Positive co-occurrence of flea infestation at a low biological cost in two rodent hosts in the Canary archipelago

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SUMMARY

Non-random assemblages have been described as a common pattern of flea co-occurrence across mainland host species. However, to date, patterns of flea co-occurrence on islands are unknown. The present work investigates, on one hand, whether the decrease in the number of species on islands affects the pattern of flea co-occurrence, and on the other hand, how the cost of higher flea burdens affects host body mass. The study was carried out in the Canary Islands (Spain) using null models to analyse flea co-occurrence on *Rattus rattus* and *Mus musculus*. Results supported aggregation of flea species in *Mus* but not in *Rattus*, probably due to the relationship between abundance and both prevalence and intensity of infection of the main flea species parasitizing *Mus*. In addition, heavy individuals of both rodent species showed the highest flea burdens as well as higher species richness, probably due to the continued accumulation of fleas throughout life and/or immunological resistance mechanisms. Whatever the mechanisms involved, it is clear that co-occurrence and high parasite intensities do not imply a detrimental biological cost for the rodents of the Canary Islands.

Key words: Canary Islands, co-occurrence, fleas, host-density, *Mus musculus*, null models, *Rattus rattus*.

INTRODUCTION

Despite intensive research efforts to describe ectoparasite assemblages (notably fleas) in small mammals around the world (Krasnov *et al*. 2006, 2010, 2011), little is known about their existence on islands. Flea assemblages are commonly non-random, with positive co-occurrence among species being the rule rather than the exception in nature (Krasnov *et al*. 2011). According to the theory of island biogeography (TIB, MacArthur and Wilson, 1967), islands show an impoverishment in terms of biodiversity and thus in the number of potential host species. Nevertheless, insular populations are affected by a set of changes known as 'insular syndrome' that influence their life-history traits (Blondel, 1995). As a consequence, populations of rodents on islands typically experience high densities in comparison to mainland populations. A decrease in interspecific competition, a low predation pressure, and a wider ecological niche appears to explain these successful populations (Alder and Levins, 1994). Accordingly, flea communities on islands should also be structured due to greater opportunities to encounter potential hosts in these small, but overcrowded, populations.

Regardless of the role of host population structure on the establishment of flea communities, activation and/or suppression of the host immune response by one flea species is one of the likely mechanisms for explaining positive co-occurrence. Within this perspective, an early infection would facilitate host-exploitation by the subsequent colonizer (Krasnov *et al*. 2005). However, co-occurrence could also be explained regardless of immune system conditions, simply assuming that some host individuals represent better habitats for fleas (or any parasite species) than others, e.g. those providing high-quality resources and lower behavioural or immunological barriers against ectoparasites (Krasnov *et al*. 2010). In addition, as previously mentioned, the most abundant host species would also be good candidates for co-infection by several flea species (Krasnov, 2008).

On the other hand, parasitized hosts generally have higher energy requirements than non-parasitized hosts due to extra energetic costs for maintaining a normal level of immune response during infection (Viney *et al*. 2005). However, and despite body mass losses due to infection (e.g. partially induced by increased daily metabolic rates) shown by certain rodent species (Khokhlova *et al*. 2002), there are no general rules concerning host energetic expenditures during infection (e.g. see Hawlena *et al*. 2006*a* for rodent species showing a constitutive immune

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response). In fact, flea infestation is more detrimental to hosts when they are not able to compensate for mass loss through increased food consumption (Hawlena *et al.* 2008). Therefore, natural selection may limit natural flea densities to minimize the negative effects of infestation. Nonetheless, little is known about the effects of ectoparasite intensities on host body mass losses in co-infection.

In this study, taking advantage of an intensive sampling of fleas infecting three rodent hosts (*Rattus rattus, Rattus norvegicus* and *Mus musculus*) throughout the Canary archipelago, we explore whether insularity shapes the patterns of co-occurrence described mainly under mainland conditions. Specifically, we tested the following predictions: although flea species found in the Canary Islands are rodent-specific (Beaucournu and Launay, 1990) and rodent communities are typically small (mostly *R. rattus* and *M. musculus*), the high rodent densities that occur on islands (Alder and Levins, 1994) will favour flea infestation. Consequently, we predict that despite isolation, non-random flea co-occurrence will also be the rule rather than the exception in the Canary archipelago (first prediction). Thus, the higher intensities should be due to co-infection by several flea species. Therefore, higher flea burdens in co-infection would increase the cost of parasitism ultimately breaking the equilibrium that maximizes flea burdens while minimizing host body weight losses (Hawlena *et al.* 2006*b*). Hence, we expect to find higher flea burdens in hosts parasitized by a single flea species, but lower body weights in hosts carrying more than one flea species (second prediction).

MATERIALS AND METHODS

Study area and sampling procedure

The Canary Islands are located between latitudes 27°37′–29°25′ north, and longitudes 13°20′–18°10′ west. Situated in the Atlantic Ocean about 100 km from Africa's west coast, the Canaries are divided into the western islands (El Hierro, La Palma, La Gomera and Tenerife) and the eastern islands (Gran Canaria, Fuerteventura and Lanzarote) (Fig. 1). The climate of the Canary Islands is mainly subtropical varying between 22 °C in summer to 19 °C in winter. Despite average annual precipitation ranges between 300 to 600 mm, rain patterns strongly depend on island relief and orientation. Indeed, the western islands are characterized by a higher average altitude, by the constant action of trade winds and by the formation of the 'sea of clouds', leading to wetter climatic conditions. On the other hand, the eastern islands show a much lower altitude, there is no formation of 'sea of clouds', and their climate is influenced by warm air masses from the African continent. Thus, the eastern islands show more arid conditions, most significantly in Fuerteventura and Lanzarote.

Trapping was carried out from 2007 to 2012 resulting in 16 trapping campaigns: six in Tenerife, three in El Hierro, two in La Palma, and one in La Gomera, Gran Canaria, Fuerteventura, Lanzarote and La Graciosa islet. A typical sampling campaign consisted of two to six trapping sessions in which 200 live traps were placed in the late evening and collected at dawn. The sampling effort was 41 traps/day (minimum = 10, maximum = 100). Two different types of live traps were used: Ferroven traps ($n = 100$) for both *M. musculus* and *Rattus* species, and Sherman traps ($n = 100$) for *M. musculus*.

The climatic contrast between western and eastern islands results in the disposition of a great diversity of vegetative species. Sampling areas included diverse habitats located at different altitudes in order to cover the greatest range of biotopes from each island. Mainly, traps were placed in coastal habitats (0–50 m), arid habitats dominated by different botanical species but mostly belonging to the genus *Euphorbia* (50–300 m), crop fields (150–500 m), laurel forest habitats called 'monteverde' in the western islands (500–1500 m) and pine forests (400–1900 m). A total of 888 rodents (214 *R. rattus*, 14 *R. norvegicus* and 660 *M. musculus*) were captured and studied.

Flea identification and analysis

Captured hosts were brought to the laboratory, euthanased and immediately combed for flea detection. Flea species were preserved in 70% ethanol. Prior to flea species determination, each specimen was subjected to a 20% potassium hydroxide clearing treatment and a dehydration process using a series of ethanol rinses of ascending concentration up to 100%. The specimens were then mounted in Canada balsam. Flea species and sub-species were determined based on their morphological characteristics according to the systematic manual of Beaucournu and Launay (1990).

Finally, prevalence and mean intensity of flea infestation were estimated according to Bush *et al.* (1997). Parasite richness was estimated as the number of flea species actually observed per host individual. This measure is considered an appropriate proxy for parasite biodiversity in a broad range of host species (Bordes and Morand, 2009).

Statistical procedures

The community structure of fleas was studied using null models analysis. Data were organized as presence/absence matrices in which each row represented a flea species and each column represented an individual host. In a presence/absence matrix a '1' indicates that a species is present at a particular site or host, and '0' indicates that a species is absent (Gotelli, 2000).

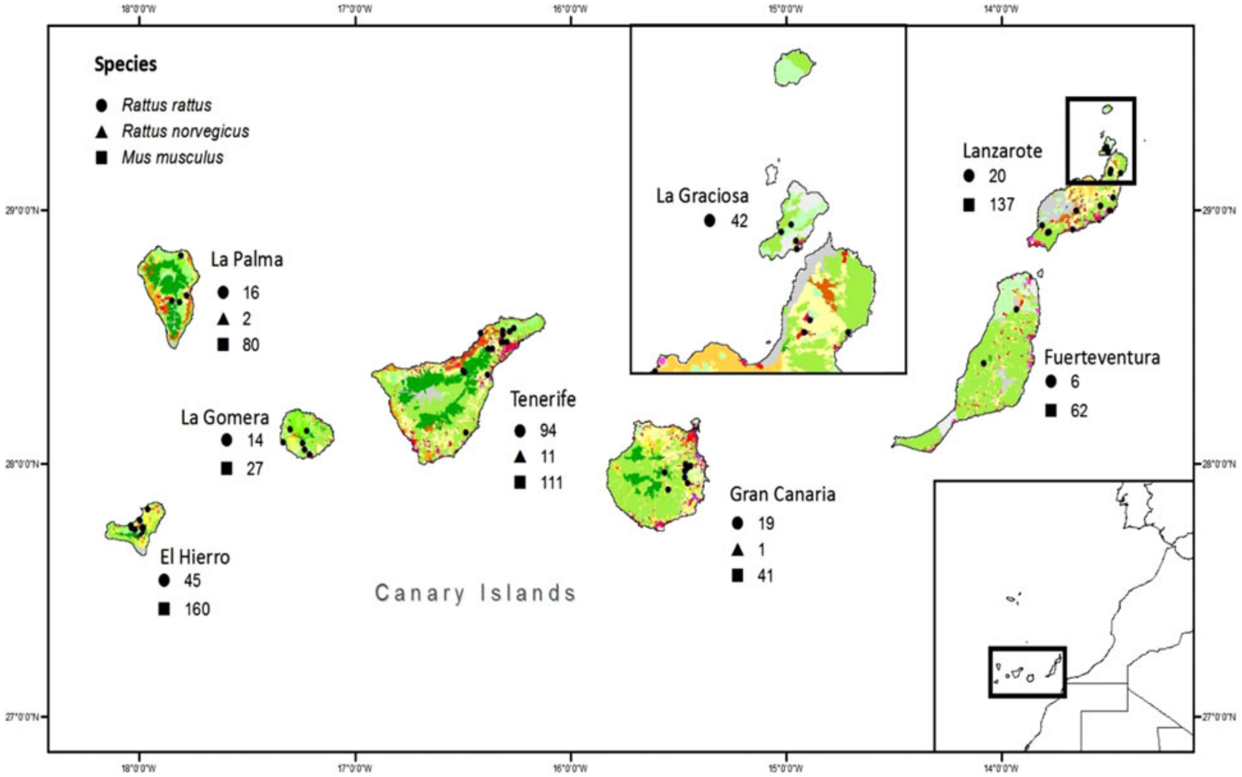

Fig. 1. Sampling areas and host species trapped on each island of the Canary archipelago, Spain.

A total of 12 matrices were created separately for *Rattus* sp. and *Mus*, representing for each the presence/absence of flea species in every individual host on each of the Canary Islands.

There are four indices for the analysis of co-occurrence patterns as well as nine possible algorithms for a null model analysis (Gotelli, 2000). In the present work, the C-score, as a co-occurrence index (Stone and Roberts, 1990) and the FE algorithm (fixed row-equiprobable column) have been chosen for analysing the results obtained. The C-score measures the average number of checkerboard units between all possible pairs of species. In a competitively structured community, the observed C-score should be significantly larger than expected by chance (O > E). That community will tend to show negative co-occurrence and to be segregated. Otherwise, a C-score smaller than expected by chance (O < E) indicates positive co-occurrence, and thus species in that community will tend to be aggregated (Gotelli and McCabe, 2002). The C-score has been used in diverse null models as a powerful tool to measure animal assemblages and ectoparasite co-occurrences (Gotelli and Rohde, 2002; Krasnov *et al.* 2006, 2010, 2011; Both *et al.* 2011).

The FE algorithm describes how the rows and columns of the matrix have been treated in the null model. With the fixed row, the observed row totals are maintained in the simulation, i.e. the number of occurrences of each species in the null communities is the same as in the original data. Using the equiprobable column, each site (host individual) is equally likely to be represented (Gotelli, 2000). Choosing the FE algorithm the null model is quite similar to what happens in reality considering that uninfected hosts could be used by parasites, but, by chance, are not colonized (Gotelli and Rohde, 2002). Uninfected hosts (empty sites) are included in the input matrices because they are considered suitable by fleas to be parasitized.

The observed C-score was calculated for each presence/absence matrix and compared with the expected C-score calculated for 5000 randomly assembled null matrices by Monte Carlo procedures.

The analysis was carried out using the software EcoSim 7·72 (Entsminger, 2012).

In addition, to compare the degree of co-occurrence across data, we calculated a standardized effect size (SES) for each matrix. The SES measures the number of standard deviations that the observed index (C-score) is above or below the mean index of the simulated communities. Whether or not the average SES measured differed from zero was tested by a *t*-test (Gotelli and McCabe, 2002). Since flea aggregation would experience modifications among years, we first preformed our co-occurrence analysis by island and year. However, since we got similar results we pooled all years in the same co-occurrence analysis, thus increasing our sample size.

After this analysis, we used linear mixed models (LMM) to check whether this expected high density of hosts is related to both prevalence and intensity of flea infestation. Specifically, in such mixed models, the number of rodents trapped by location (a proxy for rodent abundance, see Tobin and Sugihara, 1992) was used as an explanatory variable, the term 'island' was considered as a random factor and flea prevalence or flea infestation as response variables. Only trapping locations with more than five captures were included in the analysis. Concerning host species, only *Mus* records were analysed since neither the number of trapped *R. norvegicus* nor *R. rattus* were large enough to fit any model. With regards to flea species, only the most abundant flea species (i.e. those showing prevalences higher than 5·0%) of *Mus* were considered. The best random structure (e.g. no random term except for the ordinary residuals, random intercept or random intercept and slope model) was selected according to the Zuur *et al.* (2009) protocol.

Finally, to explore whether the maximum intensity of flea parasitization was influenced by both ectoparasite richness and host body weight (higher flea burdens at lower body weights) we fitted a set of host-specific (one for *M. musculus* and the other for *R. rattus*) independent generalized linear models (GLM) in which flea burden (as a response variable) was explained by the single effects of flea richness, host body weight and their two-way interactions. Models were fitted using Poisson errors and the log link function. Flea intensity of infection was log-transformed only for graphic representations and only host individuals parasitized by at least one flea were retained for this analysis.

We then performed a model selection procedure based on the information-theoretic approach and Akaike's Information Criterion corrected for small sample sizes (AICc, see Burnham and Anderson, 2002; Johnson and Omland, 2004). In short, competing models are ranked in relation to the difference between their Akaike scores and the score of the best model (Δi), which has the lowest AICc. Models with $\Delta i < 2$ units have substantial support for explaining the observed variability in the variable of interest. Subsequently, we estimated the Akaike weight (w_{i}), defined as the relative probability that a given model is the best model for those being compared, and the relative importance of each variable (R_i, the sum of w_i over the subset of models that include the variable; Burnham and Anderson, 2002). Once the best model had been selected, the explained deviance (ED) was calculated as a measure of explained variability for each response variable (Zuur *et al.* 2007). All statistical analyses were performed using R software version 3.0.1. (R Development Core Team, 2013), including prevalence estimated by the 'epiR' version 0.9–48 package (Stevenson *et al.* 2013).

RESULTS

Flea prevalence and flea intensities of infection

A total of 843 flea specimens belonging to 5 genera represented by 7 species and 1 subspecies were found in the Canary Islands. *Echidnophaga gallinacea* (Westwood, 1875) was represented by 8 specimens collected from *Rattus* and 1 from *Mus; Xenopsylla cheopis* (Rothschild, 1903) was collected from *Rattus* (34 specimens) and *Mus* (10 specimens); *Stenoponia tripectinata tripectinata* (Tiraboschi, 1902) was detected on *Rattus* (3 specimens) and *Mus* (492 specimens); *Leptopsylla segnis* (Schonherr, 1811) was present in *Rattus* (58 specimens) and *Mus* (42 specimens) and finally *Nosopsyllus barbarus* (Jordan & Rothschild, 1912) was collected from *Rattus* (48 specimens) and *Mus* (49 specimens). Fleas only detected in a single host species were: *Echidnophaga murina* (Tiraboschi, 1903), 45 on *Mus; Xenopsylla brasiliensis* (Baker, 1904), 5 on *Rattus*; and *Xenopsylla guancha* (Beaucournu, Alcover & Launay, 1989), 48 on *Mus*. No flea species were found on *R. norvegicus*.

In terms of prevalence of flea infection, 22·9% of *R. rattus* (95% CI = 17·8–29·0, $n = 214$) and 30·9% of *Mus* (95% CI = 27·5–34·5, $n = 660$) were parasitized by fleas. The most prevalent flea species on *R. rattus* was *L. segnis* with 11·9% (95% CI = 7·9–17·0), with Gran Canaria being the island on which this flea species displayed the highest prevalence. Concerning *Mus*, the most prevalent flea species was *S. t. tripectinata* with 17·5% (95% CI = 14·6–20·5) again on Gran Canaria also being the most abundant of all the species found (Table 1). Four flea species were host-specific: *X. brasiliensis* for *Rattus, E. murina, X. guancha* and *S. t. tripectinata* for *Mus*. The flea species *N. barbarus* and *L. segnis* appeared in both rodents but *Rattus* was revealed as its principal host. Prevalence of *N. barbarus* in *Rattus* and *Mus* was 9·6% (95% CI = 6·0–14·4) and 6·0% (95% CI = 4·3–8·1) respectively, while prevalence of *L. segnis* was 11·9% (95% CI = 7·9–17·0) in *Rattus* and 4·3% (95% CI = 2·9–6·2) in *Mus*. In regards to mean intensity, *S. t. tripectinata* was the flea with the highest value of 4·2 (95% CI = 3·07–5·40) in infected *Mus*, and Tenerife the island with the greatest value, 5·4 (95% CI = 4·40–6·40, see Table 2).

Null models

Results of the null models show that the observed C-score is smaller than expected by chance ($O \leqslant E$) in every presence/absence matrix except for *Rattus* on Tenerife and Lanzarote islands (Table 3). The values are only significant ($P \leqslant 0·05$) in the flea community of El Hierro for *Rattus* and in the flea community of La Palma for *Mus*. Nevertheless, we should not reject the other values since they show a tendency for flea communities to be aggregated. The SES values for the averaged C-score differed significantly from zero for *Mus* ($t = -3·88$, $P = 0·008$) but not for *Rattus* ($t = -1·23$, $P = 0·28$). The C-scores for *Rattus* from Tenerife and Lanzarote were greater than expected by chance, perhaps because of the low flea prevalence.

Table 1. Prevalence of flea parasitization and 95% confidence intervals (CI) in 214 *Rattus rattus* (*R. r.*) and 660 *Mus musculus* (*M. m.*) from the Canary Islands

	Prevalence (95% CI)							
	El Hierro		La Palma		La Gomera		Tenerife	
Flea species	*R. r.* (n = 45)	*M. m.* (n = 160)	*R. r.* (n = 16)	*M. m.* (n = 80)	*R. r.* (n = 14)	*M. m.* (n = 27)	*R. r.* (n = 94)	*M. m.* (n = 111)
E. gallinacea	–	–	–	–	–	–	–	–
E. murina	–	5·0 (2·5–9·5)	–	–	–	–	–	–
X. cheopis	13·3 (6·2–26·2)	2·5 (1·0–6·2)	–	–	–	–	–	0·9 (0·1–4·9)
X. brasiliensis	–	–	–	–	7·1 (1·3–31·5)	–	–	–
X. guancha	–	–	–	–	–	–	–	–
S. tripectinata	–	28·8 (22·3–36·2)	–	26·2 (17·8–36·8)	–	37·0 (21·5–55·8)	–	17·1 (11·2–25·2)
L. segnis	24·4 (14·2–38·7)	7·5 (4·3–12·6)	–	11·2 (6·0–20·0)	7·1 (1·3–31·5)	3·7 (0·6–18·3)	3·2 (1·1–9·0)	0·9 (0·1–4·9)
N. barbarus	8·9 (3·5 –20·7)	9·4 (5·8–14·9)	6·3 (1·1–28·3)	15·0 (8·8–24·4)	14·3 (4·0–39·9)	–	6·4 (2·9–13·2)	3·6 (1·4–8·9)

	Prevalence (95% CI)						
	Gran Canaria		Fuerteventura		Lanzarote		La Graciosa
Flea species	*R. r.* (n = 19)	*M. m.* (n = 41)	*R. r.* (n = 6)	*M. m.* (n = 62)	*R. r.* (n = 20)	*M. m.* (n = 137)	*M. m.* (n = 42)
E. gallinacea	15·8 (5·5–37·6)	–	–	1·6 (0·3–8·6)	–	–	–
E. murina	–	–	–	3·2 (0·9–11·0)	–	1·5 (0·4–5·2)	–
X. cheopis	21·0 (8·5–43·3)	2·4 (0·4–12·6)	33·3 (9·7–70·0)	4·8 (1·6–13·3)	5·0 (0·1–24·8)	–	–
X. brasiliensis	5·3 (0·9–24·63)	–	–	–	10·0 (1·2–31·7)	–	–
X. guancha	–	–	–	6·4 (2·5–15·4)	–	19·7 (13·9–27·1)	11·9 (5·2–25·0)
S. tripectinata	10·5 (2·9–31·4)	48·8 (34·2–63·5)	–	–	–	–	–
L. segnis	52·6 (31·7–72·7)	4·9 (1·3–16·1)	–	3·2 (0·9–11·0)	5·0 (0·1–24·8)	1·5 (0·4–5·2)	–
N. barbarus	42·1 (23·1–63·7)	14·6 (6·9–28·4)	–	3·2 (0·9–11·0)	–	0·7 (0·1–4·0)	–

Table 2. Mean intensity and 95% confidence intervals (CI) of flea parasitization in 214 *Rattus rattus* (*R. r.*), and 660 *Mus musculus* (*M. m.*), from the Canary Islands

	Mean intensity (95% CI)							
	El Hierro		La Palma		La Gomera		Tenerife	
Flea species	*R. r.* (n = 45)	*M. m.* (n = 160)	*R. r.* (n = 16)	*M. m.* (n = 80)	*R. r.* (n = 14)	*M. m.* (n = 27)	*R. r.* (n = 94)	*M. m.* (n = 111)
E. gallinacea	–	–	–	–	–	–	–	–
E. murina	–	1·25 (0·86–1·63)	–	–	–	–	–	–
X. cheopis	3·5 (0–7·52)	1·25 (0·45–2·04)	–	–	–	–	–	1·0
X. brasiliensis	–	–	–	–	1·0	–	–	–
X. guancha	–	–	–	–	–	–	–	–
S. tripectinata	–	3·72 (2·57–4·87)	–	3·57 (1·35–5·79)	–	4·5 (0–9·05)	–	5·4 (0–11·24)
L. segnis	1·73 (0·88–2·58)	1·25 (0·96–1·53)	–	2·0 (1·06–2·94)	3·0	1·0	3·3 (0–13·36)	1·0
N. barbarus	3·0 (0·76–5·24)	1·13 (0·94–1·32)	2·0	1·5 (1·07–1·92)	3·0	–	2·33 (0–5·27)	1·0

	Mean intensity (95% CI)						
	Gran Canaria		Fuerteventura		Lanzarote		La Graciosa
Flea species	*R. r.* (n = 19)	*M. m.* (n = 41)	*R. r.* (n = 6)	*M. m.* (n = 62)	*R. r.* (n = 20)	*M. m.* (n = 137)	*M. m.* (n = 42)
E. gallinacea	2·33 (0–5·19)	–	–	1·0	–	–	–
E. murina	–	–	–	1·5 (0–7·89)	–	16·0 (0–168·47)	–
X. cheopis	1·75 (0·22–3·28)	1·0	2·5 (0–8·88)	1·0	1·0	–	–
X. brasiliensis	1·0	–	–	–	1·5 (0–7·89)	–	–
X. guancha	–	–	–	1·5 (0·58–2·42)	–	1·37 (1·12–1·62)	1·0
S. tripectinata	1·5 (0–7·89)	4·9 (2·75–7·05)	–	–	–	–	–
L. segnis	2·5 (1·53–3·46)	1·5 (0–7·89)	–	1·0	1·0	1·0	–
N. barbarus	1·75 (0·78–2·72)	1·17 (0·74–1·60)	–	1·0	–	1·0	–

Table 3. Observed (O) and expected by chance (E) values of the C-score for presence/absence matrices of flea communities on 214 *Rattus rattus* and 660 *Mus musculus* sampled in the Canary archipelago, Spain. Negative values of the standardized effect size (SES) indicates that O < E, whereas positive values indicate the contrary. The asterisk indicates significant *P*-value <0·05

	R. rattus				*M. musculus*			
	C-score				C-score			
Island	O	E	*P*	SES	O	E	*P*	SES
El Hierro	17·00	31·49	0·01*	−2·35	161·50	166·27	0·36	−0·33
La Palma					92·33	122·36	0·04*	−1·75
La Gomera	1·00	1·49	0·22	−1·53	0·00	6·85	0·31	−1·47
Tenerife	18·00	16·40	0·81	0·48	10·50	16·83	0·07	−2·03
Gran Canaria	8·00	8·89	0·27	−0·60	15·17	16·79	0·43	−0·34
Fuerteventura					4·80	4·91	0·33	−0·36
Lanzarote	1·67	1·50	0·75	0·53	14·67	19·33	0·21	−1·15

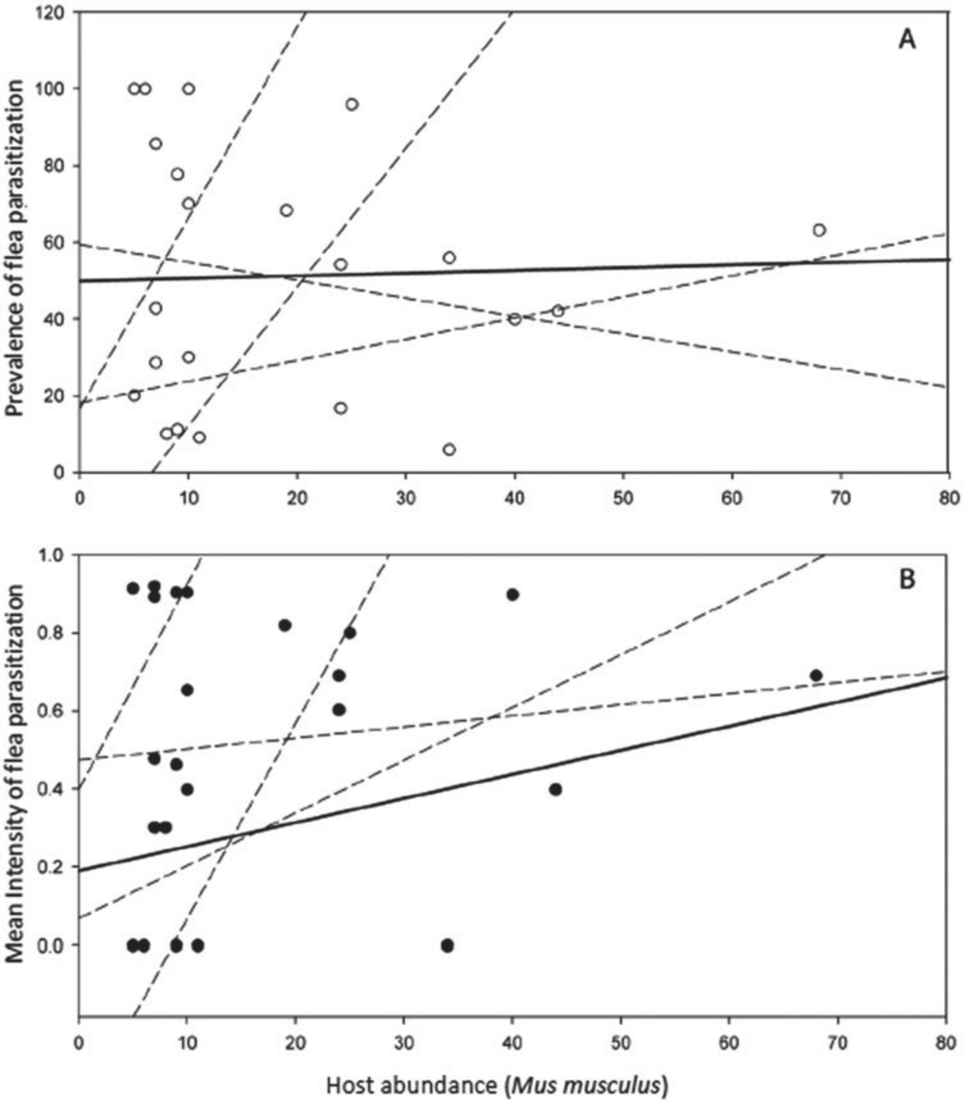

Fig. 2. Influence of host abundance (number of rodents captured in 200 live traps) at trapping locations on both prevalence (white dots A) and mean intensity (solid dots, B) of flea parasitization of the most abundant flea species (*S. t. tripectinata, L. segnis, N. barbarus and X. guancha*) in *Mus musculus* from Gran Canaria, Tenerife, El Hierro and La Palma. Broken lines represent islands whereas solid lines represent the average trend. Only trapping locations with more than five captures were considered.

Table 4. Model selection for the intensity of flea infection in 660 *Mus musculus* and 214 *Rattus rattus* trapped in the Canary archipelago, Spain.
Mo = null model only including the intercept term, K = number of parameters, AICc = Akaike Information Criterion corrected for small samples sizes, Δi = difference of AICc with respect to the best model, w_i = Akaike weight. In bold, models with substantial support

Biological models	K	AICc	Δi	w_i
Mus musculus				
Body weight + Flea richness	3	1099·16	0	0·73
Body weight* Flea richness	4	1101·19	2·03	0·26
Flea richness	2	1110·61	11·45	0·01
Body weight	2	1187·09	87·93	< 0·001
Mo	1	1193·83	94·68	< 0·001
Rattus rattus				
Body weight + Flea richness	3	206·95	0	0·77
Body weight* Flea richness	4	209·40	2·45	0·22
Flea richness	2	216·96	10·01	0·01
Body weight	2	233·32	26·37	< 0·001
Mo	1	240·79	33·84	< 0·001

On the other hand, we found a positive relationship between *Mus* abundance and both prevalence ($\beta_{\text{abundance}} = 0{\cdot}28$, S.E. = 0·24, *t*-value = 1·16, Fig. 2A) and intensity of infection ($\beta_{\text{abundance}} = 0{\cdot}04$, S.E. = 0·01, *t*-value = 2·4, Fig. 2B) of the most abundant flea species (i.e. *S. t. tripectinata, L. segnis, N. barbarus* and *X. guancha*). Between 0·01 to 20% of observed variability in both prevalence and intensity of flea infection in *Mus*, was respectively explained by differences among trapping locations.

Evaluating the biological cost of flea co-occurrence

The chosen model selection showed that the best model for explaining the observed variability in flea burden in both host species were those that included the additive effects of flea richness and body weight (Table 4). For *Mus* ($w_{\text{Flea richness + Body Weight}} = 0{\cdot}73$), this model explained 13·2% of the observed variability in flea intensity of infection, whereas the value was 49% in *Rattus* ($w_{\text{Flea richness + Body Weight}} = 0{\cdot}77$). For both host models, ectoparasite richness ($\beta_{\text{Flea richness}} = 1{\cdot}03$, S.E. = 0·09, *z*-value = 14·4 for *Mus* and $\beta_{\text{Flea richness}} = 4{\cdot}08$, S.E. = 0·65, *z*-value = 6·1 for *Rattus*, see Fig. 3A and B respectively) and body weight, had positive effects on flea intensity of infection indicating that optimum ectoparasite intensity of infection peaked in the heaviest hosts carrying more than one flea species. In fact, *Mus* carrying two flea species had in average of 2·8 more fleas than those parasitized by a single flea species. In *Rattus*, individuals infected by two species carried an average of three times more fleas than mono-parasitized hosts. Finally, higher flea loads did not result in body weight impairment. Heavier *Mus* individuals ($\beta_{\text{Body weight}} = 0{\cdot}05$, S.E. = 0·01, *z*-value = 3·53, Fig. 4A) and *Rattus* ($\beta_{\text{Body weight}} = 0{\cdot}002$, S.E. = 0·005, *z*-value = 0·5, Fig. 4B) showed higher flea burdens, contrary to our predictions.

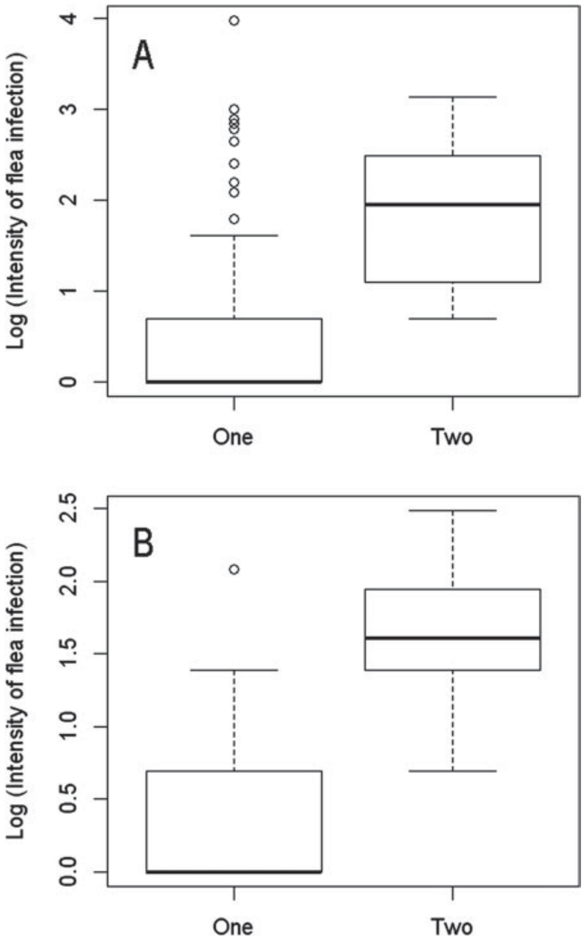

Fig. 3. Box plot showing the relationship between flea richness and intensity of flea parasitization in 204 box-trapped *Mus musculus* (A) and 48 *Rattus rattus* (B) from the Canary archipelago, Spain.

DISCUSSION

Different patterns of co-occurrence in Mus *and* Rattus

The results showed a tendency for aggregation rather than segregation of flea assemblages in the Canary Islands, as described in other continental areas (in accordance with our first prediction). Such assemblages, however, were host-specific and were non-random in *Mus* but random in *Rattus*.

Body size has been tested frequently as a parameter of parasite diversity with the assumption that larger body-sized hosts are likely to sustain richer flea assemblages because they provide more space and

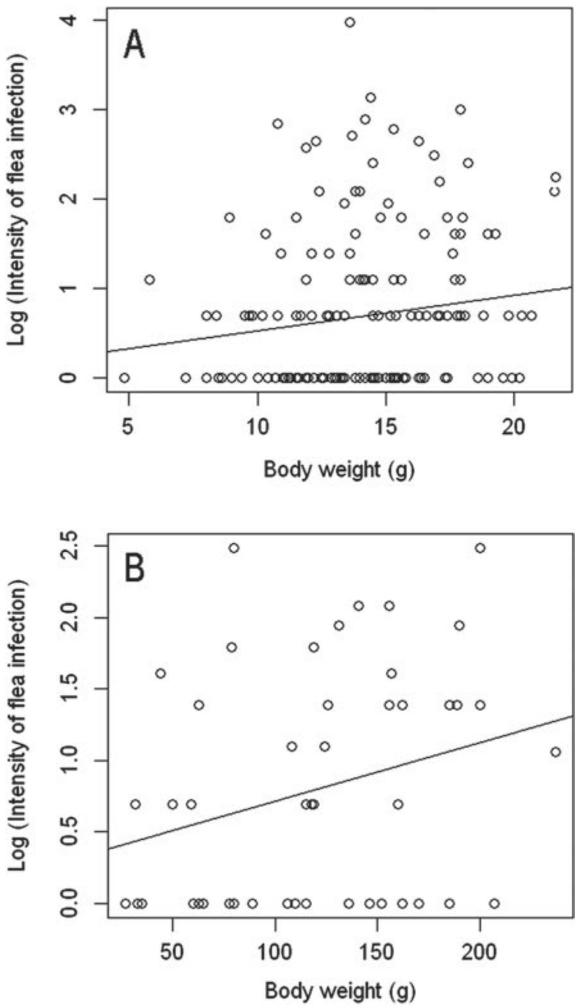

Fig. 4. Relationship between host body weight and flea intensity of infection in *Mus musculus* (A) and *Rattus rattus* (B) from the Canary archipelago, Spain.

a greater variety of niches (Kuris *et al.* 1980). Nonetheless, although in ungulates (Ezenwa *et al.* 2006) and bats (Bordes *et al.* 2008), positive correlation between host body size and parasite species richness appears to be the rule, rodents (Feliu *et al.* 1997) and primates (Nunn *et al.* 2003) are clear exceptions. In this study, host abundance rather than body size explains these patterns of co-occurrence.

Both rodents can be found in the seven islands of the Canary archipelago in a broad range of ecosystems, from sea level to the high peaks (Nogales *et al.* 2006). In this work, *Rattus* and *Mus* were captured at low and high altitudes (<100 and >1300 m) even often sharing the same habitats. However, it can be postulated that successive rat control campaigns in order to preserve native fauna and avoid detrimental effects caused by *R. rattus*, resulted in an increase in the *Mus* population (Rando, 2009). Regarding *R. norvegicus*, the low numbers of this rat species captured throughout the study (only 14 specimens), is the main reason for the lack of fleas found in this species. This rodent species is found mostly in urban areas living in dumps, sewers and basements and is frequently found in harbours (Blanco, 1998). In the current study, the trapping locations were primarily in rural and wild habitats where *R. rattus* is favoured. Hence, despite that habitats were sampled proportionally and that half of the live traps were specific for each host species, *Mus* individuals were captured three times more than *Rattus*. This biased abundance in favour of *Mus* would guarantee more constant resource availability and thus high reproductive success for fleas and the appearance of flea assemblages, in particular for the most abundant species (e.g. *Stenoponia tripectinata tripectinata* and *Xenopsylla guancha*, Table 3). The positive relationship between *Mus* abundance and prevalence and mean intensity of flea infestation, mainly for the most abundant species, is in line with this hypothesis.

In accordance with the idea that host abundance rather than host size drives flea assemblages, we observed that most of the generalist *S. t. tripectinata* were collected from *Mus* rather than from *Rattus*. This flea species parasitizes a wide range of rodents (*Apodemus, Arvicanthis, Meriones, Microtus* and *Pitymys*, see Hopkins and Rothschild, 1962) and even non-rodent species (e.g. *Crocidura russula*, see Jordan, 1958), and, at least in theory, would be expected in *Mus* and *Rattus* in a similar proportion. However, of the total 495 specimens of *S. t. tripectinata* observed, only three specimens (0·4% of records) were detected in *Rattus*, suggesting an accidental parasitization. This can all be considered clear evidence of the fact that high host densities are more influential than body size for some flea species. Along the same line, in the islands where *S. t. tripectinata* was not detected, the non-random assemblage seems to be due to *X. guancha*, to date the only endemic species of flea in the archipelago (Beaucournu and Launay, 1990), and with the highest prevalence in *Mus* in the absence of *Stenoponia* (Table 3).

According to Krasnov *et al.* (2005), when a certain flea species parasitizes a host it may facilitate the exploitation by other flea species due to the depression of the immune system. The positive co-occurrence (aggregation) of flea species in *Mus* and not in *Rattus* may be due principally to the great number of *S. t. tripectinata* and to a lesser extent to *X. guancha*, which may favour infection by other flea species (Fig. 4).

High tolerance to co-infection

Contrary to our expectations, the higher flea intensities occurred in the heaviest individuals (Fig. 4). In addition, such heavily infected individuals commonly carried more than one flea species (Fig. 3) indicating that the cost of multiple infections is low for both host species. There are two possible

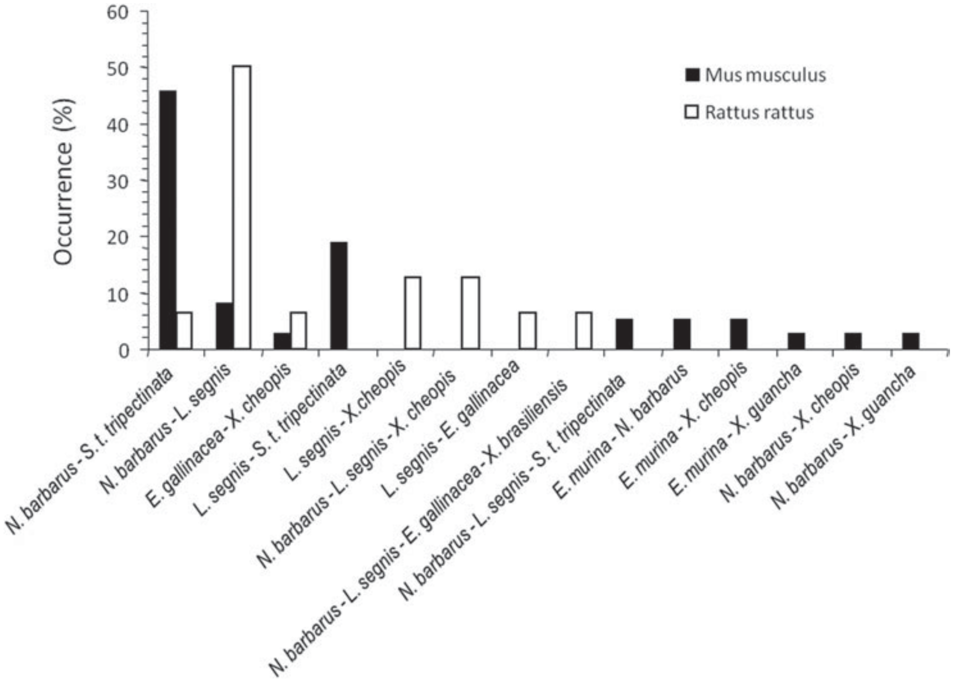

Fig. 5. Frequency of appearance (%) of flea pairs in *Mus musculus* and *Rattus rattus* from the Canary archipelago, Spain. Only individuals carrying more than one flea species are represented.

explanations for the positive correlation between host body mass (weight in the absence of body length) and intensity of flea parasitization in *Mus* (Figs 3A and 4A) and *Rattus* (Figs 3B and 4B). The first is based on the fact that older individuals harbour more parasite species than younger ones as a consequence of the continued parasite accumulation throughout life (Morand, 2000). In fact, since body weight is a common proxy for age in rodents, heavy, and hence older, individuals will show higher infestation loads. Nevertheless, exceptions to this pattern are common (Stanko *et al*. 2002; Krasnov *et al*. 2004). On the other hand, the apparent tolerance of flea infection found in our host system could be due to a phenomenon known as cross-reactivity due to similarity of anti-clotting, antiplatelet and vasodilatory substances contained in the saliva of the different parasite taxa (Mans *et al*. 2002). Experiments developed by Khokhlova *et al*. (2004*a*, *b*) demonstrate the existence of cross-reactivity between several flea species in wild rodents. Even though the flea species in these studies belong to the family Pulicidae and cross-reactivity has been described for congeneric rather than heterogeneric ectoparasites (McTier *et al*. 1981), the similarity of salivary components within a parasite taxon can lead to cross-reactivity of a host against closely related parasites (den Hollander and Allen, 1986). Cross-reactivity may lead the host to tolerate a multi-infection, explaining why heavier hosts are exploited by different flea species. Thus, in this study, immunosuppression resulting from parasitization by multiple fleas throughout life and cross-reactivity could explain the aggregation of fleas on heavier host individuals. Whatever the mechanism involved, it is clear that the energetic cost of flea infestation in the main rodent species in the Canary Islands is low.

In summary, as a result of this study, two conclusions can be drawn. First, patterns of flea co-occurrence in insular areas depend mainly on the host density. Apparently, non-randomness is shaped by host-specific fleas that favour the exploitation of non-specific fleas. Second, it appears that co-infection does not affect the host negatively with some tolerance to parasitization by different flea species. However, due to the observational nature of this study, further experimental research on the role of early infections on the success of infection of new colonizers in the absence of food restrictions are necessary to better understand the low cost of flea infestation in insular habitats.

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