

## RESEARCH ARTICLE

# Morphological asymmetry and habitat quality: using fleas and their rodent hosts as a novel experimental system

Elizabeth M. Warburton<sup>1,\*</sup>, Irina S. Khokhlova<sup>2</sup>, Daniel Kiefer<sup>1</sup> and Boris R. Krasnov<sup>1</sup>

## ABSTRACT

Morphological asymmetry is widely used to measure developmental instability and higher levels of asymmetry often correlate with decreased mating success, increased inbreeding, increased stress and decreased habitat quality. We studied asymmetry and relationships between asymmetry and host identity in two flea species, host generalist *Xenopsylla ramesis* and host specialist *Parapulex chephrenis*, and asked: (1) what the level of asymmetry was in their femurs and tibiae; (2) which type of asymmetry predominates; and (3) whether fleas that fed on host species distantly related to their principal host species produced offspring that exhibited greater asymmetry compared with offspring of fleas that fed on their principal host species. We found fluctuating asymmetry in femurs and tibiae of *X. ramesis* and in the tibiae of *P. chephrenis* as well as significantly left-handed directional asymmetry in the femurs of *P. chephrenis*. Host species identity significantly impacted asymmetry in leg segments of *P. chephrenis* but not in those of *X. ramesis*. Offspring asymmetry increased when mother fleas fed on a host that was distantly related to the principal host. Fleas parasitizing multiple host species might compensate for developmental instability when utilizing a novel host species; therefore, host-switching events in host-specific parasites could be constrained by the relatedness between a novel and a principal host species.

**KEY WORDS:** Parasite, Host switching, *Parapulex chephrenis*, Rodentia, Siphonaptera, *Xenopsylla ramesis*

## INTRODUCTION

Bilateral symmetry evolved multiple times in various taxa and over 99% of modern animal species possess this body plan (Finnerty, 2005). Facilitation of directional locomotion (Ruppert et al., 2004; Holló and Novák, 2012) and internal anatomical circulation (Finnerty, 2005) could have provided selective advantages to bilaterally symmetric animals. Bilateral symmetry is pervasive; however, it is not perfect. Symmetry can break down during development as a result of factors arising from inconsistencies within the cell (e.g. Freeman and Lundelius, 1982) or from the external environment (e.g. Govind, 1989). Bilateral traits are rarely exactly identical on both sides of the body; therefore, the

processes behind asymmetry are as ubiquitous as bilateral symmetry itself.

Asymmetry arising from environmental or cellular sources can occur in three different forms: fluctuating asymmetry, directional asymmetry and antisymmetry. In fluctuating asymmetry, differences between left and right trait values (L–R) in a population are randomly clustered around a mean of zero that represents perfect symmetry (Palmer and Strobeck, 1986; Palmer, 1994). However, in directional asymmetry, the mean difference between trait values in a population is not equal to zero and positive values indicate sinistral bias while negative values represent dextral bias (Palmer, 1994; Graham et al., 1998). Antisymmetry exhibits a bimodal or platykurtic distribution, where differences between left and right trait values are randomly distributed around two peaks (Palmer, 1994). Asymmetry, especially fluctuating asymmetry, is widely used as a measure of developmental instability (e.g. Palmer and Strobeck, 1992; Graham et al., 1998). This instability often extends to ecological studies, where levels of asymmetry indicate a metric of individual quality. Thus, ecologists may examine correlations between asymmetry and mating success (Markow and Ricker, 1992; Møller, 1992; Watson and Thornhill, 1994) or use asymmetry to determine levels of stress, inbreeding (Réaale and Roff, 2003; Ludoški et al., 2014) and habitat quality (Cuervo and Restrepo, 2007; Soto et al., 2008; Benítez et al., 2014) that their study organisms experience.

These links between asymmetry and environmental quality or habitat perturbation provide a unique experimental opportunity to parasite ecologists. Parasites use their host as a resource patch or a habitat (Bush et al., 1997) and quantifying parasite asymmetry can provide a novel context for host–parasite relationships. Thus, the environmental quality a parasite experiences could be related to the physiological and/or immunological processes of the host. Novel host species, especially ones distantly related to the principal host species, could represent a lower quality resource for a given parasite (Khokhlova et al., 2014) and thus increase the developmental instability of the parasites forced to utilize them. Co-evolution, host switching and host specificity all focus on evolutionary relationships between hosts and parasites (Combes, 2001; Poulin, 2011). In addition, host switching may be constrained by the relatedness of the host species in question (Krasnov et al., 2004a). Thus, using parasite asymmetry to examine proximate effects of host quality can provide insight into the ultimate causes of these events.

Here, we explored asymmetry and the relationships between asymmetry and host identity in two flea species, *Xenopsylla ramesis* and *Parapulex chephrenis*, parasitic on rodents and sharply different in their degree of host specificity. *Xenopsylla ramesis* naturally parasitizes a variety of rodents; however, because it attains highest abundance and prevalence (*sensu* Bush et al., 1997) on *Meriones crassus* (Sundevall's jird), this is considered as the principal host of this flea (Krasnov et al., 1999). In contrast, *P. chephrenis* almost

<sup>1</sup>Mitrani Department of Desert Ecology, Swiss Institute for Dryland Environmental and Energy Research, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben-Gurion 8499000, Israel. <sup>2</sup>Wyler Department of Dryland Agriculture, French Associates Institute for Agriculture and Biotechnology of Drylands, Jacob Blaustein Institutes for Desert Research, Ben-Gurion University of the Negev, Sede Boqer Campus, Midreshet Ben-Gurion 8499000, Israel.

\*Author for correspondence (warburte@post.bgu.ac.il)

 E.M.W., 0000-0001-8125-2741

exclusively exploits its principal host species *Acomys cahirinus* (Egyptian spiny mouse) and is only rarely found on other rodent species (Krasnov et al., 1999). Fleas are a convenient model parasite for laboratory studies because, while the adults are obligate blood-feeding insects, they can alternate on-host periods with off-host periods to oviposit in the substrate of the host's burrow. Fleas are holometabolous and their non-haematophagous larvae usually reside in the burrow where they pupate and then emerge as adult fleas. Thus, fleas are easily monitored in the laboratory during all life stages. Both rodent hosts and fleas can be easily maintained in the laboratory to allow for experimental host switching and analysis of its effects on the resulting offspring. We examined flea femurs and tibiae for asymmetry because flea leg morphology is crucial for locomotion. Femurs are directly attached to the structures that power jumping while tibiae transfer the force generated from these structures to the ground (Rothschild et al., 1973; Rothschild et al., 1975; Sutton and Burrows, 2011). The ability of fleas to jump properly influences their ability to successfully find a host and to avoid host grooming, both of which directly impact flea survival. Therefore, any significant asymmetry would alter their ability to jump straight towards a target and is likely to impact an individual flea's probability of survival (Rothschild et al., 1973).

To better understand how the host, a parasite's habitat, influences parasite asymmetry, we maintained *X. ramesis* and *P. chephrenis* on multiple species of rodents of varying relatedness to the principal host of a given flea. Our experiments had three main goals: (1) to assess levels of asymmetry in the femurs and tibiae of each flea species; (2) to identify which type of asymmetry was present in each flea species; and (3) to determine whether fleas that fed on host species distantly related to their principal host species produced offspring that exhibited greater asymmetry than those that fed on their principal host species. We predicted that in both flea species: (1) asymmetry would be more pronounced in tibiae because femurs directly connect to structures that power the flea's jump (Rothschild et al., 1975); (2) fluctuating asymmetry would be more common than directional asymmetry; and (3) asymmetry would be greater in offspring if maternal fleas exploited a host species distantly related to the principal host species.

## MATERIALS AND METHODS

### Study animals

We used two different sets of hosts for each flea species. Hosts for *X. ramesis* (Rothschild 1904) included *M. crassus* (principal host), *Gerbillus nanus*, *Gerbillus pyramidum*, *A. cahirinus* and *Mesocricetus auratus*. Hosts for *P. chephrenis* (Rothschild 1903) included *A. cahirinus* (principal host), *A. russatus*, *M. crassus* and *M. auratus*. *Mesocricetus auratus* is distantly related to other host species (it belongs to the family Cricetidae, whereas the remaining hosts belong to Muridae). In addition, all rodent species, except for *M. auratus*, inhabit the same geographical area as fleas used in this study. In other words, both *X. ramesis* and *P. chephrenis* have the opportunity to encounter all of these potential hosts with the exception of *M. auratus*. Thus, *M. auratus* is a totally novel host species for *X. ramesis* and *P. chephrenis* from both evolutionary and ecological standpoints. Fleas and rodents, except for golden hamsters (*M. auratus*), originated from our laboratory colonies, whereas golden hamsters were commercially available. Specific details regarding rearing procedures and colony maintenance are published elsewhere (e.g. Krasnov et al., 2002; Khokhlova et al., 2009).

Prior to experiments, rodents were individually housed in plastic cages (33×23×13 cm at 25±1°C and 12 h:12 h dark:light) with

wood shavings as bedding material. They were fed whole millet seeds *ad libitum* and fresh alfalfa as a water source. Drinking water was not offered. All rodents were weighed weekly to assess their condition and no experimental animals had poor or deteriorating condition. During experimental infestations with fleas, each rodent was placed in an individual plastic cage (33×23×13 cm) with the bottom covered by a 1 cm sand layer and a set of three wire screens separating the animal from the sandy floor. This design created a refugium for fleas that provided them with a suitable environment for oviposition as well as larval development and allowed adult fleas to hide between blood meals.

### Experimental design

Fleas were randomly selected from laboratory colonies and used to infest a single individual host of each rodent species in groups of 30–90 fleas (one-third males and two-thirds females). We used a single individual of each host species to avoid additional sources of variation unrelated to the question at hand. Although variation among individual conspecific hosts may result in variation of flea responses, this variation is lower than variation resulting from interspecific host differences. For example, the number of eggs produced by a female *X. ramesis* varies less among individuals of the same host (*M. crassus*) [coefficient of variation (CV)=0.33 versus 0.93, respectively; calculated from data of Khokhlova et al., 2010a, 2012a]. Furthermore, in our study an experimental unit was an individual flea, whereas a treatment group was a group of fleas fed on a host belonging to a given species. In other words, we manipulated host identity and kept all other factors constant as accepted by the principles of experimental design (e.g. Cochran and Cox, 1992).

The number of fleas per animal was appropriate for the size of each rodent species, with approximately 1 flea per 1 g of host body mass. Fleas were allowed to feed on rodents uninterrupted for 72 h. After 72 h, we collected fleas from the rodents' bodies, via brushing with a toothbrush, and from the sand within the cage. After all fleas were recovered, we placed sand, containing eggs that adult female fleas had oviposited, from each individual rodent cage into separate plastic boxes (20×10×10 cm) and added larvae nutrient medium (94% dry bovine blood, 5% millet flour, 1% ground faeces of the principal host species). The amount of medium was at least double the food requirements of larvae as per our earlier observations. These plastic boxes were placed in an incubator (FOC225E, Velp Scientifica srl, Milano, Italy) and maintained at 25°C with 92–95% relative humidity as monitored by Fisherbrand Traceable Humidity/Temperature Pen with Memory (Fisher Scientific International, Somerville, NJ, USA). We then checked these boxes daily according to the minimum development time of each flea species (35 days post-infestation for *P. chephrenis* and 24 days post-infestation for *X. ramesis*; Khokhlova et al., 2010b) until 2 weeks after the last new imago emerged. All experimental protocols met the requirements of the 1994 Law for the Prevention of Cruelty to Animals (Experiments on Animals) of the State of Israel and were approved by the Ben-Gurion University Committee for the Ethical Care and Use of Animals in Experiments (permit IL-72-10-2012).

### Leg measurement

After new flea imagoes emerged, they were placed in individual vials filled with 70% ethanol for preservation until they were photographed. Femurs and tibiae were photographed under ×940 magnification using a Moticam 2000 digital microscope camera with Motic Images Plus 2.0 software (Motic, Speed Fair Ltd, Causeway Bay, Hong Kong) that was calibrated with an object-micrometer prior to use. After fleas were photographed, we measured the maximal length of the right and left

**Table 1. Results of mixed-model ANOVA for asymmetry in *Xenopsylla ramesis***

Leg segment	Type of variation	<i>F</i>	d.f. <sub>1</sub>	d.f. <sub>2</sub>	<i>P</i>
Femur	Fluctuating asymmetry/ antisymmetry	3.652	138	556	<0.001
	Directional asymmetry	1.342	1	138	0.249
	Variation due to trait size	2.685	138	138	<0.001
Tibia	Fluctuating asymmetry/ antisymmetry	1.404	138	556	<0.001
	Directional asymmetry	0.1714	1	138	0.026
	Variation due to trait size	8.903	138	138	<0.001

femurs and tibiae to 0.001 mm for each individual flea (139 *X. ramesis*, 110 *P. chephrenis*). Each leg segment was measured in triplicate by the same investigator, who rotated the image 90 deg between each measurement to reduce error (see Table S1). Morphological landmarks present on femurs and tibiae facilitated consistent measurement between replicates. Femurs and tibiae were measured separately because they contribute differently to locomotion.

### Statistical analyses

We used a mixed-model analysis of variance (ANOVA) specifically developed to determine the presence and type of asymmetry (Palmer and Strobeck, 1986; Palmer, 1994) to analyse asymmetry in each flea species separately. This analysis includes leg segment length as a dependent variable, side (right versus left) as a fixed factor and identification number of an individual flea as a random factor (Palmer, 1994; Palmer and Strobeck, 2003; Réaale and Roff, 2003). This allowed us to determine the significance of three effects with one test: the effect of individual (i.e. variation among individual fleas), the effect of side (i.e. directional asymmetry), and the interaction between individual and side (i.e. non-directional asymmetry composed of fluctuating asymmetry and antisymmetry). This method is also advantageous because it assesses significance relative to measurement error, thus providing an unbiased evaluation of each asymmetry type (Palmer, 1994; Palmer and Strobeck, 2003; Réaale and Roff, 2003). For traits that exhibited directional asymmetry, we tested the handedness of the direction using a *t*-test in which the signed differences between mean length of the left and right sides ( $L_{\text{mean}} - R_{\text{mean}}$ ) were compared with zero (i.e. perfect symmetry). For traits that exhibited non-directional asymmetry, we visually inspected the distribution of trait values around the mean to ensure that it was not bimodal or platykurtic, thus confirming that fluctuating asymmetry rather than antisymmetry was present (Palmer, 1994).

After determining the type of asymmetry present for femurs and tibiae in each species, we then sought to uncover underlying trends in asymmetry related to flea sex and host relatedness to the principal

**Table 2. Results of mixed-model ANOVA for asymmetry in *Parapulex chephrenis***

Leg segment	Type of variation	<i>F</i>	d.f. <sub>1</sub>	d.f. <sub>2</sub>	<i>P</i>
Femur	Fluctuating asymmetry/ antisymmetry	1.262	109	440	0.054
	Directional asymmetry	163.339	1	109	<0.001
	Variation due to trait size	725.953	109	109	<0.001
Tibia	Fluctuating asymmetry/ antisymmetry	4.364	109	440	<0.001
	Directional asymmetry	1.458	1	109	0.229
	Variation due to trait size	62.500	109	109	<0.001

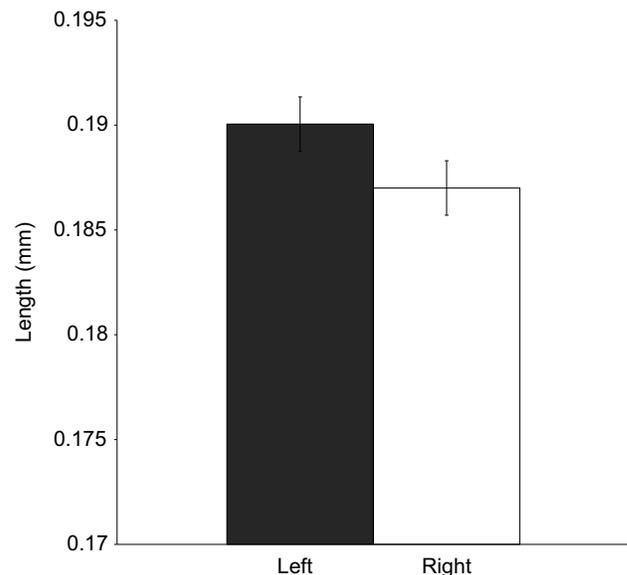
**Table 3. Effects of host relatedness rank (HR) and flea sex (FS) on absolute length differences between left and right leg segments in *X. ramesis***

Segment	Source	SS	d.f.	MS	<i>F</i>	<i>P</i>
Femur	Intercept	0.001	1	0.001	26.648	<0.001
	HR	0.00006	4	0.00002	0.082	0.518
	FS	0.00003	1	0.00003	1.599	0.208
	HR×FS	0.00008	4	0.00002	0.954	0.437
	Error	0.003	128	0.00002		
Tibia	Intercept	0.001	1	0.001	45.135	<0.001
	HR	0.00009	4	0.00002	0.789	0.534
	FS	0.00001	1	0.00001	0.369	0.545
	HR×FS	0.00005	3	0.00001	0.478	0.752
	Error	0.004	128	0.00003		

host of a flea. Fleas exhibit sexual size dimorphism with females being larger than males. Therefore, we wanted to account for the possibility of sexually dimorphic asymmetry. To determine host effect, we ranked hosts according to their relatedness to the principal host of a given flea based on a previously published phylogenetic supertree (Khokhlova et al., 2012b), assigning the principal host a rank of 1 (*M. crassus* for *X. ramesis* and *A. cahirinus* for *P. chephrenis*). The remaining hosts for *X. ramesis* were ranked as *G. nanus* (2), *G. pyramidum* (3), *A. cahirinus* (4) and *M. auratus* (5). The remaining hosts for *P. chephrenis* were ranked as *A. russatus* (2), *M. crassus* (3) and *M. auratus* (4). We then performed two-way ANOVA for each species and each leg segment using the absolute amount of asymmetry present in each flea ( $L_{\text{mean}} - R_{\text{mean}}$ ) as the dependent variable and flea sex and host relatedness rank as fixed factors. Traits that indicated significant effects of flea sex, host relatedness rank, or the interaction between flea sex and host relatedness rank were subjected to multiple comparisons using Hochberg's GT2 test. All statistical analyses were performed using SPSS version 22 (IBM Corporation 2013).

### RESULTS

We found fluctuating asymmetry in femurs ( $F_{138,556}=3.652$ ,  $P<0.001$ ) and tibiae ( $F_{138,556}=1.404$ ,  $P=0.004$ ) of *X. ramesis* (Table 1) as well as in the tibiae of *P. chephrenis* ( $F_{109,440}=4.364$ ,

**Fig. 1. Mean ( $\pm$ s.e.m.) length of left and right femurs of *Parapulex chephrenis*.**

**Table 4. Effects of host relatedness rank (HR) and flea sex (FS) on absolute length differences between left and right leg segments in *P. chephrenis***

Segment	Source of variation	SS	d.f.	MS	F	P
Femur	Intercept	0.00008	1	0.00008	71.653	<0.001
	HR	0.00002	3	0.00001	1.685	0.181
	FS	<0.00001	1	<0.00001	0.015	0.903
	HR×FS	<0.00001	3	<0.00001	0.219	0.883
	Error	0.00090	101	<0.00001		
Tibia	Intercept	0.00100	1	0.00100	89.407	<0.001
	HR	0.00013	3	0.00004	2.726	0.048
	FS	<0.00001	1	<0.00001	0.001	0.984
	HR×FS	0.00004	3	0.00002	0.949	0.420
	Error	0.00200	101	0.00002		

$P<0.001$ ; Table 2). We also detected directional asymmetry ( $F_{109,440}=163.339$ ,  $P<0.001$ ; Table 2) in the femurs of *P. chephrenis* only (Fig. 1). This directional asymmetry in *P. chephrenis* was significantly left-handed ( $t=5.325$ , d.f.=109,  $P<0.001$ ).

Fluctuating asymmetry in the femurs and tibiae of *X. ramesis* was not affected by either flea sex or host relatedness rank (Table 3). The same was true for directional asymmetry in femurs of *P. chephrenis* (Table 4). In contrast, fluctuating asymmetry in tibiae of this species were significantly affected by host relatedness rank ( $F=2.726$ , d.f.=3,  $P=0.048$ ). These data exhibited equality of variances (Levene's  $F_{3,96}=0.960$ ,  $P=0.415$ ) and subsequent multiple comparisons showed that asymmetry was significantly different ( $P=0.049$ ; Table 5) between offspring of fleas infesting *A. cahirinus* (host relatedness rank=1) and those infesting *M. auratus* (host relatedness rank=4), with fleas infesting *M. auratus* exhibiting greater levels of asymmetry (Fig. 2).

## DISCUSSION

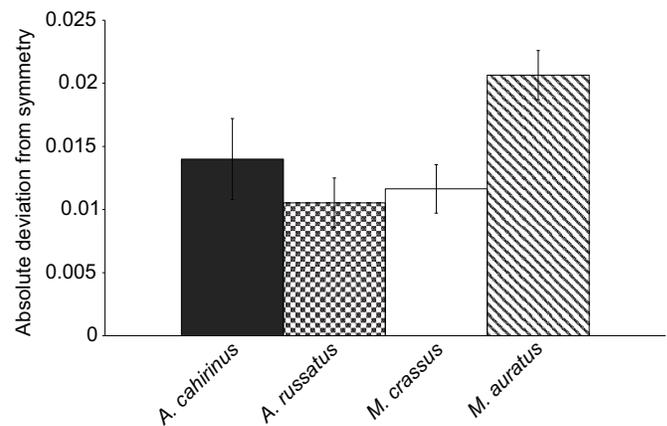
We found significant asymmetry in both species of flea; however, our results only partially support our predictions. Both femurs and tibiae exhibited significant asymmetry and, as predicted, fluctuating asymmetry was more common than directional asymmetry. Tibiae of both species had significant fluctuating asymmetry; however, fluctuating asymmetry was found in *X. ramesis* femurs while directional asymmetry was found in *P. chephrenis* femurs. Asymmetry was affected by host identity in *P. chephrenis* but not *X. ramesis*. Offspring asymmetry increased in *P. chephrenis* when mother fleas fed on a host distantly related to the principal host.

It was somewhat unexpected to find significant directional asymmetry in one of the flea species; however, some features of *P. chephrenis* and the rodents they infest might account for this type of asymmetry. Firstly, *P. chephrenis* is a so-called 'body flea'

**Table 5. Multiple contrasts using Hochberg's GT2 test for effects of host relatedness rank on asymmetry in *P. chephrenis* tibiae**

Host relatedness rank I	Host relatedness rank J	Mean (±s.e.m.) difference (I–J)	P
1	2	−0.0012±0.0010	0.798
	3	−0.0022±0.0011	0.299
	4	−0.0030±0.0011	0.049
2	3	−0.0009±0.0010	0.934
	4	−0.0018±0.0010	0.407
3	4	−0.0009±0.0011	0.970

Hosts were ranked in the following manner: *Acomys cahirinus* (1, N=25), *Acomys russatus* (2, N=35), *Meriones crassus* (3, N=24) and *Mesocricetus auratus* (4, N=24).

**Fig. 2. Mean (±s.e.m.) absolute values of deviation from symmetry (L–R) of tibiae of the offspring of *P. chephrenis* feeding from different hosts.** Host species: *Acomys cahirinus* (principal host), *Acomys russatus*, *Meriones crassus* and *Mesocricetus auratus*.

(Krasnov et al., 2004b), spending most of its time on a host and only leaving the host to oviposit. This contrasts sharply with *X. ramesis*, which is a 'nest flea' that frequently alternates between on- and off-host periods. Nest fleas only engage with a host long enough to take a blood meal and then return into the burrow's substrate (Krasnov, 2008). In addition, fleas generally prefer areas with less fur and/or areas that are difficult to groom, such as ear pinnae or areas around the head and neck (Krasnov, 2008). These site choices are influenced by interactions with hosts and are not caused by programmed preferences or interspecific interactions between ectoparasites (Pilosof et al., 2012). Egyptian spiny mice (*A. cahirinus*), the principal host of *P. chephrenis*, lack fur on their pinnae and they do not have their characteristic spines around their head, so fleas are readily observed feeding in these areas. Finally, most individual rodents are right-pawed (Agulova et al., 2010; Kutlu et al., 2012; Ribeiro-Carvalho et al., 2010; Ribeiro et al., 2014) and begin their grooming sequence at the right side of their head (Berridge, 1990). Thus, a speculative but logical sequence of events related to flea avoidance of host grooming could explain directional asymmetry favouring the sinistral side in *P. chephrenis*. In this scenario, fleas are on the head of the rodent and oriented so that they face the caudal end of their host. The host begins grooming on the right side of the head (Berridge, 1990) and the flea relocates to its right side (the rodent's left side) to avoid being killed. Being a body flea, *P. chephrenis* would probably move to a different area on the host, rather than leave the host like a nest flea would. Then, when the host finishes grooming the right side, the flea turns and moves to its right, which is also now the rodent's right, to return to its preferred feeding location. Again, although logical, this scenario is speculative and needs to be tested experimentally.

We expected that fluctuating asymmetry not only would be more common than directional asymmetry in fleas but also would be higher if the host on which the maternal fleas fed was not closely related to the flea's principal host. This was the case for *P. chephrenis* and increased asymmetry indicates that *M. auratus* could be a poorer resource for this flea species. Increased fluctuating asymmetry and, in turn, increased developmental instability might be a result of decreased offspring quality following maternal feeding on *M. auratus*, despite the ability of *P. chephrenis* to take blood meals from this host and produce eggs thereafter (Khokhlova et al., 2012a,b). Although the difference in the level of asymmetry between offspring of fleas fed on *A. cahirinus* and *M. auratus* was not especially large,

it was nevertheless significant. This suggests that the reason behind lower quality offspring produced by mothers exploiting *M. auratus* could be the nutritional unsuitability of golden hamster blood for *P. chephrenis*. Interestingly, we found that this transgenerational effect occurs in fleas, a taxon in which developing larvae and pupae have no direct contact with the host. Fluctuating asymmetry in the generalist flea species *X. ramesis* did not depend on host identity and feeding performance of *X. ramesis* indicates that it can successfully infest completely alien hosts such as bats (Korine et al., 2012). Our results tentatively suggest that, at least in our host–parasite system, a generalist lifestyle enables fleas to somehow compensate for developmental instability when exploiting novel host species. One of the ways in which successful host-switching events might be separated from unsuccessful ones is by the level of developmental instability, as indicated by asymmetry, that a flea exhibits on the new host species. To further confirm this, we suggest that more alien host taxa be used in future experiments investigating host effects on parasite asymmetry.

To successfully switch hosts, parasites must pass through an encounter filter and a compatibility filter (Holmes, 1987; Combes, 2001). Encounter filters determine whether transfer from one host to another is ecologically possible via coincidence in space and time, while compatibility filters, such as host defences and nutritional quality, determine whether the parasite can survive on or in the novel host (Combes, 2001). Selective pressures associated with multiple compatibility filters could provide these generalist fleas with a suite of characteristics that enables them to more readily switch hosts. Thus, encounter and compatibility filters suggest that co-occurring, phylogenetically close hosts might facilitate host switching for specialist fleas but not necessarily generalist fleas. However, not all host switches need to be between closely related hosts. Switching between unrelated hosts occurred in the evolutionary history of multiple parasites. Ecological fitting, whereby traits relevant to host–parasite interactions evolved with different species or under different conditions, is the most common explanation for these phenomena (see Agosta et al., 2010, for a recent review). However, the potential for ecological fitting could differ among parasitic species.

Our research provides novel insight into the effect of host quality on parasite development. Generalist parasites might be able to cope with the developmental instability that comes with a lower quality resource because they have already passed through multiple compatibility filters. Host-specific parasites, in contrast, might require a potential host that is more similar to their principal host because they have not been subjected to the selective pressures that arise from multiple compatibility-filtering events. Thus, parasites with different levels of specialization could differ in their potential to broaden their host spectra and/or switch hosts. The ability to exploit multiple hosts confers the advantages of greater resource availability and stability, without a concomitant decrease in abundance, allowing parasites to resist local perturbations in their host populations (Krasnov et al., 2004c). Broadening of the host spectrum could therefore be advantageous but, depending on the parasite species, might be constrained by phylogenetic relatedness within the local host community.

In addition, morphological asymmetry due to developmental disruption in parasites could arise as a response not only to an unfavourable host species but also to an unfavourable host individual, although the effect of intraspecific host variability is likely to be weaker than the effect of interspecific host variability (see above). Indeed, intraspecific host variation has been shown to affect various reproductive traits in parasites including, for example,

duration of development (e.g. Khokhlova et al., 2010a) and thus might result in variation in the degree of asymmetry. Furthermore, variation in morphological asymmetry among fleas collected from the same host species has been reported (Korzun and Nikitin, 1997). Interestingly, this variation was associated with variation in their ability to transmit plague via differential patterns of formation of gut blockage following bacterial multiplication (see Gage and Kosoy, 2005, for details), although the mechanism of this association is completely unknown. However, the effect of intraspecific host variation on the manifestation of morphological asymmetry of parasites has never been studied and warrants further investigation.

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#### Competing interests

The authors declare no competing or financial interests.

#### Author contributions

B.R.K. and I.S.K. conceived and designed the experiments. I.S.K. conducted the experiments. E.M.W. and D.K. collected data. E.M.W. performed statistical analyses. All authors drafted the manuscript.

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#### Supplementary information

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