounting for ~83% of observed variance (GLM, $z = 8.952$, $p < 0.0001$; Fig. 4A); neither the proportional volume of the right kidney nor host size (snout–vent length or stage) accounted for significant additional variation (ΔAIC > 2). Scaling the difference in kidney position by host kidney size (i.e. dividing by the average length of the host's left and right kidneys) did not strongly alter its
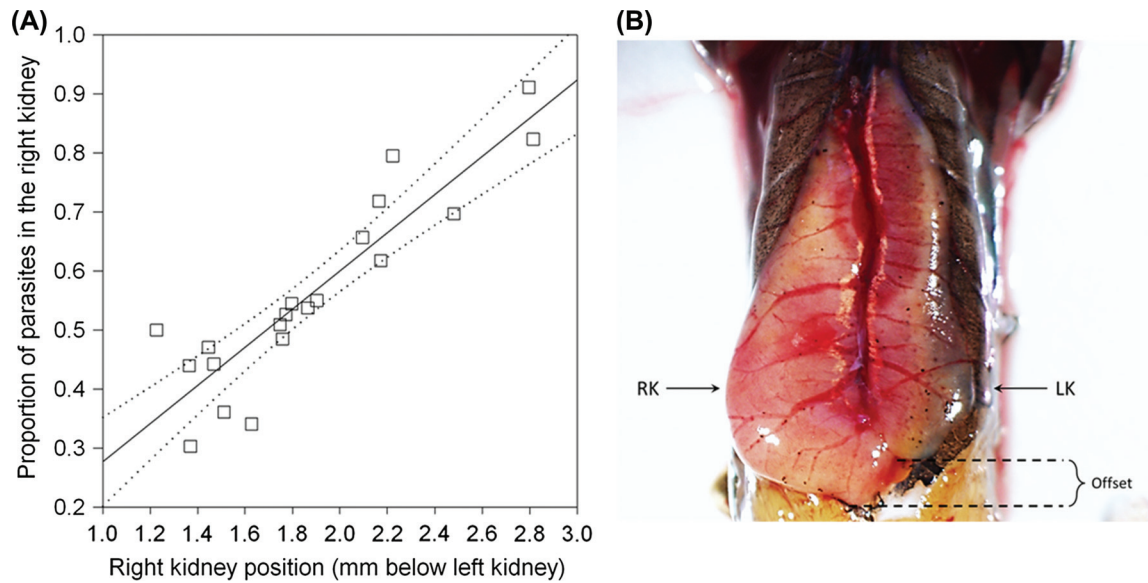
Figure 4. (A) Proportion of echinostome parasites in the right kidney of *L. catesbeianus* in relation to the positional difference between the kidneys (see 'offset' in (B)) (pseudo-$R^2 = 0.83$, n = 20). Here, position refers to how much lower (in mm) the right kidney is along the anteroposterior axis relative to the left. (B) Representative coronal image of amphibian kidneys illustrating the position difference (i.e. offset) between the left kidney (LK) and the right kidney (RK).

influence on the degree of infection bias ($R^2 = 0.58$; z = 7.635, p < 0.0001); however, the raw difference in position may better reflect what a parasite encounters given that cercariae will not change in size, regardless of host size. The results were also insensitive to whether we used estimates of kidney volume (calculated as described above or direct measurements of kidney mass, which exhibited correlation coefficients between 0.68 and 0.77).

Discussion

Our results provide insights into the factors contributing to asymmetric infections of amphibian kidneys and their implications for host tolerance. In particular, we establish a consistent and definitive pattern of echinostome parasite aggregation, identify an influential mechanism for the right-side bias, and highlight the potential importance of this aggregation for host condition. Using the largest assembled data set of amphibian macroparasite infections (>6000 hosts representing 29 species across 424 wetlands), we find a remarkably consistent bias in favor of infections in the right kidney. Even after accounting for the effects of host species, wetland, and total infection, right kidneys exhibited an approximately 50% higher likelihood of infection than their left-side counterparts, which mirrors previous work done in this system for particular host species or geographic regions (Thiemann and Wassersug 2000, Holland et al. 2007, Holland 2009). Experiments involving three different anuran species from divergent parts of North America yielded a strongly similar estimate of right-side bias, illustrating both the consistency of this pattern and its reproducibility under laboratory conditions. Collectively, the robust patterns established by these field and experimental observations shift attention

toward understanding the mechanisms underlying this pervasive trend and identifying its implications for host-parasite interactions.

Previous work on parasite aggregation within hosts has advanced three primary groups of hypotheses to explain consistent infection biases: 1) initial infections are symmetric but subsequent mortality of host or parasites leads to bias (Housa 1963, Thiemann and Wassersug 2000, Holland 2009, Szuroczki and Richardson 2009); 2) subsequently colonizing parasites follow (or avoid) cues established by earlier infections leading to density-dependent bias (Treat 1957, Rau et al. 1979, Graczyk 1991); and 3) congenital asymmetries involving host morphology or parasite directional preferences cause uneven infection probabilities (Treat 1957, Payne et al. 1966, Bilong-Bilong 1995, Lee et al. 2012). Our data failed to support hypotheses 1) and 2) as major contributors to observed patterns. Based on laboratory experiments, the percentage of administered parasites recovered as meta-cercariae was unaffected by their distribution between kidneys, suggesting differential establishment success of parasites was not necessary to account for bias. Similarly, host survival in experiments was high and independent of the proportion of parasites in the right kidney. This conclusion was also supported by field data, which yielded a similar degree of right-bias as experiments and did not show an increase in bias with infection intensity, as would be predicted by differential host mortality or the 'follow-the-leader' hypothesis. Perhaps more importantly, experimental infections with fluorescently labeled parasites indicated that the initial pattern of parasite colonization between the kidneys had no effect on the distributional bias of subsequently administered parasites; while hosts varied in the degree of right-side bias, this discrepancy did not change between infection events.

The final hypothesis involves inherent asymmetry on the part of the host or parasite. Based on the parasite choice assays, we found no evidence that cercariae congenitally preferred to move to the right, as sometimes demonstrated for other taxa (Cooper et al. 2011). Instead, parasites responded to host-derived cues, generally moving toward the maze arm containing kidney extract rather than one with water, regardless of whether it was on the left or right side. Correspondingly, when kidney extract was infused into both arms, parasites moved preferentially toward the side with a higher concentration, consistent with previous work detailing parasite chemotactic and navigational ability within hosts (Grabe and Haas 2004). Although experimental mazes differed from the anatomy of a host's nephric system, these results nonetheless suggest that the right-side bias derives from consistent asymmetries in signal strength after parasites enter the host cloaca. Intuitively, one might expect this would owe to raw differences in the volumes of the left and right kidneys (Thiemann and Wassersug 2000); indeed, our field data indicated that the right kidney was generally ~5–8% larger than the left across species. However, the degree of volumetric difference between the kidneys was often relatively minor compared to the observed differences in infection (~50% greater in right), and kidney volume explained a trivial amount of variance in our analyses, both in the field and in the lab.

As an alternative to volume, we propose that stronger cues associated with the right kidney stem from positional differences between the two kidneys. Owing in part to pressure from the liver, the right kidney sits lower (more posterior) in the body of many vertebrates. In humans, for instance, the right kidney is ~2 to 8 cm below the left kidney as a result of the liver position (which is on the right) relative to the spleen (which is on the left). In our own measurements of larval bullfrogs, the right kidney was ~2 mm lower than the left, or ~11% of total kidney length. This places the right kidney closer to the host cloaca where colonizing cercariae enter the host, which we suggest functions to increase the relative signal strength from the right branch of the nephric duct and thereby causes more parasites to make the corresponding directional decision to travel rightward. Based on detailed anatomical measurements collected from a subset of larval bullfrogs with broad variation in both infection load and body size, we found that the positional difference between the two kidneys accounted for ~83% of the variation in right kidney bias. In contrast, detailed measurements of the volumetric differences between the kidneys failed to improve model fit, regardless of whether we used estimates of kidney volume or measurements of kidney mass. Given that the proportion of parasites in the right kidney ranged from 30% to > 90% among hosts, the explanatory power provided by kidney position is remarkable, although we note that further work is needed to evaluate the strength of this effect in other amphibian species and relative to other anatomical features (e.g. the density of collecting ducts). Interestingly, liver position also functions prominently in the extreme right bias of giant kidney worm infections: nematode worms, which are acquired through diet, travel from the digestive system to the kidney by way of the liver (Measures 2001), leading to an almost exclusive right bias in mammalian infections (> 99%).

Beyond the mechanistic underpinnings driving the right kidney bias, one of the most interesting questions focuses on the ecological (and evolutionary) consequences of asymmetric infection patterns. In particular, do uneven parasite distributions reduce the fitness impacts associated with infection and thereby improve host tolerance? This idea has most frequently been advanced in connection with infection of paired structures such as eyes, ears, and kidneys, in which parasite-driven impairment of both structures has the capacity to reduce host fitness and, by extension, parasite fitness (depending on the transmission mode of the parasite). For instance, parasitic mites *Myrmonyssus phaltenodectes* often destroy one but never both tympanic organs of noctuid moths, which researchers have argued may adaptively reduce the risk that moths – unable to hear the high pitched sounds of bats – are consumed along with their mites (Treat 1957, Payne et al. 1966). Here, we show that the degree of right kidney infection bias correlated positively with measures of host condition in experimentally exposed chorus frogs and wood frogs. Consistent with previous work, total echinostome infection reduced host growth or final size/stage, likely due to impacts on osmoregulatory function (Fried et al. 1997, Schotthoefer et al. 2003, Holland et al. 2007, Holland 2009). However, the proportion of parasites in the right kidney associated positively with host growth; in chorus frogs, this was a main effect independent of total infection, whereas in wood frogs it was evident only when infection was high. Although difficult to determine whether this relationship was causal (i.e. given the challenges of manipulating the degree of aggregation itself), these data are suggestive of a link between intra-host aggregation and host condition. Given the widespread occurrence of echinostome infections in natural amphibian populations and the established link between larval growth rate (time to metamorphosis) and amphibian survival or fecundity (Wilbur and Collins 1973, Berven and Gill 1983), infection could therefore constitute a selective force maintaining kidney asymmetry in amphibians. Such a bias could also benefit echinostomes by increasing the probability that amphibian hosts survive to metamorphosis, assuming that frogs are more likely to encounter suitable final hosts (birds and mammals) than are tadpoles.

Despite the ubiquity of bilateral symmetry in the natural world, many organisms exhibit biological asymmetries that range from slight to substantial (Fujinaga 1997, McManus 2004). In some cases, handedness of organ arrangement is hypothesized to enhance physiological functions such as optimal packing, fluid dynamics, or exchange surface area. In others, it may be influenced by sexual selection (e.g. chelae size in fiddler crabs) or interactions between predators and prey (Norberg 1977, Lee et al. 2012). For example, fish with greater brain lateralization are better able to capture prey in the presence of a predator than are fish without lateralization, whereas the two groups forage equivalently in the absence of predation threat (Dadda and Bisazza 2006). Overall morphological symmetry is sometimes cited as sexual signal in mate selection indicative of high fitness, including absence of disease (reviewed in Møller 1996, 2006), yet the notion that asymmetry could

mitigate the impacts of infection offers a different perspective for why morphological deviation away from symmetry may be selectively reinforced rather than selected against. If asymmetries help to localize the effects of infection, thereby reducing host or parasite fitness costs, they could represent an important form of host tolerance (i.e. the ability of a host to limit damage caused by infection). Strategies involving tolerance – rather than resistance, or the ability of a host to limit infection – have very different evolutionary implications (Råberg et al. 2009). Resistance often stimulates a co-evolutionary arms race between host and pathogen, sometimes leading to increased virulence, whereas tolerance can function to dampen host-parasite conflict and the amount of pathology (Råberg et al. 2009). Given the general importance of symmetry for organismal fitness and the oft-cited premise that natural enemies select against host/prey asymmetries, understanding deviations and their causes will elucidate the circumstances under which asymmetry in host structures or parasite distribution may be beneficial, as well as broaden our understanding of structural defenses in general. If, as found here, the distribution of parasites between structures within a host affects potential metrics associated with fitness, simple quantifications of infection load may inadequately capture the effects of parasites on hosts.

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