Variation within and among host species in engorgement of larval trombiculid mites

C.-C. KUO[1,2]*§, H.-C. WANG[2] *and* C.-L. HUANG[2]

[1] *Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA*
[2] *Research and Diagnostic Center, Centers for Disease Control, Department of Health, No. 6, Linsen S. Road, Taipei, Taiwan, ROC*

SUMMARY

We recovered larval trombiculid mites (i.e. chiggers), vectors of scrub typhus, from small mammal hosts in Taiwan, and compared the relative degree of engorgement (RDE) of the dominant chigger (*Leptotrombidium imphalum*) from different hosts. Naturally occurring chiggers recovered from *Rattus losea* and *Bandicota indica* were 1·4x and 1·3x as engorged as those from *Apodemus agrarius*. Within each host species, RDE was negatively related to chigger loads, but was mostly unrelated to gender or to body or reproductive condition of hosts. We documented significant variation in chigger engorgement both within and among host species; to the extent that RDE is a proxy for fitness, this contradicts predictions of the Ideal Free Distribution (IFD) that the *per capita* fitness of vectors should be similar among hosts. Failure to meet predictions of the IFD may reflect the limited mobility of chiggers, which consequently must be less selective in the hosts on which they feed. Further disease control efforts should consider vector feeding success in addition to vector abundance and may be able to capitalize on the unsuitability of certain hosts in supporting disease vectors.

Key words: disease control, feeding success, Ideal Free Distribution, *Leptotrombidium imphalum*, *Orientia tsutsugamushi*, trombiculid mites, scrub typhus.

INTRODUCTION

The persistence of any vector-borne disease requires the co-existence of the pathogen, vector, and host. Arthropod vectors associated with transmission of human disease normally are not limited to single host species (Ostfeld and Keesing, 2000), and feeding success, growth, and development of vectors can vary with host species. For example, engorged larval black-legged ticks (*Ixodes scapularis*) recovered from the striped skunk, red and eastern gray squirrel, and white-tailed deer had higher moulting success than ticks foraging on birds and the northern raccoon (LoGiudice *et al*. 2003). Unfed larval *I. scapularis* placed on the white-footed mouse had much higher feeding success than those placed on the Virginia opossum (Keesing *et al*. 2009). Assassin bugs (*Triatoma infestans*) were heavier and more engorged when feeding on domestic dogs than on cats or chickens (Gürtler *et al*. 2009), and bloodmeal size and egg production by fleas (*Parapulex chephrenis* and *Xenopsylla* spp.) differed with hosts (Krasnov *et al*.

2004; Khokhlova *et al*. 2008). Finally, survival and reproduction of many mosquito species also vary with vertebrate hosts (Lyimo and Ferguson, 2009).

Given these observations, assessing host suitability has important implications for prevention of vector-borne diseases, as it allows disease control efforts to more clearly target appropriate hosts. Moreover, vectors on unsuitable hosts may have much lower chances of surviving, moulting, or laying eggs so that the inclusion of such hosts in the community may reduce vector abundance (Keesing *et al*. 2009), a phenomenon resembling the 'dilution effect', in which the presence of incompetent hosts can reduce the prevalence of disease among vectors (Ostfeld and Keesing, 2000; LoGiudice *et al*. 2003).

The observation that vectors feed on unsuitable hosts is, nevertheless, inconsistent with the evolutionary perspective that vectors should endeavour to maximize fitness. The Ideal Free Distribution (IFD; Fretwell and Lucas, 1970) predicts that competition for resources will impel animals to disperse themselves among resource patches so that at equilibrium, *per capita* fitness should be similar across patches. The IFD recently has been proposed to explain the marked heterogeneity of vectors distributed among hosts, and it predicts that vectors should disperse themselves such that per capita fitness is similar across hosts (Kelly *et al*. 1996; Kelly and Thompson,

* Corresponding author: Department of Wildlife, Fish, & Conservation Biology, University of California, Davis, One Shields Avenue, Davis, CA 95616, USA. Tel: +886-4-23226940. Fax: +886-4-23232146. E-mail: ccckuo@ucdavis.edu
§ Current address: Department of Zoology, National Museum of Natural Science, Taichung 404, Taiwan.

2000; Kelly, 2001). However, most studies on variation in host suitability have constrained access to only one or a few selected hosts (Kelly, 2001; but see LoGiudice *et al.* 2003; Gürtler *et al.* 2009). As such, these studies were unable to demonstrate if unrestrained vectors would feed on unsuitable hosts and distribute themselves according to the IFD. This is notable because the IFD assumes that the cost of assessing the quality of a resource patch is negligible (Fretwell and Lucas, 1970). The IFD may thus hold for vectors with greater mobility (e.g., mosquitoes), but not for less vagile vectors (e.g., ticks, mites) due to the high costs associated with host switching.

Scrub typhus (*Orientia tsutsugamushi* ; OT) is an acute human infectious disease prevalent mainly in the western Pacific (Kawamura *et al.* 1995). Scrub typhus is vectored by larval trombiculid mites (i.e., chiggers), which are very limited in mobility (<45 cm, Philip *et al.* 1949), normally waiting on debris or twigs for hosts passing by (Traub and Wisseman, 1974). *Leptotrombidium* chiggers are the primary vectors of OT, and murine rodents, especially *Rattus* species, are the predominant hosts of chiggers in regions with endemic scrub typhus (Traub and Wisseman, 1974; Kawamura *et al.* 1995). Trombiculid mites are the only reservoirs of OT, while vertebrate hosts provide chiggers with food resources, but play little role in transmitting OT (Kawamura *et al.* 1995).

Between 2007 and 2008, we trapped rodents and collected trombiculid chiggers in a region of Taiwan with prevalent scrub typhus (Kuo, 2010). *Apodemus agrarius* was the primary host (47·82% of recovered), followed by *Rattus losea* (40·77%) and *Bandicota indica* (10·42%). *Leptotrombidium imphalum* dominated both among (84·47% of identified) and within host species (>80%, except *Mus musculus*), and is the potential vector in transmitting local inhabitants scrub typhus (Kuo, 2010). We quantified and compared the relative degree of engorgement (RDE) of *L. imphalum* recovered from different host species and different individuals of the same host species. We addressed 3 questions. First, we asked whether RDE of *L. imphalum* differed among host species across the study region under natural conditions. Second, because feeding success of vectors can be related to some intrinsic factors of hosts, such as age, gender, and body condition (Krasnov *et al.* 2005; Hawlena *et al.* 2007a; Khokhlova *et al.* 2009), we evaluated whether RDE of *L. imphalum* was affected by host gender, as well as host body and reproductive condition. We also evaluated whether negative density-dependent feeding success occurred under natural conditions. Finally, we tested whether RDE of *L. imphalum* was similar within and among host species in each trapping plot, and discussed the compliance with the IFD of this disease vector.

MATERIALS AND METHODS

Study area

We studied chiggers and their hosts in abandoned agricultural fields in the lowlands of central Hua-lien County of eastern Taiwan (23°47′N, 121°29′E). Although one of Taiwan's least populated counties, Hua-lien had the country's second highest number of human cases of scrub typhus between 1998 and 2007 (after Kin-men Island; CDC, URL: http://nidss.cdc.gov.tw/).

Small mammal trapping and collection of chiggers

Small mammals were trapped monthly from January to March 2007, and from August 2007 to March 2008 (except November 2007). In the former period, we sampled 14 plots, deploying 2 parallel transect lines containing 10 Sherman traps ($26·5 \times 10 \times 8·5$ cm) at 10 m intervals, and 2 hand-made live traps ($27 \times 16 \times 13$ cm) at 50 m intervals. Hand-made traps were used to target the less abundant, but larger *B. indica*. Adjacent transect lines were separated by 10 m. In the latter period, we increased trapping effort, sampling 35 additional fields with 3 parallel transect lines containing 10 Sherman traps at 10 m intervals, and 3 hand-made live traps at 30 m intervals in each plot; adjacent transect lines were separated by 10 m. Six plots were surveyed in both periods. Plots were separated by at least 500 m. Traps were opened and baited in the evening and checked for captures early in the morning. Sites were surveyed for 3 consecutive nights throughout the course of this study.

Trapped small mammals (rodents and shrews) were transferred to a clean nylon mesh bag; bags were carefully examined to ensure that no arthropod vectors remained from earlier captures. Rodents were anaesthetized with Zoletil 50 (Fa. Virbac. Carros, France) and examined for gender and reproductive status. Reproductive activity was inferred for males if testes were scrotal, and for females if vaginae were perforate and/or nipples were swollen; otherwise, animals were considered reproductively inactive. Animals were weighed to the nearest 0·5 g. Head + body length (snout to anus) and tail length (anus to tip of tail) were measured (mm), and the length of the ear and hind foot were recorded (0·1 mm). Morphological measurements were implemented by the same researcher to minimize individual differences in measurement bias. Rodents were examined for ectoparasites by combing their fur carefully. Skin with attached chiggers was detached with minimal injury to the animals and preserved in vials; chiggers released themselves from the skin and were transferred to 70% ethanol after 2 days. We counted the number of chiggers recovered from each host animal. Rodents were identified by fur clips and released at least 5 km away from the study areas. Shrews (*Crocidura attenuata, C. suaveolens*, and *Suncus*

murinus) were screened for ectoparasites; those with chiggers were euthanized with an overdose of Zoletil 50, and blood collected via heart puncture. Those free of ectoparasites or infected only with ticks were identified with fur clips and released outside the study areas without collecting blood. Length of body, tail, ear, and hind-foot were measured as for rodents. Sex of each shrew was also recorded as above. Reproductive activity was determined only for females by the existence of swollen nipples. All procedures were approved by the University of California, Davis Animal Use and Care Administrative Advisory Committee and met guidelines recommended by the American Society of Mammalogists (Gannon *et al.* 2007).

Chigger identification and measurement

We randomly selected at least one-fifth of chiggers from each host individual for species identification. Chiggers were soaked in deionized water for 2–3× 30-min baths, then slide-mounted in Berlese fluids (Asco Laboratories, Manchester, UK). Slides were dried at 40 °C for at least 1 night, and chiggers were examined under a light microscope and identified with published keys (Wang and Yu, 1992; Li *et al.* 1997).

Leptotrombidium imphalum was the most common chigger identified (84·47%), and was recovered from most small mammal host individuals (>82·65%, except *Mus musculus*) (Kuo 2010); consequently, we measured RDE only for this chigger species. Feeding leads to enlargement of the idiosoma (the body part bearing the legs) of chiggers. The idiosoma is elliptical in shape, and we measured both the length and width with an ocular micrometer. The area of the idiosoma was calculated as π(length/2)(width/2). Because the thickness of the idiosoma is difficult to assess, we only calculated area. We defined RDE as the increase in idiosoma area relative to the smallest idiosoma area measured, which was assumed to be similar to that of an unfed chigger.

Because recovering fully engorged chiggers requires caging hosts in the laboratory, which would greatly limit the number of animals that can be sampled, we detached feeding chiggers directly from the hosts. Additionally, the very small size of even highly engorged chiggers (length *ca.* 0·5 mm) hindered their recovery from the collecting water pans placed under the holding cage. Consequently, chiggers recovered in this study were in different feeding stages, with some close to repletion while others were just preparing to feed. To avoid confounding our conclusions by including non- or little engorged chiggers, we analysed only chiggers with RDE in the upper 25th percentile within each host individual (analyses with all chiggers included got qualitatively similar results, see Appendix 1 for details). Additionally, we excluded hosts with <2 measured

chiggers in the upper 25th percentile (or <8 measured chiggers).

Statistical analysis

Individual hosts were treated as replicates, except where otherwise mentioned. Differences in RDE among host species were first compared across the entire study region. RDE for each individual host was defined as the mean RDE of chiggers recovered from that host. We confirmed normality and homogeneity of variance with Shapiro-Wilk and Levene tests, respectively, and transformed the data if necessary. We applied analysis of variance (ANOVA) or Kruskal-Wallis tests, depending on whether assumptions were or were not fulfilled. When necessary, these were followed by Tukey tests (ANOVA) or by pair-wise Mann-Whitney U tests after Bonferroni adjustment ($\alpha = 0.05/n$ comparisons).

For each of the major hosts (*A. agrarius*, *R. losea*, *B. indica*), we examined whether RDE of each individual host was related to its gender, reproductive condition, and body condition. We also examined the association of RDE to chigger loads to evaluate any negative density dependence. Body condition was defined as the residual of ordinary least-squares regressions between log(body weight) and log(length of body) (Schulte-Hostedde *et al.* 2005), after controlling for the effect of gender, reproductive condition, and season. Season was categorized for each month of trapping effort as above *vs* below the long-term (1971–2000; Central Weather Bureau; URL: http://www.cwb.gov.tw/) mean temperature of Hua-lien (23·3 °C). Pregnant females were excluded from analysis. RDE was expressed as a linear combination of explanatory variables: RDE $= \alpha + \beta_1{}^*$(gender) $+ \beta_2{}^*$(reproductive condition) $+ \beta_3{}^*$(body condition) $+ \beta_4{}^*$(chigger load) $+ \varepsilon_i$. Because heteroscedasticity could not be corrected for even after transformation, we estimated the 95% confidence interval for each explanatory variable by bootstrapping our data (1999 iterations, with replacement).

We tested whether RDE of chiggers was similar both among and within host species within trapping plot, considering each individual host as a habitat patch and plots as replicates, and assuming chiggers could encounter all rodents trapped within the same plot. Six plots were sampled in both sampling periods, and therefore may not be independent; we excluded these 6 plots from analysis of the first time-period but retained them for analysis of the second time-period because trapping effort was greater and more hosts were trapped in the second period. For tests among host species but within plot, we first calculated the mean RDE for each individual host, and then calculated the mean RDE for each host species. Because *B. indica* was very uncommon in most plots (7 plots with >1 *B. indica*), we only

compared *A. agrarius vs R. losea*. Because normality and homoscedasticity could not be fulfilled even after transformation, we used a non-parametric Wilcoxon signed rank test to assess if RDE differed between the two host species (*A. agrarius* and *R. losea*) within plots.

To evaluate any intra-host species variation within plots in RDE, each host species was separately analysed with a nested ANOVA; because normality and homogeneity of variance could not be fulfilled even after transformation, we used ranked input rather than raw data, making this a non-parametric analysis. RDE of chiggers across all plots was ranked, and rank was then treated as the dependent variable, with plots as the fixed factor, and host individual as a random factor nested within plots as in parametric nested ANOVA (Conover and Iman, 1981). Mean values are followed by ±1 s.e. All procedures were implemented in SPSS 16.0 (SPSS Inc. Chicago, Illinois, USA).

RESULTS

We captured 1393 small mammals belonging to 8 species, from which we recovered 110 760 chiggers. We identified 26 274 chiggers (23·72% of the sample), and measured (length and width of idiosoma) 19 464 *L. imphalum*. This sample included 10 475 *L. imphalum* from 483 *A. agrarius*, 7101 from 82 *R. losea*, 1744 from 35 *B. indica*, 74 from 4 *C. attenuata*, 65 from 5 *C. suaveolens*, and 5 from 1 *S. murinus*. The smallest *L. imphalum* (idiosoma $8·5 \times 10^3 \mu m^2$) was recovered from *A. agrarius*.

We excluded *S. murinus*, *C. attenuata*, and *C. suaveolens* from further analysis because we captured few individuals infested with *L. imphalum*. After considering only chiggers with RDE in the upper 25th percentile within each host individual, and excluding hosts with <2 measured chiggers in the upper 25th percentile, a total of 4600 *L. imphalum* was analysed. This included 2519 *L. imphalum* from 391 *A. agrarius*, 1667 from 79 *R. losea*, and 414 from 31 *B. indica*.

Variation in RDE across host species and across study region

RDE was significantly different among *A. agrarius*, *R. losea*, and *B. indica* ($H = 132·1$, D.F. = 2, $P < 0·001$). Pairwise Mann-Whitney U tests followed by Bonferroni adjustment (3 tests, $\alpha = 0·017$) revealed that engorgement differed significantly among these groups (Fig. 1). Mean RDE from chiggers on *R. losea* and *B. indica* was about 1·4x and 1·3x that from *A. agrarius* (Fig. 1).

A plot of engorgement against the proportion of chiggers with greater engorgement, considering each *L. imphalum* (instead of host) as an independent
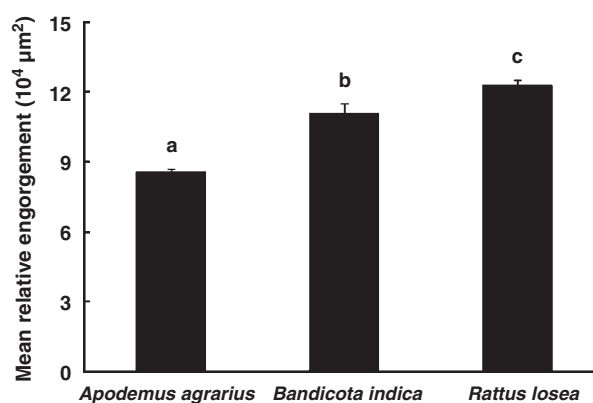

Fig. 1. A comparison of mean (+ 1 s.e., $10^4 \mu m^2$) relative degree of engorgement ($x10^4 \mu m^2$) of larval *Leptotrombidium imphalum* recovered from different host species. Letters above bars denote significant differences ($P < 0·05$) and are based on a Kruskal-Wallis test followed by pair-wise Mann-Whitney U tests after Bonferroni adjustment ($\alpha = 0·05/n$ comparisons) (only included chiggers with relative degree of engorgement in the upper 25th percentile within each host individual).
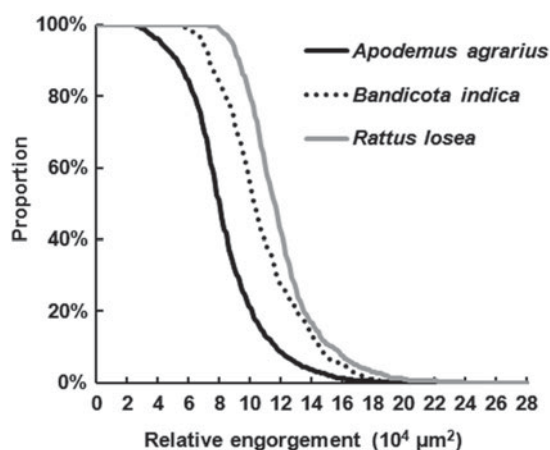

Fig. 2. A comparison of the proportion of larval *Leptotrombidium imphalum* (*y*-axis) greater than certain relative degree of engorgement ($x10^4 \mu m^2$, *x*-axis) among 3 host species (only included chiggers with relative degree of engorgement in the upper 25th percentile within each host individual).

sample, also revealed differences among the 3 host species (Fig. 2). For instance, 50·0% of chiggers from *R. losea* engorged more than $1·2 \times 10^5 \mu m^2$, compared with only 10·0% of chiggers from *A. agrarius* (Fig. 2).

Relation of RDE with chigger loads and intrinsic factors of hosts

Chigger loads varied greatly within *A. agrarius* (0 to 918, mean = 105·3 ± 5·3), *R. losea* (17 to 2082 544·1 ± 49. 8), and *B. indica* (0 to 1607 288·6 ± 58·1). Within *A. agrarius*, bootstrap analysis revealed a negative association between RDE and chigger loads, and no relationship with gender or with reproductive or

Table 1. Mean and 95% confidence interval of intercept (α) and explanatory variables (β_i) in relation to the relative degree of engorgement (μm^2, square rooted transformed) of larval *Leptotrombidium imphalum* recovered from 3 major host species using 1999 bootstrap replicates with replacement (only included chiggers with relative degree of engorgement in the upper 25th percentile within each host individual)

Host species	Number of host individuals	Intercept	Gender[a]	Reproductive condition[b]	Body condition	Chigger load
Apodemus agrarius	382	**307·44*** (295·17–319·72)	3·47 (−5·75–12·69)	−7·75 (−18·03–2·53)	−4·64 (−15·12–5·85)	**−0·14*** (−0·22 to −0·05)
Rattus losea	78	**369·08*** (341·71–396·45)	−4·50 (−17·10–8·10)	0·49 (−27·16–28·15)	−3·07 (−21·22–15·08)	**−0·03*** (−0·05 to −0·02)
Bandicota indica	30	**317·00*** (284·63–349·36)	**31·87*** (6·05–57·68)	14·57 (−15·58–44·71)	0·78 (−23·87–25·43)	**−0·04*** (−0·06 to −0·02)

[a] Females designated as 0; males as 1.
[b] Inactive reproduction designated as 0; active reproduction as 1.
* Means significantly different from 0.

body condition (Table 1, Fig. 3A). Similarly, for *R. losea*, RDE was negatively associated with chigger loads, but not to other factors (Table 1, Fig. 3B). RDE recovered from *B. indica* was also negatively associated with chigger loads (Table 1, Fig. 3C). Male *B. indica* also hosted more engorged chiggers than did females, while engorgement of chiggers was not associated with reproductive or body condition of *B. indica* (Table 1).

A test of similarity of RDE among and within host species

(a) Variation in RDE across host species and within plots. For this analysis we only analysed plots with >1 individual of each host species. As such, we compared only *A. agrarius* and *R. losea* (19 plots) due to the rarity of *B. indica* in most plots (7 plots with >1 *B. indica*). *A. agrarius* and *R. losea* averaged 8·6 ± 1·4 and 3·4 ± 0·8 ind. per plot, respectively. Mean numbers of measured chiggers were 120·7 ± 16·6 for each plot. Chiggers were always more engorged when retrieved from *R. losea* than from *A. agrarius* (Wilcoxon signed rank test, Z = 3·82, P < 0·001).

(b) Variation in RDE within host species and within plots. All 3 host species exhibited significant variation in chigger engorgement within plots. Forty-three plots had >1 captures of *A. agrarius*, with an average of 9·1 ± 0·9 individuals, and 58·5 ± 8·5 measured chiggers per plot. RDE differed among *A. agrarius* within plots (F = 8·6, D.F. = 347, P < 0·001, nested ANOVA using ranked data). Similarly, *R. losea* had an average of 3·4 ± 0·8 individuals across 19 plots, and these supported 69·9 ± 8·3 chiggers each plot, with significant variation in engorgement (F = 10·00, D.F. = 45, P < 0·001). Finally, *B. indica* averaged 2·4 ± 0·2 individuals, and 28·9 ± 6·6 chiggers per plot, and RDE also differed significantly among *B. indica* within plots (F = 2·90, D.F. = 10, P = 0·002).

DISCUSSION

RDE of larval trombiculid mites differed among host species. Larval *L. imphalum* recovered from *R. losea* and *B. indica* were 1·4x and 1·3x as engorged, respectively, as those from *A. agrarius*. In the current study, RDE was calculated based on the area of the idiosoma rather than its volume. The difference in RDE of the two hosts, however, should be even greater if we considered volume, since the latter increases as the cube of linear dimensions whereas area increases only as their square. It is therefore possible that even though *R. losea* hosted fewer chiggers than *A. agrarius* (40·77% *vs* 47·82% of total chiggers), the much more engorged chiggers recovered from the former species may contribute more to the subsistence of trombiculid mites. Besides, less engorged chiggers from *A. agrarius* may contribute disproportionally less to the chigger population such that an increase of this host species could reduce chigger numbers, especially when there is competition between *A. agrarius* and *R. losea*. A similar 'dilution effect' (LoGiudice *et al.* 2003) may occur in vectors with less mobility and thus more limited host selection (e.g., ticks), as opposed to vectors with more mobility (e.g., mosquitoes, fleas). More studies can corroborate this hypothesis.

Unlike differences in RDE within host species, those among host species did not appear to reflect negative density dependence. More engorged chiggers were recovered from host species with higher chigger loads (mean chigger loads: *R. losea* > *B. indica* > *A. agrarius*, with significant difference, Kuo *et al.* unpublished data). In host-parasite interactions, which generally are analogous to host-vector interactions, it is well documented that hosts vary in their defensive responses against parasites (Combes, 2001). Our study is consistent with predictions from one such defence, grooming behaviour, which is very effective against disease vectors (Hart *et al.* 1992; Kelly *et al.* 1996; Hawlena *et al.* 2007c;
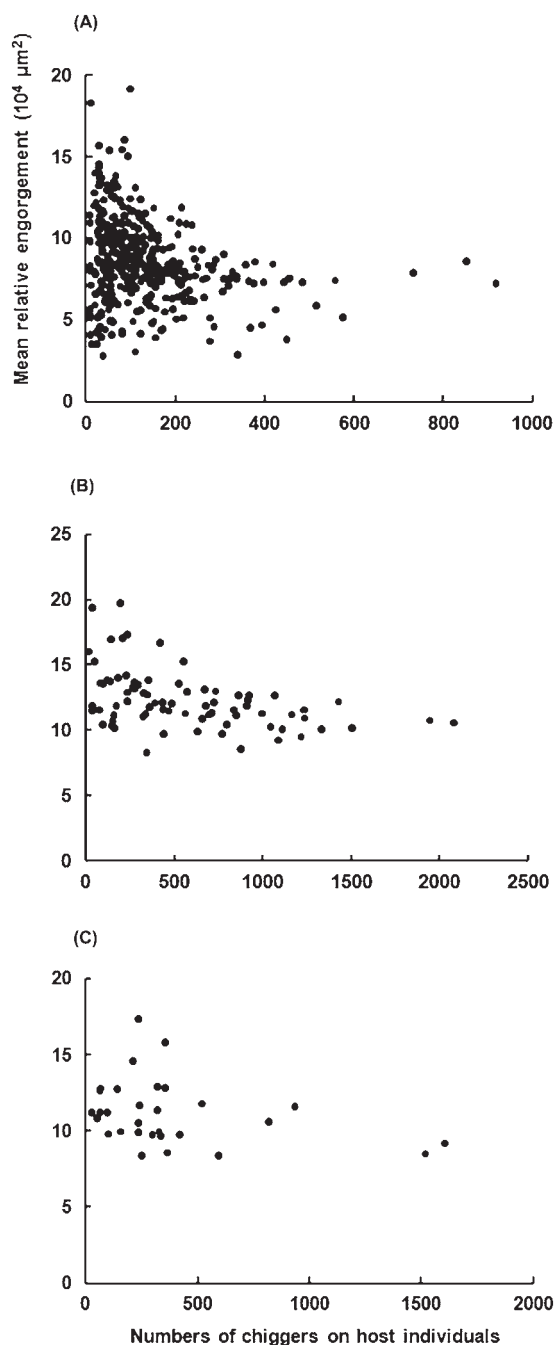
Fig. 3. Distribution of mean relative degree of engorgement (x10^4 μm^2) of larval *Leptotrombidium imphalum* (*y*-axis) against chigger loads (*x*-axis) in individuals of (A) *Apodemus agrarius*, (B) *Rattus losea*, (C) *Bandicota indica* (only chiggers with relative degree of engorgement in the upper 25th percentile within each host individual were plotted).

Slowik and Lane, 2009). Due to the larger amount of energy reserve, larger host species are more tolerant of vector infestation, while small species are more inclined to interrupt any infestation (Hart *et al*. 1992; Olubayo *et al*. 1993). Larger species should therefore host more chiggers; additionally, chiggers are allowed to engorge longer, thus becoming more fully engorged. This agrees with our findings that larger species (*B. indica*: *ca*. 300 g; *R. losea*: *ca*. 120 g) generally hosted more chiggers, and chiggers were more engorged, while small hosts (*A. agrarius*: ca. 30 g) harboured fewer, and less engorged chiggers. The 2 smallest rodent species in our study site were not highly parasitized with chiggers (*M. musculus*, *ca*. 10 g, mean chigger load = 0·06; *M. caroli*, *ca*. 15 g, mean chigger load = 0·01; Kuo, 2010). Similarly, other than vector competition for resource (Hawlena *et al*. 2007b), negative density-engorgement relations within three major host species may be due to increased grooming activity in response to an increased vector burden (Kelly, 2001). Grooming behaviour may therefore be one of the mechanisms leading to the difference in RDE.

We found that chiggers did not have similar RDE, both within and among host species. Because a positive relation of the amount of food intake to egg production has been widely reported among disease vectors (Balashov, 1972; Sonenshine, 1991; Kelly *et al*. 1996; Takken *et al*. 1998), RDE likely is a useful proxy for fitness, although at least one ectoparasite – the flea *Xenopsylla conformis* – engorged more but produced fewer eggs on one host (*Gerbillus dasyurus*) than another (*Meriones crassus*) (Krasnov *et al*. 2004). Nevertheless, the significant difference in RDE suggested that the prediction of the IFD that the *per capita* fitness of vectors should be similar among hosts was likely to be violated in our study.

The very limited mobility of chiggers (Philip *et al*. 1949), combined with their susceptibility to desiccation (Traub and Wisseman, 1974), may render host switching costly, considering the risks of exposure when questing for hosts and the uncertainty of acquiring suitable hosts. Chiggers may therefore be less selective of hosts, remaining on the hosts even when energetic returns are relatively low, leading to violation of predictions of the IFD. This may also cause the negative density-engorgement relations observed in the 3 host species studied here. That ticks naturally recovered from some hosts had much lower moulting success (LoGiudice *et al*. 2003) could be due to similar reasons, although some species have remarkable mobility (Balashov, 1972), and host choice can be assisted by ascending to certain questing height (Randolph, 2004) . In fact, the distribution of some more mobile vectors, such as sandflies, also did not abide by IFD, but was determined more by mating opportunities (Kelly *et al*. 1996). Our study provided another example of possible lack of compliance with IFD. Whether the IFD holds for other disease vectors under natural conditions warrants additional research.

REFERENCES

Balashov, Y. S. (1972). Bloodsucking ticks (Ixodoidea)-vectors of diseases of man and animals. *Miscellaneous Publications of the Entomological Society of America* **8**, 163–376.

CDC, Centers for Disease Control, Taiwan. Notifiable Infectious Disease Statistics System. [online] URL: http://nidss.cdc.gov.tw/. Accessed 12–6–2008

Combes, C. (2001). *Parasitism: the Ecology and Evolution of Intimate Interactions*. The University of Chicago Press, Chicago, IL, USA and London, UK.

Conover, W. J. and Iman, R. L. (1981). Rank transformations as a bridge between parametric and nonparametric statistics. *American Statistician* **35**, 124–129.

CWB, Central Weather Bureau, Taiwan. Statistical Data. [online] URL: http://www.cwb.gov.tw/V6/index. htm. Accessed 5–11–2009

Fretwell, S. D. and Lucas, H. L. Jr. (1970). On the territorial behavior and other factors influencing habitat distribution in birds. *Acta Biotheoretica* **19**, 16–36.

Gannon, W. L., Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. (2007). Guidelines of the American Society of Mammalogists for the use of wild mammals in research. *Journal of Mammalogy* **88**, 809–823. doi: 10.1644/06-MAMM-F-185R1.1.

Gürtler, R. E., Ceballos, L. A., Ordóñez-Krasnowski, P., Lanati, L. A., Stariolo, R. and Kitron, U. (2009). Strong host-feeding preferences of the vector *Triatoma infestans* modified by vector density: implications for the epidemiology of Chagas disease. *PloS Neglected Tropical Diseases* **3**, e447. doi:10.1371/journal.pntd.0000447.

Hart, B. L., Hart, L. A., Mooring, M. S. and Olubayo, R. (1992). Biological basis of grooming behaviour in antelope: the body-size, vigilance and habitat principles. *Animal Behaviour* **44**, 615–631.

Hawlena, H., Abramsky, Z. and Krasnov, B. R. (2007a). Ultimate mechanisms of age-biased flea parasitism. *Oecologia* **154**, 601–609. doi: 10.1007/s00442-007-0851-7.

Hawlena, H., Abramsky, Z., Krasnov, B. R. and Saltz, D. (2007b). Host defence versus intraspecific competition in the regulation of infrapopulations of the flea *Xenopsylla conformis* on its rodent host *Meriones crassus*. *International Journal for Parasitology* **37**, 919–925. doi: 10.1016/j.ijpara.2007.01.015.

Hawlena, H., Bashary, D., Abramsky, Z. and Krasnov, B. R. (2007c). Benefits, costs and constraints of anti-parasitic grooming in adult and juvenile rodents.

Ethology **113**, 394–402. doi: 10.1111/j.1439-0310.2007.01332.x.

Kawamura, A., Tanaka, H. and Takamura, A. (1995). *Tsutsugamushi Disease: an Overview.* University of Tokyo Press. Tokyo, Japan.

Keesing, F., Brunner, J., Duerr, S., Killilea, M., LoGiudice, K., Schmidt, K., Vuong, H. and Ostfeld, R. S. (2009). Hosts as ecological traps for the vector of Lyme disease. *Proceedings of the Royal Society of London, B* **276**, 3911–3919. doi: 10.1098/rspb.2009.1159.

Kelly, D. W. (2001). Why are some people bitten more than others? *Trends in Parasitology* **17**, 578–581. doi: 10.1016/S1471-4922(01)02116-X.

Kelly, D. W. and Thompson, C. E. (2000). Epidemiology and optimal foraging: modelling the ideal free distribution of insect vectors. *Parasitology* **120**, 319–327. doi: 10.1017/S0031182099005442.

Kelly, D. W., Mustafa, A. and Dye, C. (1996). Density-dependent feeding success in a field population of the sandfly, *Lutzomyia longipalpis*. *Journal of Animal Ecology* **65**, 517–527.

Khokhlova, I. S., Serobyan, V., Krasnov, B. R. and Degen, A. A. (2009). Is the feeding and reproductive performance of the flea, *Xenopsylla ramesis*, affected by the gender of its rodent host, *Meriones crassus*? *Journal of Experimental Biology* **212**, 1429–1435. doi: 10.1242/jeb.029389.

Khokhlova, I. S., Ghazaryan, L., Krasnov, B. R. and Degen, A. A. (2008). Effects of parasite specificity and previous infestation of hosts on the feeding and reproductive success of rodent-infesting fleas. *Functional Ecology* **22**, 530–536. doi: 10.1111/j.1365-2435.2008.01393.x.

Krasnov, B. R., Khokhlova, I. S., Arakelyan, M. S. and Degen, A. A. (2005). Is a starving host tastier? Reproduction in fleas parasitizing food-limited rodents. *Functional Ecology* **19**, 625–631. doi: 10.1111/j.1365-2435.2005.01015.x.

Krasnov, B. R., Khokhlova, I. S., Burdelova, N. V., Mirzoyan, N. S. and Degen, A. A. (2004). Fitness consequences of host selection in ectoparasites: testing reproductive patterns predicted by isodar theory in fleas parasitizing rodents. *Journal of Animal Ecology* **73**, 815–820. doi: 10.1111/j.0021-8790.2004.00860.x.

Kuo, C. C. (2010). Ecology of scrub typhus in Taiwan: the impact of environment, rodent community, and socio-economy. Ph.D. dissertation. University of California, Davis, CA, USA.

Li, J., Wang, D. and Chen, X. (1997). *Trombiculid Mites of China: Studies on Vector and Pathogen of Tsutsugamushi Disease*. Guangdong Science and Technology Publishing, Guangzhou, China.

LoGiudice, K., Ostfeld, R. S., Schmidt, K. A. and Keesing, F. (2003). The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proceedings of the National Academy of Sciences, USA* **100**, 567–571. doi: 10.1073/pnas.0233733100.

Lyimo, I. N. and Ferguson, H. M. (2009). Ecological and evolutionary determinants of host species choice in mosquito vectors. *Trends in Parasitology* **25**, 189–196. doi: 10.1016/j.pt.2009.01.005.

Olubayo, R. O., Jono, J., Orinda, G., Groothenhuis, J. G. and Hart, B. L. (1993). Comparative differences in densities of adult ticks as a function of body size on some East African antelopes. *African Journal of Ecology* **31**, 26–34. doi: 10.1111/j.1365-2028.1993.tb00515.x.

Ostfeld, R. S. and Keesing, F. (2000). The function of biodiversity in the ecology of vector-borne zoonotic diseases. *Canadian Journal of Zoology* **78**, 2061–2078. doi: 10.1139/cjz-78-12-2061.

Philip, C. B., Traub, R. and Smadel, J. E. (1949). Chloramphenicol (chloromycetin) in the chemoprophylaxis of scrub typhus (tsutsugamushi disease): epidemiological observations on hyperendemic areas of scrub typhus in Malaya. *American Journal of Hygiene* **50**, 63–74.

Randolph, S. E. (2004). Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors. *Parasitology* **129** (Suppl.) S37–S65. doi: 10.1017/S0031182004004925.

Schulte-Hostedde, A. I., Zinner, B., Millar, J. S. and Hickling, G. J. (2005). Restitution of mass/size residuals: validating body condition indices. *Ecology* **86**, 155–163. doi: 10.1890/04-0232.

Slowik, T. J. and Lane, R. S. (2009). Feeding preferences of the immature stages of three western north American ixodid ticks (Acari) for avian, reptilian, or rodent hosts. *Journal of Medical Entomology* **46**, 115–122. doi: 10.1603/033.046.0115.

Sonenshine, D. E. (1991). *Biology of Ticks, Vol. 1*. Oxford University Press. Oxford, UK.

Takken, W., Klowden, M. J. and Chambers, G. M. (1998). Effect of body size on host seeking and blood meal utilization in *Anopheles gambiae* sensu stricto (Diptera: Culicidae): the disadvantage of being small. *Journal of Medical Entomology* **35**, 639–645.

Traub, R. and Wisseman, C. L. Jr. (1974). The ecology of chigger-borne rickettsiosis (scrub typhus). *Journal of Medical Entomology* **11**, 237–303.

Wang, D. Q. and Yu, Z. Z. (1992). Chigger mites of the genus *Leptotrombidium*: key to species and their distribution in China. *Medical and Veterinary Entomology* **6**, 389–395.

APPENDIX 1

(*All* chiggers were included in the analyses instead of only upper 25th percentile engorged)

Relative degree of engorgement (RDE) across host species

A total of 19 320 *L. imphalum* were analysed, including 10 475 from 483 *A. agrarius*, 7101 from 82 *R. losea*, and 1744 from 35 *B. indica*. RDE was significantly different among *A. agrarius, R. losea*, and *B. indica* ($H = 123 \cdot 8$, D.F. = 2, $P < 0 \cdot 001$). Pairwise Mann-Whitney U tests followed by Bonferroni adjustment (3 tests, $\alpha = 0 \cdot 017$) revealed that engorgement differed significantly among these groups except between *R. losea* and *B. indica* (Fig. S1, online version only). Mean RDE from chiggers on *R. losea* and *B. indica* was about 1·4x and 1·3x that from *A. agrarius* (Fig. S1).

A plot of engorgement against the proportion of chiggers with greater engorgement, considering each *L. imphalum* (instead of host) as an independent sample, also revealed differences among the 3 host species (Fig. S2, online version only). For instance, 50·0% chiggers from *R. losea* engorged more than 8×10^4 μm^2, compared with only 16·8% of chiggers from *A. agrarius* (Fig. S2).

Relation of RDE to chigger loads and intrinsic factors of hosts

Chigger loads varied greatly within *A. agrarius* (0 to 918), *R. losea* (17 to 2082), and *B. indica* (0 to 1607). Within *A. agrarius*, bootstrap analysis revealed a negative association between RDE and chigger loads, but no relationship to gender or to reproductive or body condition (Table S1, Fig. S3A, online version only). Similarly, for *R. losea*, RDE was negatively associated with chigger loads, but not to other factors (Table S1, Fig. S3B). RDE recovered from *B. indica* was also associated only with chigger loads, but not with the other factor (Table S1, Fig. S3C).

A test of similarity of RDE among and within host species

(a) Variation in RDE within plots and across host species. Only plots with >1 individual of each host species were analysed. As such, we compared only *A. agrarius* and *R. losea* (19 plots) due to the infrequent occurrence of *B. indica* in most plots (7 plots with >1 *B. indica*). *A. agrarius* and *R. losea* averaged $10 \cdot 3 \pm 2 \cdot 4$ and $3 \cdot 4 \pm 0 \cdot 8$ ind. per plot, respectively. Mean numbers of measured chiggers were $491 \cdot 6 \pm 112 \cdot 8$ for each plot. Wilcoxon signed rank test revealed significant within-plot difference in RDE between *A. agrarius* and *R. losea* ($Z = 3 \cdot 82$, $P < 0 \cdot 001$). Chiggers were always more engorged when retrieved from *R. losea* than from *A. agrarius*.

(b) Variation in RDE within plot and within host species. Forty-three plots had >1 captures of *A. agrarius*, with an average of $10 \cdot 8 \pm 1 \cdot 1$ individuals, and $242 \cdot 4 \pm 32 \cdot 8$ measured chiggers per plot. RDE differed among *A. agrarius* within plots (F = 7·02, D.F. = 424, $P < 0 \cdot 001$, nested ANOVA using ranked data). Similarly, RDE differed among *R. losea* (F = 8·30, D.F. = 45, $P < 0 \cdot 001$) within 19 plots. Abundance of *R. losea*, and numbers of measured chiggers averaged $3 \cdot 4 \pm 0 \cdot 8$, and $283 \cdot 9 \pm 33 \cdot 7$, respectively. Only 7 plots yielded >1 *B. indica*, with a mean of $2 \cdot 4 \pm 0 \cdot 2$ individuals, and $116 \cdot 1 \pm 26 \cdot 6$ measured chiggers per plot. RDE also differed significantly among *B. indica* within plots (F = 11·67, D.F. = 10, $P < 0 \cdot 001$).
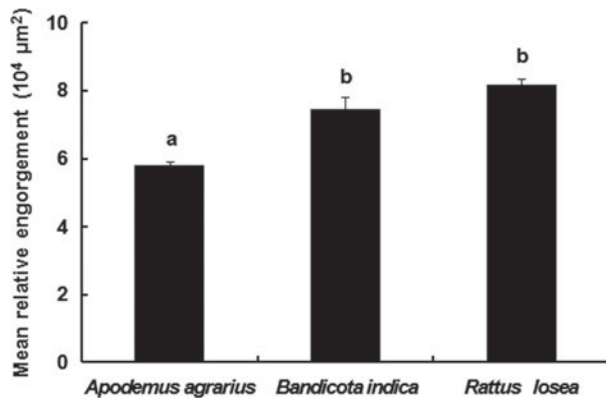
Fig. S1. A comparison of mean (+1 S.E., 10^4 μm^2) relative degree of engorgement (x10^4 μm^2) of larval *Leptotrombidium imphalum* recovered from different host species. Letters above bars denote significant differences ($P<0\cdot05$) and are based on a Kruskal-Wallis test followed by pair-wise Mann-Whitney U tests after Bonferroni adjustment ($\alpha=0\cdot05/n$ comparisons).
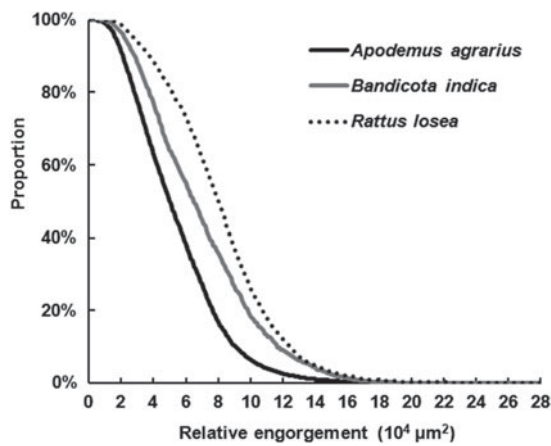

Fig. S2. A comparison of the proportion of larval *Leptotrombidium imphalum* (*y*-axis) greater than certain relative degree of engorgement (x10^4 μm^2, *x*-axis) among 3 host species.
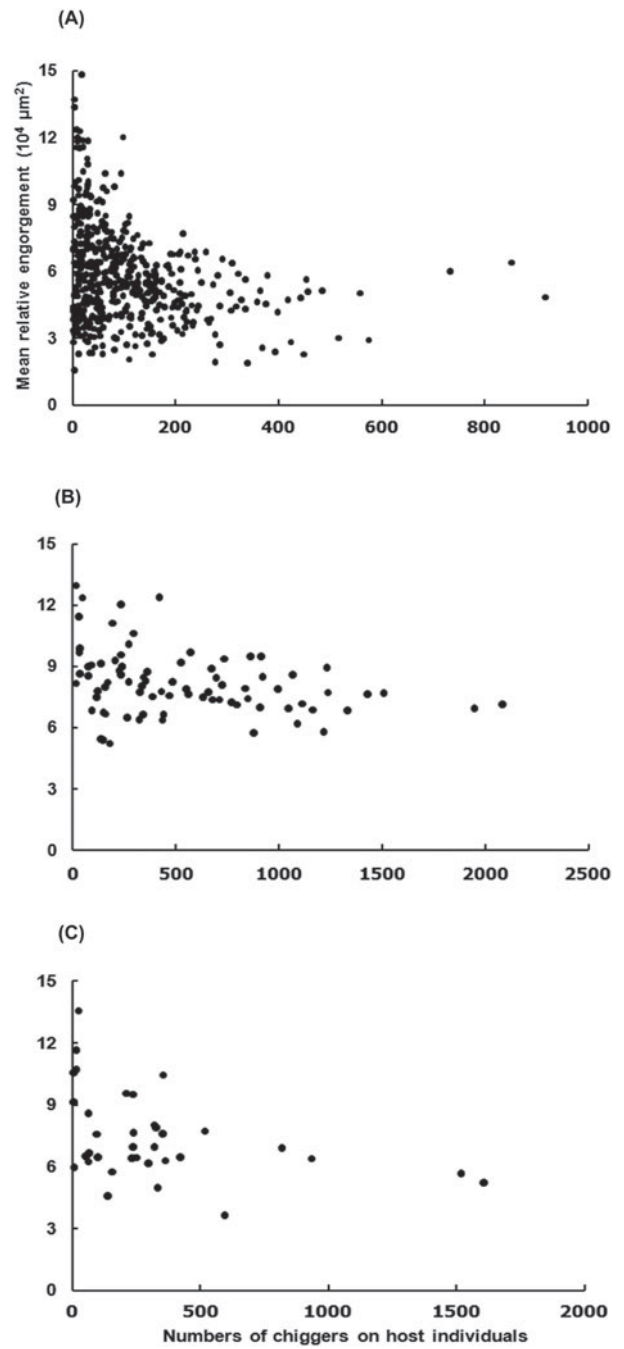

Fig. S3. Distribution of mean relative degree of engorgement (x10^4 μm^2) of larval *Leptotrombidium imphalum* (*y*-axis) against chigger loads (*x*-axis) in individuals of (A) *Apodemus agrarius*, (B) *Rattus losea*, (C) *Bandicota indica*.

Table S1. Mean and 95% confidence interval of intercept (*α*) and explanatory variables (*β$_i$*) in relation to the relative degree of engorgement of larval *Leptotrombidium imphalum* recovered from three major host species using 1,999 bootstrap replicates with replacement

Host species	Number of host individuals	Intercept	Gender	Reproductive condition	Body condition	Chigger load
Apodemus agrarius	473	**257·25** (246·83–267·66)	2·18 (−5·93–9·56)	−9·06 (−18·20–0·28)	−9·41 (−18·87–0·02)	**−0·19*** (−0·26 to −0·12)
Rattus losea	80	**288·02** (271·67–304·38)	−1·29 (−13·84–10·66)	9·87 (−4·76–26·13)	−3·11 (−23·96–17·98)	**−0·02*** (−0·04 to −0·01)
Bandicota indica	35	**270·92** (234·84–307·10)	12·21 (−19·32–37·73)	10·65 (−17·67–37·63)	13·80 (−4·61–40·55)	**−0·05*** (−0·10 to −0·02)

* Significantly different from 0.