Canadian Science Publishing
NRC Research Press journals | Publishing Services

Canadian Journal of Zoology
Revue canadienne de zoologie

Population structure in two geographically sympatric and congeneric ectoparasites: *Cimex adjunctus* and *Cimex lectularius*, in the North American Great Lakes region

Journal:	*Canadian Journal of Zoology*
Manuscript ID	cjz-2017-0014.R1
Manuscript Type:	Article
Date Submitted by the Author:	27-Apr-2017
Complete List of Authors:	Talbot, Benoit; Western University, Department of Biology Vonhof, Maarten; Western Michigan University, Department of Biological Sciences Broders, Hugh; University of Waterloo, Department of Biology Fenton, M.B.; Western University, Department of Biology Keyghobadi, Nusha; Western University, Department of Biology
Keyword:	Bat bug, Cimex adjunctus, Cimex lectularius, Common bed bug, Genetic differentiation, Genetic diversity, Host

Population structure in two geographically sympatric and congeneric ectoparasites: *Cimex adjunctus* and *Cimex lectularius*, in the North American Great Lakes region

Benoit Talbot (btalbot4@uwo.ca)[1*],
Maarten J. Vonhof (maarten.vonhof@wmich.edu)[2],
Hugh G. Broders (hbroders@uwaterloo.ca)[3],
Brock Fenton (bfenton@uwo.ca)[1]
and Nusha Keyghobadi (nkeyghob@uwo.ca)[1]

[1]Department of Biology, University of Western Ontario, 1151 Richmond Street, London, Ontario, Canada, N6A 3K7;
[2]Department of Biological Sciences, Western Michigan University, 1903 W Michigan Avenue, Kalamazoo, Michigan, United States, 49008-5410;
[3]Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, Ontario, Canada, N2L 3G1.
*Author for correspondence

Population structure in two geographically sympatric and congeneric ectoparasites: *Cimex adjunctus* and *Cimex lectularius*, in the North American Great Lakes region

Benoit Talbot, Maarten J. Vonhof, Hugh G. Broders, Brock Fenton and Nusha Keyghobadi

Abstract

Subdivided populations can be described by different models of population structure that reflect population organisation, dynamics and connectivity. We used genetic data to investigate population structure in two geographically sympatric, congeneric species of generalist ectoparasites of warm-blooded animals. We characterized the spatial genetic structure of *Cimex adjunctus* Barber, 1939, an understudied and fairly abundant species, using microsatellite markers at a spatial scale representing contemporary dispersal of the species. We found seven genetic clusters, global G'_{ST} of 0.2, 33% of genetic variation among sites, and non-significant isolation-by-distance. We compared these results with *Cimex lectularius* L., 1758, a closely related but conversely well-known species, in the same geographic area. We found stronger genetic structuring in *C. lectularius* than in *C. adjunctus*, with eleven genetic clusters, G'_{ST} of 0.7, 57% of genetic variation among sites, and significant but weak isolation-by-distance ($R^2 = 0.09$). These results suggest that while both species can be described as having classic metapopulation structure, *C. adjunctus* leans more towards a patchy population and *C. lectularius* leans more towards a non-equilibrium metapopulation. The difference in population structure between these species may be attributable to differences in movement potential and extinction-colonization dynamics.

Keywords: Bat bug, *Cimex adjunctus*, *Cimex lectularius*, Common bed bug, Genetic differentiation, Genetic diversity, Host.

Résumé

Les sous-populations peuvent être décrites par plusieurs modèles de structure populationnelle qui reflètent organisation, dynamique et connectivité. Nous avons utilisé des données génétiques pour investiguer la structure populationnelle chez deux ectoparasites généralistes, géographiquement sympatriques et congénériques d'animaux à sang chaud. Nous avons caractérisé la structure génétique spatiale de *Cimex adjunctus* Barber, 1939, une espèce peu étudiée et abondante, en utilisant des marqueurs microsatellites, à une échelle spatiale représentative de la dispersion contemporaine de l'espèce. Nous avons trouvé sept groupes génétiques, un G'_{ST} global de 0.2, 33% de la variation génétique entre les sites, et un isolement-par-la-distance non significatif. Nous avons comparé ces résultats avec *Cimex lectularius* L., 1758, une espèce apparentée mais connue, dans la même aire géographique. Nous avons trouvé une plus forte structure génétique chez *C. lectularius* que chez *C. adjunctus*, avec onze groupes génétiques, un G'_{ST} de 0.7, 57% de la variation génétique entre les sites, et un isolement-par-la-distance significatif mais faible ($R^2 = 0.09$). Ces résultats suggèrent que bien que les deux espèces possèdent une structure classique de métapopulations, *C. adjunctus* penche plus vers une population parcellaire et *C. lectularius* penche plus vers une métapopulation en déséquilibre. La différence de structure populationnelle entre ces deux espèces peut être attribuable à des différences de potentiel de mouvement et de dynamique d'extinctions-colonisations.

Introduction

Natural populations are often subdivided, most commonly as a result of landscape heterogeneity (Storfer et al. 2007). Individuals may move from one patch to the other, but usually will not settle or breed in intervening areas. Different species often exhibit contrasting levels of connectivity among subpopulations, as well as different local dynamics (Mimet et al. 2013). These, in turn, affect population persistence, and genetic diversity and differentiation (Neel 2008; Andreakis et al. 2009). The fundamental organisation and dynamics of spatially subdivided populations are described by models (Harrison 1991) that provide predictions of population genetic structure and differentiation (Mayer et al. 2009; Table 1). At one end of a continuum of population structure, patchy populations are characterised by high connectivity among subpopulations, effectively constituting a single panmictic population. Genetic differentiation among subpopulations in a patchy population is essentially non-existent, and the subpopulations would be expected to form a single genetic cluster and not display isolation-by-distance (IBD). At the other extreme, in non-equilibrium metapopulations, subpopulations are disconnected from each other. Non-equilibrium metapopulations are characterized by high differentiation among subpopulations, with almost all subpopulations predicted to each form a separate genetic cluster. IBD is also not expected in this case because of the predominance of genetic drift over gene flow in determining genetic differentiation (Hutchison and Templeton 1999). Finally, classic metapopulations are intermediate in the continuum between patchy populations and non-equilibrium metapopulations (Hanski 1998). Classic metapopulations have some, but limited, connectivity among subpopulations, and connectivity is usually distance dependent. In classic metapopulations

genetic differentiation is intermediate between patchy populations and non-equilibrium metapopulations; there should be several distinct genetic clusters, but the number of such clusters is expected to be less than the number of occupied habitat patches. Also, because more distant subpopulations are connected by lower dispersal and gene flow, isolation-by-distance should be present in a classic metapopulation (Bohonak 1999), but not in populations following the other two models (Mayer et al. 2009). Real populations in nature may show characteristics of a classic metapopulation in combination with some attributes of either a patchy population or a non-equilibrium metapopulation (Rasic and Keyghobadi 2012).

Understanding population structure is important because it predicts regional dynamics and persistence (Harrison 1991; Hanski 1998). However, key ecological variables determining population structure may be difficult to quantify directly. For example, movements among subpopulations can be very be difficult to track in species that are small or cryptic, such as ectoparasites. Because each population model makes specific genetic predictions (Mayer et al. 2009), a snapshot of patterns of genetic differentiation can provide an assessment of population structure. In our study, we used genetic data to investigate population structure of two congeneric parasite species living in sympatry, but associated with different hosts, in the Great Lakes region of North America.

The genus *Cimex* (Order: Hemiptera, Class: Insecta) is characterized by species that are temporary ectoparasites of warm-blooded animals, mostly bats. Species of *Cimex* typically remain in the hosts' roosts, emerging from cracks in the walls only to obtain blood meals (Usinger 1966; Cooper et al. 2015). *Cimex* species are hypothesized to have low inherent capacity for dispersal between contiguous structures, rather depending on

their hosts for dispersal (Usinger 1966). In central and eastern North America, *Cimex adjunctus* Barber, 1939, is a widespread ectoparasite of North American bats, although it is also known to bite people visiting or residing near bat roosting sites (Goddard et al. 2012). This species occurs from the east coast to the Rocky Mountains, and from Labrador and Northwest Territories to Texas (Usinger 1966). Talbot et al. (2016) found high levels of continent-wide spatial genetic structure in *C. adjunctus*, although with evidence of multiple potential instances of long-distance dispersal. The big brown bat (*Eptesicus fuscus*) and the little brown myotis (*Myotis lucifugus*) are two key hosts of *C. adjunctus* that frequently roost in buildings (Furlonger et al. 1987; Ellison et al. 2007; Pearce and O'Shea 2007). The big brown bat is known to frequently switch roosts during the summer due to temperature and parasite density (Ellison et al. 2007). The common bed bug (*Cimex lectularius* L., 1758) is a congener that is a public health concern in many countries (Goddard 2009; Criado et al. 2011). This ectoparasite feeds primarily on humans and is most commonly found associated with humans in their dwellings, although it is also known to feed on a range of other animals, including chickens and bats (Usinger 1966). *Cimex lectularius* is regularly observed in association with bats in Europe (Balvín et al. 2012; Booth et al. 2015), although has never been recorded with bats in North America. Pesticides have been used for many decades on bed bug infestations around the world. The effect of DDT was particularly strong on *C. lectularius* populations, effectively eliminating them from households (Adelman et al. 2011). Use of DDT on populations of other *Cimex* species that associate with bats was likely not as intense or widespread as it was for *C. lectularius* populations associated with humans. In recent years, *C. lectularius* has experienced a resurgence in many parts of the world (Davies et al. 2012).

In *C. lectularius,* limited human-mediated gene flow and colonization, along with local extinctions driven by pest control practices, result in a mixture of classic and non-equilibrium metapopulation attributes (Harrison 1991; Fountain et al. 2014). There is significant genetic differentiation among infestation locations, and typically either no or very weak isolation-by-distance (Saenz et al. 2012). Interestingly, genetic differentiation indices (F_{ST}) are much higher among human-associated *C. lectularius* subpopulations than among bat-associated conspecifics collected from roosts (Booth et al. 2015), indicating an effect of host-association on population structure. While the genetic attributes and population structure of *C. lectularius* have been addressed in several studies (Booth et al. 2012; Saenz et al. 2012; Fountain et al. 2014; Booth et al. 2015), the characteristics of populations of other *Cimex* species that typically associate with bats have received little attention (but see Talbot et al. 2016). An understanding of the structure and genetics of populations of these insects could provide insight into their propagation and potential impact on bat populations. *Cimex adjunctus* is of particular importance because it is the most widespread cimicid parasite of bats in North America (Usinger 1966), and its key hosts, the big brown bat and the little brown myotis, are currently threatened by the fungus causing White-Nose Syndrome (Blehert et al. 2009).

We first investigated the population structure of *C. adjunctus*, an understudied and fairly abundant species of *Cimex* in North America. *C. adjunctus* is concentrated in bat roosts, usually within man-made structures. We then wanted to compare population structure of *C. adjunctus* with that of its well-known and sympatric congener, *C. lectularius*. We therefore examined household infestations of *C. lectularius* in the same geographic area and at a similar spatial scale. We predicted that population structure

would differ between the two *Cimex* species and that *C. lectularius* would show higher levels of genetic structure and differentiation than *C. adjunctus* due to possibly more limited movement and more frequent extinctions.

Materials and Methods

Data collection

We examined *C. adjunctus* population structure over a spatial scale that is representative of contemporary dispersal of their hosts (Penczykowski et al. 2016). Population structure at a larger spatial scale (e.g., range wide) could reflect historic demographic processes, such as post-glacial recolonizations and secondary contact zones (Swenson and Howard 2005), and has been described in Talbot et al. (2016). From a larger *C. adjunctus* dataset (Talbot et al. 2016), we selected individuals from sites around the Great Lakes region of the United States and Canada (Fig. 1) with a median distance among sites corresponding to the estimated median translocation distances in bat hosts (Norquay et al. 2013). Most samples are from mist-netted host individuals of *E. fuscus* and *M. lucifugus* between 2005 and 2014 (Appendix A1). Mist net capture locations were adjacent to a known summer roost (house, barn, church, or school) of either of the two bat species, or within forested provincial or state lands (Appendix A1). Most mist-netted bats and the *C. adjunctus* individuals they harboured likely came from the adjacent known roost, although it is possible that a small proportion came from different roosts in the area. Overall, between 3 and 15% of mist-netted bats harboured a parasite, depending on the location. We also sampled *C. adjunctus* individuals from the interior of two summer roosts. One roost was in a church attic inhabited by *M. lucifugus*, and one was in a house attic

inhabited by *E. fuscus* (Appendix A1). Because we could be certain of the roost site in these cases, we considered these two sampling locations as distinct from their adjacent mist netting capture locations. Talbot et al. (2016) showed, in a range-wide study of *C. adjunctus* that included the samples used in this study, that there is limited or no effect of sampling year or host species on genetic clustering.

We collected *C. lectularius* samples from infested homes in the same geographic area (Fig. 1) and at a comparable spatial scale, with the help of Abell Pest Control Inc. (Toronto, Ontario, Canada) in 2014 (Appendix A2). Due to privacy reasons, we only obtained postal codes for each *C. lectularius* individual, from which we obtained centroid geographical coordinates in WGS84 datum using the public CivicSpace USA ZIP Code Database (Schuyler Erle, CivicSpace Labs Inc., San Francisco, California, United States) and the crowd-sourced Canadian Postal Code Geocoded Database (Geocoder.ca, Geolytica Inc., Ottawa, Ontario, Canada). We sampled between one and four housing units per postal code, and we considered separate households in the same postal code as different sites. Upon collection, we immediately stored each sample individually in 95% ethanol until further analyses.

Genetic analyses

We used *C. adjunctus* genotypes at nine microsatellite loci, originally developed for *C. lectularius* (Cle002, Cle003, Cle013, Cle015 from Fountain et al. 2014; Clec15, Clec21, Clec48, Clec104 and BB28B from Booth et al. 2012), from Talbot et al. (2016). We extracted DNA from the whole insect for all *C. lectularius* samples using the DNeasy Blood and Tissue Kit (QIAGEN, Germantown, Maryland, United States). We amplified

20 microsatellite loci designed specifically for that species, and including the nine loci also used for *C. adjunctus* (Cle011 and Cle021 from Fountain et al. 2014; Clec11, Clec45, Clec96, Clec97, Clec99, BB21B, BB29B, BB31B and BB38B, from Booth et al. 2012). Amplifying a larger number of microsatellite loci in *C. lectularius* allowed us to compare the statistical resolution between using a smaller versus a larger set of markers (Appendix B1). For all other analyses, we only used microsatellite markers that amplified for both *C. adjunctus* and *C. lectularius*.

We used a DNAEngine PTC-200 Thermal Cycler (BIO-RAD, Hercules, California, United States) to execute the Polymerase Chain Reaction (PCR) amplification of *C. lectularius* samples. We performed PCR using the same protocols as in Booth et al. (2012) and Fountain et al. (2014) for the loci developed by each study, respectively. We visualized PCR products by 1.5% agarose gel electrophoresis using SYBR Green (BIO-RAD, Hercules, California, United States) on a UV transilluminator to check the quality and size of amplified fragments. We then sized products on a 3730xl DNA Analyzer (Applied Biosystems, Foster City, California, United States; ABI). We called all microsatellite genotypes for each species using GeneMapper Software v.4.0 (ABI), and we checked all calls manually.

Statistical analyses

Hardy-Weinberg and linkage disequilibrium, genetic diversity, and pedigree analysis

For microsatellite loci, we tested for Hardy-Weinberg and linkage disequilibrium within each site that had data from at least two sampled individuals, using Genepop 4.2 (Raymond and Rousset 1994). For each type of test, we corrected for multiple tests using

Bonferroni correction, with a threshold α of 0.05. For sites with data from at least three sampled individuals, we calculated average number of alleles, observed and expected heterozygosity, and inbreeding coefficient, using GenoDive v2.0 (Meirmans 2012). Sampling individuals from the same family group (i.e., associated either by a parent-offspring or a full-sibling relationship) can influence results of population genetic analyses (Goldberg and Waits 2010), and sampling of family groups has been reported in *C. lectularius* (Saenz et al. 2012). Therefore, we wanted to determine the relationship of each pair of individuals in the dataset, to ascertain that relatedness is not a source of bias in our comparison of genetic structure between the two species. To this end, we used ML-Relate (Kalinowski et al. 2006), which provides maximum likelihood estimates of relationship, to assess the proportion, of all pairs of individuals, in each species, that are between individuals that are related as either parent-offspring or full-siblings and were collected from the same sampling site.

Individual-level analyses

We then tested our prediction of differing population structure between *C. adjunctus* and *C. lectularius*. To facilitate meaningful comparison between the two parasite species, we analyzed *C. lectularius* at the same seven markers as *C. adjunctus*. As a secondary analysis, we also examined *C. lectularius* at the whole panel of 20 markers, to ascertain whether using fewer markers had any effect on the results. We applied most analyses at the individual level, due to the fact that a large part of our dataset is composed of sites with only one individual sampled.

First, we looked for evidence of genetic clustering and isolation-by-distance (IBD). We conducted a Bayesian clustering analysis using Geneland v4.0.5 (Guillot et al. 2005), which takes into account geographic coordinates of samples. We used 10,000,000 iterations, thinned every 1,000th iteration, and a post-process burn-in of 2,000 after thinning, for *K* values between 1 and 20. We executed 10 runs, and kept the one with the higher posterior mean density, after burn-in. We attempted to identify the population to which each individual was assigned the most often, defined here as the population where the majority of Markov Chain Monte Carlo (MCMC) chains converged for any given individual. We also conducted a *K*-Means clustering analysis using GenoDive v2.0 on allele frequencies, for *K* values between 1 and 20, and using 50,000 simulation steps, to validate results obtained with the Geneland method. We used Bayesian Information Criterion (BIC) values to determine the most likely *K* value.

Next, we conducted an individual-level analysis of IBD, using the estimate of genetic relatedness, r_W (Wang 2002), calculated with SpaGeDi v1.5 (Hardy and Vekemans 2002). We calculated $1 - r_W$ for each pairwise relationship to obtain genetic distances. We calculated geographic distance (in km) between sample sites, corrected for sphericity of the earth, using the 'rdist.earth' function from the 'fields' package (Fields Development Team 2006) in R v3.1.3 (R Development Core Team, Vienna, Austria). We then fit pairwise genetic distance to geographic distance using Multiple Regression on distance Matrices (MRM), in the 'MRM' function from the 'ecodist' package (Goslee and Urban 2007) in R v3.1.3, which uses a Mantel test derived linear regression model. We assessed significance through a permutation procedure (9,999 replicates) that takes into account non-independence of data points in distance matrices (Legendre et al. 1994;

Lichstein 2007). An assumption of the r_W relatedness index is that individuals are in a large random mating population without population structure (Wang 2011). To correct for the population structure present in our dataset, we subsequently conditioned IBD models for genetic clustering; for each pair of individuals assigned to the same population in clustering analyses, we assigned a value of 0, and for each pair of individuals assigned to different sites, we assigned a value of 1, and then tested the effect of geographic distance, together with genetic clustering (defined as a 0/1 pairwise matrix), on genetic distance in an MRM model.

Site-level analyses

We also used some site-level analyses to complement results obtained at the individual level, using sites with at least three sampled individuals. A site is defined in our study as a single housing unit, for *C. lectularius*, or a single capture location or roost, for *C. adjunctus*. We conducted an Analysis of Molecular Variance (AMOVA), using GenoDive v2.0. We calculated expected heterozygosity averaged across sites and Hedrick's global G'_{ST} among sites (Hedrick 2005), also using GenoDive v2.0. G'_{ST} provides estimates of genetic differentiation that can be more meaningfully compared between species with different levels of genetic diversity (Meirmans and Hedrick 2011).

Results

We selected 75 individuals from the *C. adjunctus* dataset of Talbot et al. (2016) from sites an average of 387 km apart (median = 278 km, between 0 and 1413 km); 54 of those were from the body of a bat, and 21 from the interior of a roost (Appendix A1). We

sampled between one and six *C. lectularius* individuals, the common bed bug, at infested housing units in the same region (Appendix A2), leading to a collection of 73 individual *C. lectularius*, at an average of 373 km apart (median = 205 km, between 0 and 903 km). Genotype data for *C. lectularius* for the whole panel of 20 microsatellite markers is available in Appendix A3. Excluding sites with only one individual sampled (7 in *C. adjunctus*; 13 in *C. lectularius*), average sample size per site was five in *C. adjunctus* and three in *C. lectularius* (over 12 sites in *C. adjunctus* and 18 sites in *C. lectularius*).

Genetic diversity, Hardy-Weinberg and linkage disequilibrium, and pedigree analysis

Two of the nine microsatellite markers used in *C. adjunctus* were monoallelic and we therefore excluded them from the analyses on both *C. adjunctus* and *C. lectularius*. Among the remaining seven microsatellite loci, in *C. adjunctus* and *C. lectularius* respectively, we observed between three and 15, and between two and 13 alleles. Respectively for *C. adjunctus* and *C. lectularius*, average number of alleles ranged from 1.7 to 3.6 and from 1.1 to 2.8, observed heterozygosity ranged from 0.11 to 0.36 and from 0.10 and 0.67, expected heterozygosity ranged from 0.33 to 0.50 and from 0.07 to 0.63, and the inbreeding coefficient varied between -0.01 and 0.75 and between -0.67 and 0.37 (Table 2; Appendix A4 for genetic diversity indices calculated for each marker). Inbreeding coefficients in *C. lectularius* were centered around zero, with a mean of -0.05. Inbreeding coefficients in *C. adjunctus* were higher, with mostly positive values and a mean of 0.41. We found three significant cases of deviation from Hardy-Weinberg equilibrium, characterized by homozygote excess, in *C. adjunctus* (one population at Clec104 and Cle015, and another population at Clec104). Since these incidences of

317 deviation from Hardy-Weinberg equilibrium were not systematic across loci or sites, we
318 retained these two markers and two sites for our analyses. We found no significant
319 evidence, at a threshold of 0.05 after Bonferroni correction, of Hardy-Weinberg or linkage
320 disequilibrium in any marker in *C. lectularius*. We also did not find any evidence of
321 significant linkage disequilibrium in any marker, in both species. Of all pairs of
322 individuals in both species, a low proportion (2.8% in *C. adjunctus* and 2.9% in *C.*
323 *lectularius*) showed a parent-offspring or full-sibling relationship and were from the same
324 sampling site.
325
326 *Spatial genetic structure*
327 Bayesian clustering in Geneland revealed a coarser division of genetic structure in
328 *C. adjunctus* than in *C. lectularius*, with seven genetic clusters in the former versus
329 eleven for the latter (Table 3). Clusters were consistent with geographic sampling
330 location in both species (Fig. 1), and only *C. adjunctus* had clusters containing
331 individuals sampled moderately far away from each other. Clusters in *C. adjunctus* were
332 also highly consistent with those in the larger-scale study of (Talbot et al. 2016). *K*-means
333 clustering in GenoDive also resulted in $K = 7$ in *C. adjunctus*. In contrast, the lowest BIC
334 value was at $K = 10$ in *C. lectularius*, and the second lowest BIC value was at $K = 11$. We
335 observed a non-significant IBD relationship in *C. adjunctus* ($P = 0.165$, $R^2 < 0.01$), but a
336 significant relationship in *C. lectularius* ($P = 0.001$, $R^2 = 0.09$), when genetic relatedness
337 was fitted only with geographic distance (Table 3). When IBD models were conditioned
338 with genetic structure, we found significant IBD in both species ($P = 0.001$); the fit of the

model was still very low in *C. adjunctus* (R^2 = 0.04; Table 3), but increased more than two-fold for *C. lectularius* (R^2 = 0.22).

Results from the AMOVA revealed a sharp difference between the species in the proportion of genetic variation among sites (Table 4), which was lower by almost 25% in *C. adjunctus* (33.3%) than in *C. lectularius* (57.9%). We also found a significantly lower global differentiation index estimate in *C. adjunctus* (G'_{ST} = 0.233) than in *C. lectularius* (G'_{ST} = 0.750). These results indicate weaker genetic structuring in *C. adjunctus* than in *C. lectularius*. Average expected heterozygosity was slightly higher in *C. adjunctus* (H_S = 0.389) than *C. lectularius* (H_S = 0.351), although the confidence intervals for these estimates overlapped (Table 4). Finally, all analyses on *C. lectularius* did not show appreciable differences when applied on the dataset of seven microsatellite loci or on the whole panel of 20 markers, with the larger panel revealing only slightly stronger genetic differentiation and structuring (Appendix B1).

Discussion

We confirmed our prediction of different population structure in *C. adjunctus* and *C. lectularius*, two congeneric ectoparasitic insects associated with different host species. Both species showed classic metapopulation characteristics, but each tended towards either a more patchy population or non-equilibrium metapopulation structure. In *C. adjunctus* we found moderate genetic differentiation, a small number of genetic clusters and no IBD, which suggest a mixture of classic metapopulation and patchy population attributes. In *C. lectularius* we found high genetic differentiation, a larger number of genetic clusters, and weak IBD, which suggest a mixture of classic and non-equilibrium

362 metapopulation attributes. The high levels of genetic differentiation and weak pattern of
363 IBD we observed in *C. lectularius* are consistent with existing literature on the species
364 that points to a very strong genetic structure between housing units (Booth et al. 2012;
365 Fountain et al. 2014), and the presence of weak IBD in the eastern United States in a
366 study area of comparable size (Saenz et al. 2012). Our results therefore support previous
367 work in *C. lectularius* while providing new insight into the biology of the less well
368 studied *C. adjunctus*. An interesting observation is that the degree of spatial genetic
369 structuring in *C. lectularius* at a scale of a few hundred kilometers, observed here, is
370 similar to what is observed for *C. adjunctus* across its entire range of several thousands of
371 kilometers (Talbot et al. 2016).

372 Two possible caveats of our between-species comparison are a higher mean
373 number of samples per site and sampling over a longer time frame (increasing the
374 possibility of genetic differentiation among samples due to drift), in *C. adjunctus* than in
375 *C. lectularius*. However, both these factors would have a tendency to bias results towards
376 higher genetic structuring in *C. adjunctus*, which we clearly did not observe in our results.
377 We also found a weaker effect of geographic distance in *C. adjunctus* than in *C.*
378 *lectularius*, despite the fact that geographic coordinates in *C. lectularius* were much less
379 precise than *C. adjunctus* (we only had the postal code for each unit), which could lead to
380 increased noise and a dampening of any underlying spatial pattern. Finally, we found
381 similar patterns of genetic structure in *C. lectularius* when using a panel of twenty
382 available markers versus the seven that overlapped with *C. adjunctus*, indicating that the
383 seven markers used for our study provide reliable and meaningful estimates of genetic
384 structure. Higher inbreeding coefficients in *C. adjunctus* reflect a generally lower

observed than expected heterozygosity, which could be due to some pooling of individuals from separate populations (i.e., a Wahlund effect) arising from a lower certainty of roost assignment.

Host movement behaviour can be an important determinant of connectivity and population structure in hitchhiking parasites. For example, two tick host races parasitizing black-legged kittiwake and Atlantic puffin respectively showed disparate genetic differentiation patterns at some spatial scales, likely caused by different dispersal patterns of the two host species (McCoy et al. 2003). Movement and parasite transport by humans is complex and makes use of a number of different modalities. Nonetheless, it is most likely that the dispersal kernels for parasites transported by humans versus flying bats are very different. The more patchy population structure in *C. adjunctus* than in *C. lectularius* observed here could be due to more frequent transport of parasites over a scale of tens to hundreds of kilometers by bats (Roberts et al. 2012; Norquay et al. 2013; Weller et al. 2016) than by humans.

Predicted genetic consequences of the three main models of population structure result largely from differences in connectivity among sub-populations (Mayer et al. 2009). Among different species that display classic metapopulation structure, variation in the degree of genetic differentiation of subpopulations will depend on both differences in connectivity as well as local dynamics that affect genetic drift. Higher genetic differentiation in *C. lectularius* versus *C. adjunctus* could also therefore be due to stronger effects of genetic drift, mediated by smaller effective population size (Garant et al. 2007; Gandon and Nuismer 2009). Differences in effective population size between the two parasite species might, in turn, be due in part to a difference in population dynamics

mediated by pesticide use. Pesticides have been used extensively on household *C. lectularius* infestations (Davies et al. 2012), potentially causing local bottlenecks and extinctions, whereas they may be only rarely used in known bat roosts. This could possibly explain why *C. lectularius* associated with bats are genetically more diverse than *C. lectularius* associated with humans (Booth et al. 2015). Local population crashes or extinctions may also occur in bat-associated *Cimex* parasites, due to roost-switching in bats (Bartonička and Růžičková 2013). However, pesticide use is arguably much more efficient at reducing or eliminating parasite populations than is roost switching by hosts. Therefore populations of *C. lectularius* may experience higher rates of local bottlenecks and extinctions, and genetic drift may play a more important role in driving divergence among populations of human-associated versus bat-associated parasites.

To conclude, our results show appreciable differences in population structure and genetic differentiation between the two parasite species. Population structure, in turn, can have important implications for the transmission of vector-borne diseases and the eco-evolutionary dynamics between these parasites and their hosts (Vander Wal et al. 2014*a*, 2014*b*).

Acknowledgements

We thank all field assistants who offered their help in collecting samples: Alejandra Ceballos, Cal Butchkoscki, Christy Humphrey, David Elliot, Derek Morningstar, Dylan Baloun, Elizabeth Warburton, Jenna Siu, John Whitaker, Ken Luzynski, Kristin Jonasson, Laura Kaupas, Lee Johnson, Lesley Hale, Lisa Winhold, Lucas Greville, Lynne Burns, Mark Brigham, Paul Faure, Quinn Webber, Rob Valdizon,

Robert Barclay, Roger Pearce, Sara McCarthy, Shelby Bohn, Stephanie Erickson, Toby Thorne, Tony Parr, and all personnel from Abell Pest Control Inc. who contributed in collecting samples. Natural Sciences and Engineering Research Council of Canada (NSERC), Ontario Student Assistance Program (OSAP), the Schmeelk Canada Foundation and Western University funded BT during his PhD degree. NSERC funded HGB, BF and NK, and Western Michigan University funded MJV.

References

Adelman, Z.N., Kilcullen, K.A., Koganemaru, R., Anderson, M.A.E., Anderson, T.D., and Miller, D.M. 2011. Deep sequencing of pyrethroid-resistant bed bugs reveals multiple mechanisms of resistance within a single population. PLoS ONE **6**(10): e26228. doi:10.1371/journal.pone.0026228.

Andreakis, N., Kooistra, W.H.C.F., and Procaccini, G. 2009. High genetic diversity and connectivity in the polyploid invasive seaweed *Asparagopsis taxiformis* (Bonnemaisoniales) in the Mediterranean, explored with microsatellite alleles and multilocus genotypes. Mol. Ecol. **18**(2): 212–226. doi:10.1111/j.1365-294X.2008.04022.x.

Balvín, O., Munclinger, P., Kratochvíl, L., and Vilímová, J. 2012. Mitochondrial DNA and morphology show independent evolutionary histories of bedbug *Cimex lectularius* (Heteroptera: Cimicidae) on bats and humans. Parasitol. Res. **111**(1): 457–469. doi:10.1007/s00436-012-2862-5.

Bartonička, T., and Růžičková, L. 2013. Recolonization of bat roost by bat bugs (*Cimex pipistrelli*): could parasite load be a cause of bat roost switching? Parasitol. Res. **112**(4): 1615–1622. doi:10.1007/s00436-013-3316-4.

Blehert, D.S., Hicks, A.C., Behr, M., Meteyer, C.U., Berlowski-Zier, B.M., Buckles, E.L., Coleman, J.T.H., Darling, S.R., Gargas, A., Niver, R., Okoniewski, J.C., Rudd, R.J., and Stone, W.B. 2009. Bat White-Nose Syndrome: an emerging fungal pathogen? Science **323**(5911): 227–227. doi:10.1126/science.1163874.

Bohonak, A.J. 1999. Dispersal, gene flow, and population structure. Q. Rev. Biol. **74**(1): 21–45. doi:10.1086/392950.

Booth, W., Balvín, O., Vargo, E.L., Vilímová, J., and Schal, C. 2015. Host association drives genetic divergence in the bed bug, *Cimex lectularius*. Mol. Ecol. **24**(5): 980–992. doi:10.1111/mec.13086.

Booth, W., Saenz, V.L., Santangelo, R.G., Wang, C., Schal, C., and Vargo, E.L. 2012. Molecular markers reveal infestation dynamics of the bed bug (Hemiptera: Cimicidae) within apartment buildings. J. Med. Entomol. **49**(3): 535–546. doi:10.1603/ME11256.

Cooper, R., Wang, C., and Singh, N. 2015. Mark-release-recapture reveals extensive movement of bed bugs (*Cimex lectularius* L.) within and between apartments. PLOS ONE **10**(9): e0136462. doi:10.1371/journal.pone.0136462.

Criado, P.R., Belda Junior, W., Criado, R.F.J., Vasconcelos e Silva, R., and Vasconcellos, C. 2011. Bedbugs (Cimicidae infestation): the worldwide renaissance of an old partner of human kind. Braz. J. Infect. Dis. **15**(1): 74–80. doi:10.1590/S1413-86702011000100014.

Davies, T.G.E., Field, L.M., and Williamson, M.S. 2012. The re-emergence of the bed bug as a nuisance pest: implications of resistance to the pyrethroid insecticides. Med. Vet. Entomol. **26**(3): 241–254. doi:10.1111/j.1365-2915.2011.01006.x.

Ellison, L.E., O'Shea, T.J., Neubaum, D.J., and Bowen, R.A. 2007. Factors influencing movement probabilities of big brown bats (*Eptesicus fuscus*) in buildings. Ecol. Appl. Publ. Ecol. Soc. Am. **17**(2): 620–627. doi:10.1890/06-0315.

Fields Development Team. 2006. Fields: tools for spatial data. National Center for Atmospheric Research, Boulder, CO, USA.

Fountain, T., Duvaux, L., Horsburgh, G., Reinhardt, K., and Butlin, R.K. 2014. Human-facilitated metapopulation dynamics in an emerging pest species, *Cimex lectularius*. Mol. Ecol. **23**(5): 1071–1084. doi:10.1111/mec.12673.

Furlonger, C.L., Dewar, H.J., and Fenton, M.B. 1987. Habitat use by foraging insectivorous bats. Can. J. Zool. **65**(2): 284–288. doi:10.1139/z87-044.

Gandon, S., and Nuismer, S.L. 2009. Interactions between genetic drift, gene flow, and selection mosaics drive parasite local adaptation. Am. Nat. **173**(2): 212–224. doi:10.1086/593706.

Garant, D., Forde, S.E., and Hendry, A.P. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. Funct. Ecol. **21**(3): 434–443. doi:10.1111/j.1365-2435.2006.01228.x.

Goddard, J. 2009. Bed bugs (*Cimex lectularius*) and clinical consequences of their bites. J. Am. Med. Assoc. **301**(13): 1358–1366. doi:10.1001/jama.2009.405.

Goddard, J., Baker, G.T., Ferrari, F.G., and Ferrari, C. 2012. Bed bugs (*Cimex Lectularius*) and bat bugs (several *Cimex* species): a confusing issue. Outlooks Pest Manag. **23**(3): 125–127. doi:10.1564/23jun09.

Goldberg, C.S., and Waits, L.P. 2010. Quantification and reduction of bias from sampling larvae to infer population and landscape genetic structure. Mol. Ecol. Resour. **10**(2): 304–313. doi:10.1111/j.1755-0998.2009.02755.x.

Goslee, S.C., and Urban, D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Softw. **22**(7). doi:10.18637/jss.v022.i07.

Guillot, G., Mortier, F., and Estoup, A. 2005. Geneland: a computer package for landscape genetics. Mol. Ecol. Notes **5**(3): 712–715. doi:10.1111/j.1471-8286.2005.01031.x.

Hanski, I. 1998. Metapopulation dynamics. Nature **396**(5): 41–49. doi:10.1111/j.1095-8312.1991.tb00548.x.

Hardy, O.J., and Vekemans, X. 2002. Spagedi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. Mol. Ecol. Notes **2**(4): 618–620. doi:10.1046/j.1471-8286.2002.00305.x.

513 Harrison, S. 1991. Local extinction in a metapopulation context: an empirical
514 evaluation. Biol. J. Linn. Soc. **42**(1–2): 73–88. doi:10.1111/j.1095-
515 8312.1991.tb00552.x.
516 Hutchison, D.W., and Templeton, A.R. 1999. Correlation of pairwise genetic and
517 geographic distance measures: inferring the relative influences of gene flow
518 and drift on the distribution of genetic variability. Evolution **53**(6): 1898.
519 doi:10.2307/2640449.
520 Kalinowski, S.T., Wagner, A.P., and Taper, M.L. 2006. ML-Relate: a computer
521 program for maximum likelihood estimation of relatedness and relationship.
522 Mol. Ecol. Notes **6**(2): 576–579. doi:10.1111/j.1471-8286.2006.01256.x.
523 Legendre, P., Lapointe, F.-J., and Casgrain, P. 1994. Modeling brain evolution from
524 behavior: a permutational regression approach. Evolution **48**(5): 1487.
525 doi:10.2307/2410243.
526 Lichstein, J.W. 2007. Multiple regression on distance matrices: a multivariate spatial
527 analysis tool. Plant Ecol. **188**(2): 117–131. doi:10.1007/s11258-006-9126-3.
528 Mayer, C., Schiegg, K., and Pasinelli, G. 2009. Patchy population structure in a short-
529 distance migrant: evidence from genetic and demographic data. Mol. Ecol.
530 **18**(11): 2353–2364. doi:10.1111/j.1365-294X.2009.04200.x.
531 McCoy, K.D., Boulinier, T., Tirard, C., and Michalakis, Y. 2003. Host-dependant
532 genetic structure of parasite populations: differential dispersal of seabird tick
533 host races. Evolution **57**(2): 288–296. doi:10.1111/j.0014-
534 3820.2003.tb00263.x.
535 Meirmans, P.G. 2012. AMOVA-based clustering of population genetic data. J. Hered.
536 **103**(5): 744–750. doi:10.1093/jhered/ess047.
537 Meirmans, P.G., and Hedrick, P.W. 2011. Assessing population structure: F_{ST} and
538 related measures. Mol. Ecol. Resour. **11**(1): 5–18. doi:10.1111/j.1755-
539 0998.2010.02927.x.
540 Mimet, A., Houet, T., Julliard, R., and Simon, L. 2013. Assessing functional
541 connectivity: a landscape approach for handling multiple ecological
542 requirements. Methods Ecol. Evol. **4**(5): 453–463. doi:10.1111/2041-
543 210x.12024.
544 Neel, M.C. 2008. Patch connectivity and genetic diversity conservation in the
545 federally endangered and narrowly endemic plant species *Astragalus albens*
546 (Fabaceae). Biol. Conserv. **141**(4): 938–955.
547 doi:10.1016/j.biocon.2007.12.031.
548 Norquay, K.J.O., Martinez-Nuñez, F., Dubois, J.E., Monson, K.M., and Willis, C.K.R.
549 2013. Long-distance movements of little brown bats (*Myotis lucifugus*). J.
550 Mammal. **94**(2): 506–515. doi:10.1644/12-MAMM-A-065.1.
551 Pearce, R.D., and O'Shea, T.J. 2007. Ectoparasites in an urban population of big
552 brown bats (*Eptesicus fuscus*) in Colorado. J. Parasitol. **93**(3): 518–530.
553 doi:10.1645/GE-973R.1.
554 Penczykowski, R.M., Laine, A.-L., and Koskella, B. 2016. Understanding the ecology
555 and evolution of host-parasite interactions across scales. Evol. Appl. **9**(1):
556 37–52. doi:10.1111/eva.12294.
557 Prunier, J.G., Kaufmann, B., Fenet, S., Picard, D., Pompanon, F., Joly, P., and Lena, J.P.
558 2013. Optimizing the trade-off between spatial and genetic sampling efforts

in patchy populations: towards a better assessment of functional connectivity using an individual-based sampling scheme. Mol. Ecol. **22**(22): 5516–5530. doi:10.1111/mec.12499.

Rasic, G., and Keyghobadi, N. 2012. The pitcher plant flesh fly exhibits a mixture of patchy and metapopulation attributes. J. Hered. **103**(5): 703–710. doi:10.1093/jhered/ess040.

Raymond, M., and Rousset, F. 1994. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. J. Hered. **86**: 248–249. doi:10.1093/oxfordjournals.jhered.a111573.

Roberts, B.J., Catterall, C.P., Eby, P., and Kanowski, J. 2012. Long-distance and frequent movements of the flying-fox *Pteropus poliocephalus*: implications for management. PLoS ONE **7**(8): e42532. doi:10.1371/journal.pone.0042532.

Saenz, V.L., Booth, W., Schal, C., and Vargo, E.L. 2012. Genetic analysis of bed bug populations reveals small propagule size within individual infestations but high genetic diversity across infestations from the eastern United States. J. Med. Entomol. **49**(4): 865–875. doi:10.1603/ME11202.

Storfer, A., Murphy, M.A., Evans, J.S., Goldberg, C.S., Robinson, S., Spear, S.F., Dezzani, R., Delmelle, E., Vierling, L., and Waits, L.P. 2007. Putting the "landscape" in landscape genetics. Heredity **98**(3): 128–142. doi:10.1038/sj.hdy.6800917.

Swenson, N.G., and Howard, D.J. 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. Am. Nat. **166**(5): 581–591. doi:10.1086/491688.

Talbot, B., Vonhof, M.J., Broders, H.G., Fenton, B., and Keyghobadi, N. 2016. Range-wide genetic structure and demographic history in the bat ectoparasite *Cimex adjunctus*. BMC Evol. Biol. **16**(1). doi:10.1186/s12862-016-0839-1.

Usinger, R.L. 1966. Monograph of Cimicidae (Hemiptera, Heteroptera). Entomological Society of America, Annapolis, MD, USA.

Vander Wal, E., Garant, D., Calmé, S., Chapman, C.A., Festa-Bianchet, M., Millien, V., Rioux-Paquette, S., and Pelletier, F. 2014*a*. Applying evolutionary concepts to wildlife disease ecology and management. Evol. Appl. **7**(7): 856–868. doi:10.1111/eva.12168.

Vander Wal, E., Garant, D., and Pelletier, F. 2014*b*. Evolutionary perspectives on wildlife disease: concepts and applications. Evol. Appl. **7**(7): 715–722. doi:10.1111/eva.12179.

Wang, J. 2002. An estimator for pairwise relatedness using molecular markers. Genetics **160**(3): 1203–1215. Available from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1462003/ [Accessed 18 Feb 2016].

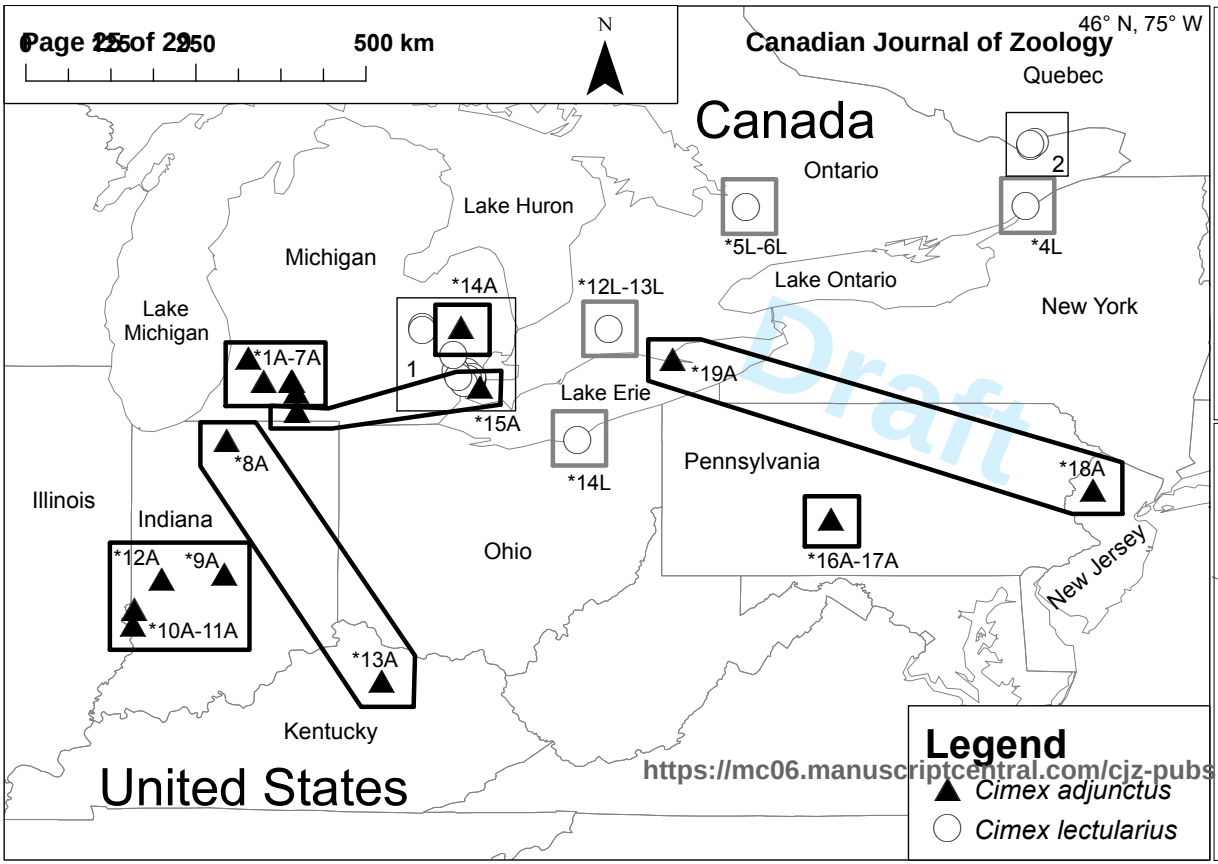
Wang, J. 2011. Unbiased relatedness estimation in structured populations. Genetics **187**(3): 887–901. doi:10.1534/genetics.110.124438.

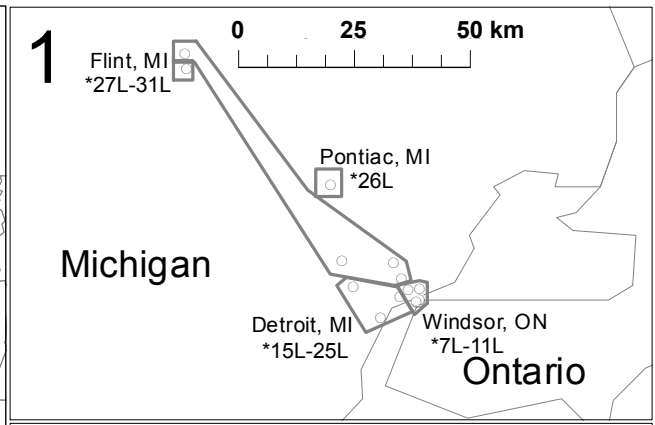
Weller, T.J., Castle, K.T., Liechti, F., Hein, C.D., Schirmacher, M.R., and Cryan, P.M. 2016. First direct evidence of long-distance seasonal movements and hibernation in a migratory bat. Sci. Rep. **6**(1). doi:10.1038/srep34585.

604 **Figure legends**

605
606 **Figure 1.** Study area showing locations of 75 *Cimex adjunctus* individuals collected from
607 bats and bat roosts (black triangles), and 73 *Cimex lectularius* individuals collected in
608 infested housing units (white circles), collected in the Great Lakes region of North
609 America, with two close-ups around the region of Detroit, MI, and Ottawa, ON. Black
610 buffers in *C. adjunctus* and grey buffers in *C. lectularius* represent genetic clusters for
611 both species. Numbers preceded by an asterisk on the map correspond to site numbers in
612 Table 2, Appendix A1 and Appendix A2, where numbers followed by an A correspond to
613 *C. adjunctus* and numbers followed by an L to *C. lectularius*.
614
615
616

Canadian Journal of Zoology





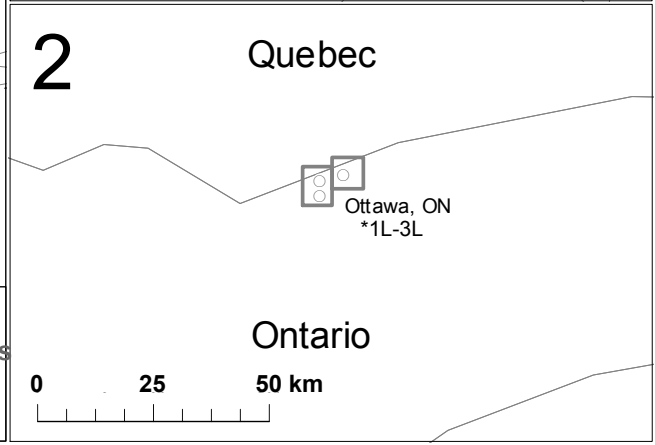


Table 1. Expected population structure characteristics based on three models on which we based our study on *Cimex adjunctus* and *Cimex lectularius*: patchy population, classic metapopulation and non-equilibrium metapopulations (based on Mayer et al. 2009).

Population structure parameters	**Patchy population model**	**Classic metapopulation model**	**Non-equilibrium metapopulation model**
Level of differentiation	None	Moderate	High
Presence of isolation-by-distance	No	Yes	No
Number of genetic clusters	One	Lower than number of subpopulations	Similar to number of subpopulations

Table 2. Genetic diversity estimates for sites with at least three sampled individuals in *Cimex adjunctus* (numbers followed by A) and *Cimex lectularius* (numbers followed by L) in the Great Lakes region of North America, averaged across seven microsatellite markers. Site numbers correspond to those in Fig. 1, Appendix A1 and Appendix A2.

Site	Average number of alleles	Observed heterozygosity	Expected heterozygosity	Inbreeding coefficient G_{IS}
1A	2.286	0.357	0.354	-0.009
2A	1.857	0.190	0.423	0.549
3A	2.143	0.119	0.467	0.745
10A	1.800	0.200	0.500	0.600
14A	2.429	0.327	0.420	0.223
15A	3.571	0.248	0.393	0.369
16A	1.714	0.190	0.333	0.429
17A	1.714	0.286	0.429	0.333
C. adjunctus mean	2.189	0.240	0.415	0.405
1L	2.833	0.667	0.625	-0.067
2L	2.000	0.429	0.382	-0.121
3L	2.571	0.405	0.393	-0.030
4L	1.286	0.190	0.114	-0.667
5L	1.286	0.143	0.167	0.143
7L	2.000	0.381	0.500	0.238
9L	1.857	0.429	0.333	-0.286
11L	2.286	0.619	0.583	-0.061
14L	1.714	0.333	0.298	-0.120
15L	2.000	0.167	0.219	0.239
21L	2.286	0.429	0.440	0.027
23L	2.143	0.286	0.452	0.368
27L	1.143	0.095	0.071	-0.333
C. lectularius mean	1.954	0.352	0.352	-0.052

Table 3. Spatial genetic structure in *Cimex adjunctus* and in *Cimex lectularius* in the Great Lakes region of North America, estimated using microsatellite markers. Most likely K = the number of genetic clusters estimated using the GeneLand method, IBD (r_W) = isolation-by-distance based on pairwise relatedness values, and IBD (r_W) + K = isolation-by-distance while correcting for population genetic structure.

Species		***Cimex adjunctus***	***Cimex lectularius***
Number of markers		7	7
Sample size		75	73
Most likely K		7	11
IBD (r_W)	P	0.165	0.001*
	R^2	< 0.01	0.09
IBD (r_W) + K	P (IBD)	0.358	0.001*
	P (K)	0.001*	0.001*
	R^2	0.04	0.22

*Statistically significant at α = 0.05

Table 4. Genetic diversity within and differentiation among sites with at least three sampled *Cimex adjunctus* and *Cimex lectularius*, in the Great Lakes region of North America, estimated using microsatellite markers. H_S = average expected heterozygosity, Hedrick's G'_{ST} = Hedrick's global G'_{ST} differentiation index (and 95% confidence intervals), and AMOVA = Analysis of Molecular Variance.

Species		***Cimex adjunctus***	***Cimex lectularius***
Number of markers		7	7
Sample size		60	50
H_S		0.389 (0.309 – 0.469)	0.351 (0.292 – 0.410)
Hedrick's G'_{ST}		0.233 (0.133 – 0.333)	0.750 (0.683 – 0.817)
AMOVA (% of variation)	Within sites	66.7	42.1
	Among sites	33.3	57.9