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Population Genetic Structure in German Cockroaches (*Blattella Germanica*): Differentiated Islands in an Agricultural Landscape

Warren Booth, Richard G. Santangelo, Edward L. Vargo, Dmitry V. Mukha, and Coby Schal

From the Department of Entomology and W. M. Keck Center for Behavioral Biology, North Carolina State University, Campus Box 7613, Raleigh, NC 27695 (Booth, Santangelo, Vargo, and Schal); and the Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow 119991, Russia (Mukha).

Address correspondence to Dr Warren Booth at the address above, or e-mail: wbooth@ncsu.edu.

Abstract

Although a number of species live syanthropically with humans, few rely entirely on humans for their survival and distribution. Unlike other cosmopolitan human commensals, the German cockroach (*Blattella germanica*), an insect of both public and livestock health concern, is considered incapable of dispersal outside human dwellings. Patterns of genetic association are therefore constrained and may not be associated with distance. Analogies with other human-commensal species are therefore impossible to draw with any degree of accuracy. In the past 2 decades, *B. germanica* has become a prominent pest within the US swine production system. Swine production is mainly carried out through contracted producers, each associated with a management company. It has been hypothesized that cockroach populations will be genetically structured based on association to a specific management company. Here, we tested this hypothesis using microsatellite genotypes (8 polymorphic loci) from 626 individual cockroaches collected from 22 farms in southeastern North Carolina representing 3 management companies. Significant genetic differentiation was detected ($F_{ST} = 0.171$), most of which was partitioned among the 22 farms rather than the 3 management groups. All pair-wise population comparisons yielded F_{ST} values significantly greater than zero. Our results reveal that structure does not correspond to management company of origin, but instead it may be regional and influenced strongly by the unintentional movement of cockroaches by farm workers.

Key words: Blattella germanica, *commensal, Genetic bottleneck, human-mediated dispersal, microsatellite DNA profiling, population structure*

The determination of patterns of dispersal and gene flow in organisms of public and livestock health concern are essential for the development of effective management strategies capable of mitigating disease spread and illness in urban and agricultural communities (Hampton et al. 2004). Gene flow between populations may be strongly influenced by both the dispersal ability of the organism and the physical landscape within which populations are distributed (Colautti et al. 2005; Therriault et al. 2005; Booth et al. 2009). Such factors therefore have the potential to significantly impede or promote a population's evolutionary and adaptive ability (Garant et al. 2007). In the absence of contiguous suitable habitat through which active dispersal can occur, population differentiation may be strongly driven by passive movement in the form of human-mediated dispersal. However, few studies document the impact of this dispersal mechanism on population genetic structure in species for which it is essentially the only means of interpopulation movement.

The German cockroach, *Blattella germanica*, represents an ideal species to study genetic diversity and connectivity among geographically separate populations linked solely by human-mediated dispersal. Recognized globally as a prominent household pest of medical, veterinary, and economic significance (Schal and Hamilton 1990; Brenner 1995; Rosenstreich et al. 1997; Gore and Schal 2007), this species exhibits a relatively unique behavior of strict human commensalism (Roth 1985; Cloarec et al. 1999; Jobet et al. 2000; Mukha et al. 2007). The dependency on humans is such that populations are not known to persist outside human-built structures (Roth 1985). The effect of this unique behavior on genetic diversity and population

differentiation in the urban environment has been addressed in a number of studies employing molecular markers. Potentially resulting from the low resolution of markers employed, early studies failed to clarify the scale at which genetic structure exists within the urban environment (Hampson and Steiner 1982; Cloarec et al. 1999; Jobet et al. 2000). Recently, however, utilizing a set of highly polymorphic microsatellite loci Crissman et al. (2010) identified genetic structure within individual apartment buildings. After factorial correspondence analysis, within apartment aggregations appeared visually to cluster to a higher degree with those of the same aggregation than to others. When considered in light of the highly gregarious nature of this species (Amé et al. 2006), its reported refuge fidelity (Denzer et al. 1988), and its tendency to utilize the most closely available food resource (Bret and Ross 1985; Silverman 1986; Rivault and Cloarec 1991; Durier and Rivault 2001), these observations strongly support a meta-population structure within buildings. Outside the building level, patterns of differentiation suggest that active dispersal is insignificant and thus dispersal is largely or solely human mediated.

Within the agricultural environment, specifically the factory-style swine production systems of the United States, *B. germanica* has emerged as a prominent pest over the past 2 decades (Gore et al. 2004). This has coincided with the movement toward artificial confinement production, a system that provides ideal conditions for the rapid growth of *B. germanica* populations (Waldvogel et al. 1999; Zurek et al. 2003; Gore et al. 2004). Under such conditions, it is not uncommon for densities exceeding 25 000 individuals per 250 m^2 farrowing room to be observed during a daytime 15-min transect count (Waldvogel et al. 1999; Zurek et al. 2003). Within this industry, animal production is highly "vertically integrated"—it is primarily carried out by contracted producers, each associated with one of only a few management companies who in turn provide both animals and farm supplies from company-specific central locations. Two potential barriers exist to the dispersal, whether active or passive, of *B. germanica* within this system. First, the location of farms within rural areas, each separated by open land, presents a landscape over which active dispersal is highly improbable (Jobet et al. 2000). Second, human movement within and between farms is strictly monitored due to bio-security concerns, with regimens implemented to prevent the movement of diseases and their vectors into and between farms. As a result, human-mediated passive movement of cockroaches into or out of farms is expected to be minimal. Results of a recent study by Mukha et al. (2007) suggest that although significant genetic differentiation exists between farms, those under the same management company share a higher level of genetic similarity. Although preliminary in nature and based on data collected from only 3 farms, we hypothesized that cockroaches may be moved unintentionally, possibly via supply trucks, between farms under a specific company's contract. This system therefore represents an ideal scenario under which to test the effect of human-mediated dispersal within a landscape through which active dispersal is prohibitive.

Using high-resolution microsatellite markers, we aim to determine levels of genetic diversity and connectivity within and between the farms of 3 major swine production companies within North Carolina. Specifically, we test 2 hypotheses presented by Mukha et al. (2007): 1) that human-mediated dispersal of cockroaches, as a result of strict bio-security measures, known transport pathways connecting producers, and a landscape unsuitable for active dispersal, is largely limited to farms within a company's contract, and 2) that local cockroach populations experience strong bottlenecks as a result of intermittent high mortality, mainly from insecticides.

Materials and Methods

Sample Collection

Collections of *B. germanica* were made from a total of 22 commercial swine farms located within the southeastern region of North Carolina, USA (Figure 1). Collections were made on farms under contract to 3 different management companies referred to here as X group ($n = 6$), Y group ($n = 12$), and Z group ($n = 4$). Collection occurred during daytime from aggregations found along walls, under mats, and around door frames.

DNA Extraction and Microsatellite Genotyping

Total genomic DNA was extracted from 25 to 30 adult *B. germanica* from each farm using the PUREGENE DNA isolation kit (Gentra Systems Inc., Minneapolis, MN). Samples were screened at 8 polymorphic microsatellite loci (*Bg*-G7, *Bg*-B12, *Bg*-1D5, *Bg*-D05, *Bg*-A7, *Bg*-D9, *Bg*-F7, and *Bg*-wb-2A) previously described by Booth et al. (2007). Polymerase chain reaction (PCR) conditions followed those outlined by the authors. Amplified products were labeled with M13F-29 IRDye infrared tags (LI-COR Biosciences, Lincoln, NE). PCR products were separated by electrophoresis on 6% polyacrylamide gels run on a LI-COR 4300 automated sequencer. An IRD-labeled size standard (MicroStep- 20a, Microzone, UK) was run every 15 samples to assist the sizing of allelic fragments. At least one control sample (i.e., a sample of known genotype) was included in each run to ensure accuracy and consistency of typing among different gels. The GeneProfiler (v4.05) software (Scanalytics, Rockville, MD) was used to collect genotypic data from the LI-COR system.

Genetic Data Analysis

Summary population statistics (allelic diversity, expected, and observed heterozygosity) and tests for departures from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were calculated using GENEPOP v4.0 (Raymond and Rousset 1995; Rousset 2008). The Markov chain parameters were set to 2000 dememorizations, 200 batches, and 2000 iterations per batch. MICRO-CHECKER v2.2.3 software (Van Oosterhout et al. 2004) was used to assess the likelihood that null alleles, scoring errors, or large allele dropout were evident at any locus screened.

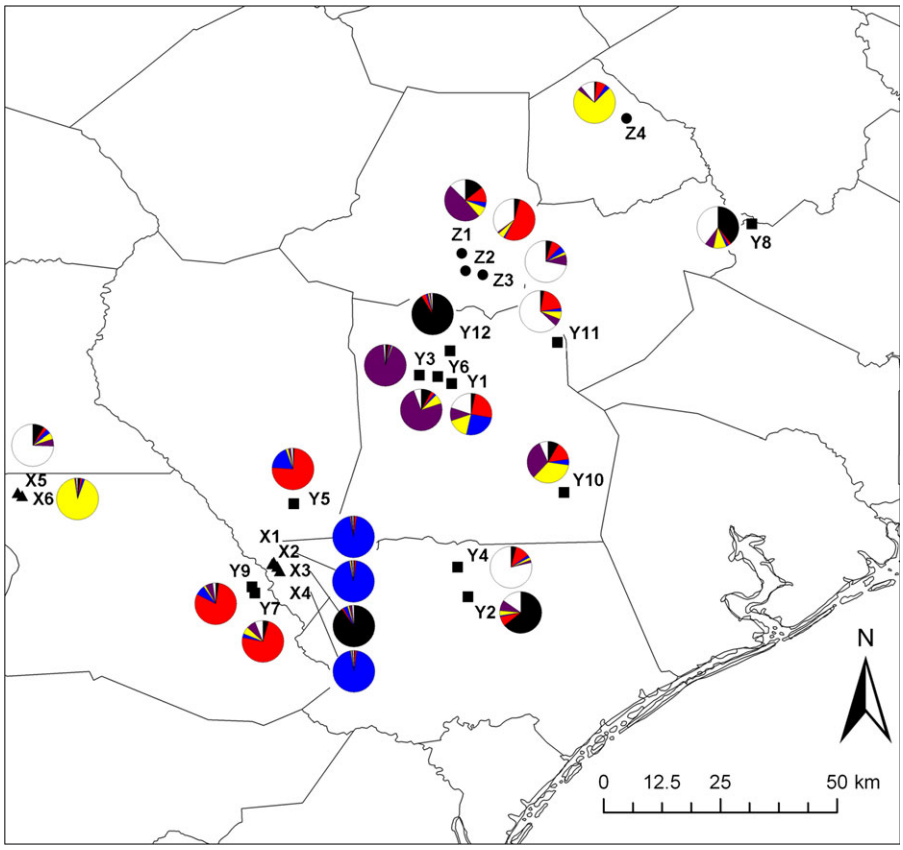

Figure 1. Results of Bayesian structure analysis, showing proportion membership of each of 22 population samples to 6 genetic groups. Sampling site locations are shown within eastern North Carolina, USA. Colored pies represent proportion membership of each population to 6 genetic groups ($K = 6$) identified in STRUCTURE. Results represent 5 sequential runs aligned using CLUMPP.

Evidence for departures from panmixia was assessed among samples using a number of independent approaches. Pairwise genotypic differentiation was tested using the log-likelihood based *G*-test (Goudet and Raymond 1996), implemented in GENEPOP. The Markov chain parameters were set to 2000 dememorizations, 200 batches, and 2000 iterations per batch. Departures from panmixia can be used to group samples into populations after the method proposed by Waples and Gaggiotti (2006), whereby samples are considered to be part of the same population when they could be connected to any other sample by a nonsignificant *G*-test. Genetic differentiation, based on F_{ST} (Weir and Cockerham 1984), both overall and between each population pair, was then estimated using FSTAT v2.9.3.2 (Goudet 2001). Significance of F_{ST} values was assessed by permutation.

The Bayesian clustering algorithm implemented in STRUCTURE v2.2.3 (Pritchard et al. 2000) was applied as an exploratory analysis to determine whether the 22 population samples could be subdivided into K clusters (were K is unknown), with no a priori assumption of population structure. Under this method, individuals are probabilistically assigned to each cluster based on the proportion of their genome that matches that cluster. STRUCTURE analysis was performed assuming the admixture model with allele frequencies correlated. Runs were based on 200 000 iterations after a 50 000 burn-in period of the Markov chain with K set from 1 to 22, replicated 5 times to check concordance of the data. The optimal value of K was calculated after the ΔK method described by Evanno et al. (2005) using the STRUCTURE HARVESTER v0.56.3 software (Earl 2009). Replicated STRUCTURE runs were aligned using the program CLUMPP (Jakobsson and Rosenberg 2007) in order to maximize each individual's membership across clusters.

In order to assess the pattern of genetic relatedness between/among the 22 population samples, an unrooted neighbor joining (NJ) tree based on the chord distance of Cavallis-Sforza and Edwards (1967) was generated using the program MICROSAT (Minch et al. 1995). This distance was chosen as it has been shown to be relatively insensitive to fluctuations in population size and mutation model and was observed to perform best when reconstructing intraspecific tree topologies based on microsatellite data (Takezaki and Nei 1996). The 5075 pseudoreplicate distance matrices generated were subjected to cluster analysis using NEIGHBOR in Phylip v3.573 (Felsenstein 1995), implementing sample randomization to construct dendrograms. The consensus tree was obtained using CONSENSE within Phylip with the

reliability of tree nodes evaluated by bootstrap analysis (Felsenstein 1985).

Analysis of molecular variance (AMOVA), performed using the ARLEQUIN V3.01 software (Excoffier et al. 2005), was used to test the hypothesis that management company association represents a barrier to gene flow between farms. We predicted that if management company association represented a barrier to gene flow or constrained gene flow to within-company movement, a greater proportion of the observed variation would be due to this company association grouping than that resulting from sampling sites alone. Finally, regression analysis was performed using Mantel's randomization test (Mantel 1967) to determine if a significant relationship existed between pairwise F_{ST} values and geographic distance. Geographic distance, estimated as both the shortest road distance and the straight-line Euclidian distance between each pair of farms, was log-transformed, and genetic distances linearized to $F_{ST}/1 - F_{ST}$. Analysis was performed using MANTEL v2 (Liedloff 1999) employing a total of 10 000 permutations. Analysis was run for each management company separately and for all samples combined.

In order to test the hypothesis that populations experience genetic bottlenecks as a result of intermittent pest control with insecticides, mean allelic richness (A_r) was calculated per farm after the rarefaction approach implemented in the FSTAT v2.9.3.2 (Goudet 2001). Under this approach, the mean allelic richness is calculated based on the smallest sample size, thus eliminating a sample size effect. Farms under the 3 management companies were divided into 2 groups. One group (X-group farms) where intensive insecticide applications were implemented by "in-house" company-employed pest control specialists, and a second group (combined Y- and Z-groups) consisting of much less intensive pest control implemented sporadically by farm workers. An analysis of variance (ANOVA) was then performed to determine if significant differences in A_r existed between the 2 groups. Finally, the program BOTTLENECK v1.2.02 (Cornuet and Luikart 1996) was employed to test for evidence of recent genetic bottlenecks or expansions. Both the Wilcoxon sign-rank test and the quantitative mode-shift analyses were performed. Given that microsatellite loci may differ in both the mode and rate of evolution (Di Rienzo et al. 1994), with allelic variation likely to follow mainly one-step mutations with a small percentage of multistep changes (Luikart et al. 1998), analysis was performed assuming a two-phase mutation model with a mix of 70:30 stepwise mutation model:infinite allele model and 30% variance.

Results

Summary Population Statistics

Unambiguous genotypes at 8 microsatellite loci were determined for a total of 626 *B. germanica* (mean = 619.63 per locus) over the 22 population samples representing 3 management companies. Given the nature of population establishment of *B. germanica*, allelic diversity and observed heterozygosity appeared moderate (allelic diversity range 3.50–8.13; observed heterozygosity range 0.487–0.699) (Table 1). After Bonferroni correction (Rice 1989), 5 populations deviated significantly from HWE (Table 1). Where deviations occurred, these resulted from a deficit of heterozygotes at loci *Bg*-1D5 and/or *Bg*-D9. MICRO-CHECKER identified these loci as potentially exhibiting null alleles; however, given that both loci exhibited large numbers of alleles, it was likely that the deficit of heterozygotes actually resulted from a sampling error (i.e., insufficient sample number). Therefore, these loci were not excluded from further analysis. After Bonferroni correction, no consistent evidence for linkage disequilibrium was detected between pairs of loci within populations.

Genetic Differentiation

Significant population differentiation was detected among *B. germanica* populations spanning 3 management companies, based on Weir and Cockerham's θ. Overall F_{ST} was 0.171 (95% confidence interval: lower 0.147, upper 0.195) after bootstrap analysis across loci. Pairwise values ranged from 0.033 (Y-group farm pair) to 0.377 (X-group farm pair). All pairwise comparisons proved significant after 7020 permutations with adjustment for multiple comparisons. Significant *G*-tests between all pairs of populations confirmed this result. The unrooted NJ tree constructed based on Cavalli-Sforza and Edwards' chord distance, in general, did not support the hypothesis that populations linked under management company association were more genetically related than to those under different management companies. Although weakly supported, the tree topology did suggest that structure might exist based on geographic location (Figure 2). Results from Structure supported the above finding, with the peak distribution of ΔK (value = 228) found at $K = 6$. Congruent with the NJ tree, structure analysis suggested that population associations might exist based on geographic location (Figure 1).

Contrary to hypothesis one, AMOVA analysis revealed that the variation component among groups (management companies) was nonsignificant (0.90%, $P = 0.0844$; Table 2). The variation component both among populations within groups and within populations was highly significant (16.03%, $P \leq 0.001$; 83.07%, $P \leq 0.001$, respectively). With the exception of X-group farms ($P = 0.015$, $r^2 = 0.372$), no pattern of isolation by distance was supported with a significant *P* value when calculated based on shortest road distance between samples or straight-line Euclidian distance.

Allelic Richness and Bottleneck Tests

Mean allelic richness per farm ranged from 3.39 to 7.18 (Table 1). When farms were grouped based on control strategy, mean allelic richness was 4.19 (standard error [SE] = 0.32) and 5.40 (SE = 0.19), for intensive (X group) and nonintensive (Y group and Z group) pest control, respectively. After ANOVA, these means were determined to be significantly different ($P = 0.004$).

Table 1 Summary statistics for German cockroach (*Blattella germanica*) population samples from 22 farms, representing 3 management companies, collected within North Carolina, USA, screened at 8 microsatellite loci

Farm population	Sample size	N_a	A_r	H_E	H_O	HWE	*P* (He)	*P* (Hd)	Mode shift	F_{ST} (95% confidence interval)
X1	30	3.88	3.73	0.601	0.570	NS	**0.002**	1.000	Shifted	
X2	30	3.50	3.39	0.581	0.615	NS	**0.006**	0.996	Normal	
X3	30	5.00	4.67	0.674	0.646	NS	**0.010**	0.994	Normal	
X4	30	3.88	3.65	0.539	0.529	NS	0.273	0.769	Normal	
X5	29	5.25	4.84	0.637	0.616	NS	0.422	0.630	Normal	
X6	29	5.25	4.87	0.557	0.576	NS	0.727	0.320	Normal	
Within X management company	29.33	4.46	4.19	0.598	0.592					0.226 (0.161, 0.293)
Y1	26	5.88	5.61	0.726	0.636	*Null	**0.002**	1.000	Normal	
Y2	26	5.38	5.23	0.629	0.577	*Null	0.273	0.769	Normal	
Y3	30	5.75	5.11	0.649	0.634	NS_{null}	0.320	0.726	Normal	
Y4	26	5.50	5.20	0.684	0.666	NS	**0.027**	0.980	Normal	
Y5	30	6.00	5.45	0.706	0.629	NS_{null}	0.230	0.808	Normal	
Y6	26	7.13	6.56	0.686	0.699	NS	0.527	0.527	Normal	
Y7	30	6.00	5.57	0.707	0.672	NS	0.125	0.902	Normal	
Y8	25	3.88	3.77	0.613	0.487	NS_{null}	**0.002**	1.000	Shifted	
Y9	30	6.13	5.52	0.697	0.694	NS	0.230	0.808	Normal	
Y10	26	4.88	4.65	0.669	0.675	NS	**0.009**	0.994	Normal	
Y11	29	7.13	6.36	0.714	0.674	*Null	0.421	0.629	Normal	
Y12	28	4.63	4.39	0.697	0.679	NS	**0.009**	0.994	Shifted	
Within Y management company	27.4	5.69	5.28	0.681	0.643					0.140 (0.120, 0.157)
Z1	30	8.13	7.18	0.749	0.654	*Null	0.628	0.421	Normal	
Z2	30	5.75	5.11	0.629	0.592	NS	0.628	0.421	Normal	
Z3	30	5.75	5.52	0.717	0.646	*Null	**0.013**	0.990	Normal	
Z4	26	5.63	5.16	0.588	0.618	NS	0.980	**0.027**	Normal	
Within Z management company	28.72	6.31	5.74	0.671	0.627					0.148 (0.107, 0.190)
Overall mean	28.16	5.47	5.07	0.657	0.626					0.171 (0.147, 0.195)

N_a, number of alleles; A_r, allelic richness; H_E, expected heterozygosity; H_O, observed heterozygosity; HWE, * indicates deviation from HWE ($P = <0.01$); NS, nonsignificant; null indicates the potential presence of null alleles; *P*(He) represents the probability of the occurrence of a genetic bottleneck under the two-phase model (TPM); and *P* (Hd) represents the probability of detecting a genetic expansion under the TPM. Significant deviation ($P < 0.05$) from equilibrium expectations in bold.

Results from the Wilcoxon test implemented in BOTTLENECK revealed significant deviations from mutation-drift equilibrium within 9 populations spanning all management companies, resulting from an excess of heterozygosity (Table 1). Evidence of recent reductions in genetic diversity was further supported by the mode-shift qualitative test. A shifted (i.e., collapsed) allelic frequency distribution, characterized by poor representation of low-frequency allelic classes, was detected within 3 populations (X1, Y8, Y12). A significant deficit of heterozygosity was detected within a single population (Z4), suggesting a recent population expansion event.

Discussion

The objectives of this research were 2-fold. First, we aimed to test a hypothesis put forward earlier by our group (Mukha et al. 2007) that as a result of strict human commensalism, the implementation of stringent bio-security practices, and a landscape of unsuitable habitat for active dispersal, cockroach populations occupying farms under a given management company will be genetically more similar to each other than to those under different companies. This pattern would arise as a result of the inadvertent movement of cockroaches on supply trucks between farms under the same management company. Then second, to test the hypothesis that populations infesting farms implementing more stringent cockroach control with insecticides would exhibit the genetic signatures of a reduction in allelic diversity and/or bottlenecks.

Determinants of Genetic Structure

The involuntary movement of *B. germanica* along supply chains has been the subject of numerous anecdotal reports and law suits. Despite this, studies documenting this form of dispersal are rare. Mallis et al. (1961) described the passive movement of cockroaches in supply boxes along a manufacturer to customer chain. Studies investigating the genetic association of dispersers to putative source populations are, however, absent. Our findings, obtained through analysis of highly polymorphic microsatellite markers, provide a somewhat contradictory result to that of Mukha et al. (2007) and

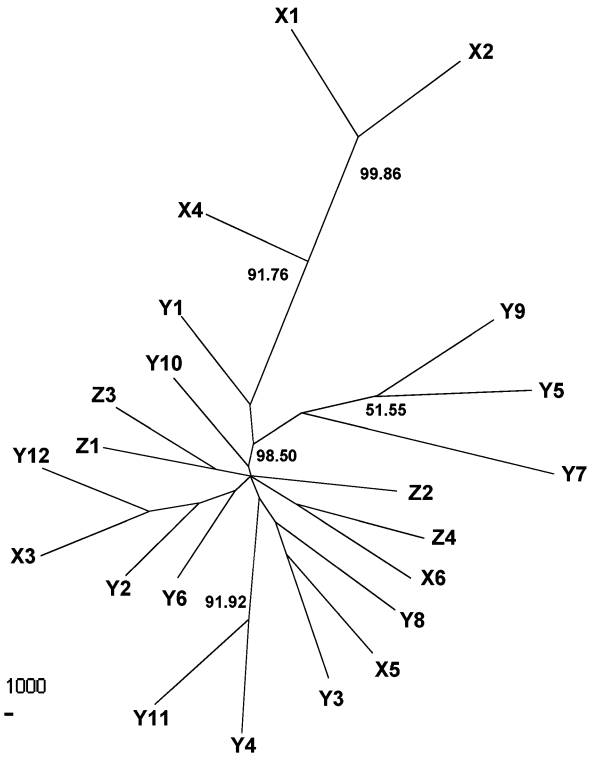

Figure 2. The NJ tree based on genetic distance calculated as chord distance at 8 microsatellite loci for 22 population samples of *Blattella germanica*. Values on nodes represent percentage bootstrap support after 1000 replicates. Only values more than 50% are shown.

give a unique insight into the genetic variability and population structure of this human-mediated pest within an economically important agricultural environment. Although we find some evidence of shared ancestry between farms under the same management company, no conclusive evidence of a distinct management company-specific genetic signature exists. Population pairwise analysis revealed that all populations were significantly genetically distinct from each other. Furthermore, overall F_{ST} values were not significantly lower among farms under a given management company than across all farms. Thus, genetic associations between populations under the same management company, as indicated by a reduced level of genetic differentiation between company farms, were absent. AMOVA analysis confirms this finding, with the majority of diversity existing at the population level.

Isolation by distance is expected when the spatial scale over which samples are collected exceeds the average dispersal distance of the study organism (Wright 1943; Slatkin 1985). Human-mediated dispersed organisms, however, are unlikely to conform to this as passive dispersal distance can be highly variable (Wilson et al. 1999; Therriault et al. 2005; but see Crissman et al. 2010). Assuming dispersal is linked solely to human-mediated movement via supply trucks, a pattern of isolation by distance is theoretically possible if farms are visited in a sequential manner based upon proximity (i.e., shortest road distance between farms). With one exception, this pattern was not observed. The exception, X-group farms, may have arisen as an artifact of sample collection as all but 2 farms (X5 and X6) exist within a multi-farm complex, with buildings separated by between 500 and 2160 m. Although all pairwise population F_{ST} and G-test comparisons are significant, it is likely that some movement of cockroaches has occurred ancestrally. Indeed, according to farm managers, deliberate movement of materials did occur up to as recently as 5 years ago among farms X1 through X4. Recent movement is not considered possible as workers are restricted to specific farms within the complex with no movement between buildings and deliberate movement of materials no longer occurring. This multifarm complex system is unique within the sampling range and therefore may represent an exceptional case of ancestral movement followed by subsequent differentiation.

If movement of cockroaches from a management company-specific source did occur, this had been ancestral with subsequent contemporary reintroductions from the same source and/or movement among management-company farms via supply trucks sufficiently rare to prevent homogenization of allele frequencies at a company-specific level. As a result, we are confident that based on our sampling, the hypothesis of management company association proposed by Mukha et al. (2007) can be rejected. In its place, we propose an alternative hypothesis, placing greater importance on the role of local, human-mediated dispersion of cockroaches. Specifically, we suggest that farm workers serve as vectors for cockroach introductions into farms, back into the local human community, as well as to other local farms outside the workers' "bio-security zone." Although impossible to test within the scope of this study, results of Bayesian structure analysis, when presented visually in a geographic context (Figure 1), suggest that gene flow within the local communities may play a greater role than previously expected in shaping the genetic structure of farm populations. Farm workers often live in

Table 2 AMOVA calculated at 3 levels of hierarchy: among groups (among management companies), among sites within groups (among farms within management companies), and within sites (individual farms)

Among groups			Among populations within groups			Within populations		
V_a	Percentage	P	V_b	Percentage	P	V_c	Percentage	P
0.0278	0.90	0.0844	0.4962	16.03	<0.0000	2.5723	83.07	<0.0000

close proximity to their farm of employment. Within communities, and even within single households, there is a mingling of individuals employed by different farms, or indeed, different management companies. As a result, the opportunity arises for unconstrained movement of cockroaches at the community level through the exchange of infested materials, and subsequently to and from the farm workers' employment site. Given bio-security practices, this exchange is likely to be rare. Moreover, cockroach populations within farms are sufficiently large that rare introductions from the neighboring communities will have limited effect in homogenizing gene frequencies. However, 2 forces may increase the likelihood of local allele sharing across management companies, and thus the assignment observed to given genetic clusters at the local level. First, even relatively infrequent introductions of cockroaches may contribute, over time, to substantial homogenization of allele frequencies. Second, frequent disturbance of the cockroach population with insecticide applications, evacuation of pigs and feed, and power washes and disinfection of barns, can lead not only to 1) temporary reduction in the population, favoring establishment of introduced alleles, and 2) forced movement of cockroaches to new aggregation sites, some of which may be more conducive to "hitchhiking" on workers (e.g., in the cafeteria, cloths lockers, showers). Given STRUCTURE results presented here, it is therefore possible that despite bio-security practices implemented to prevent the movement of diseases and their vectors into farms, cockroaches may have been introduced through unintentional transfer from farm workers living in the surrounding community and potentially from local outsourced suppliers. Due to worker anonymity, samples could not be collected from workers' homes to address this alternate hypothesis within the realm of this study.

Genetic Diversity and Population Bottlenecks

Due to their poor survival ability outside human built structures, large areas of open land, fields, and forests represent significant barriers to active dispersal of *B. germanica* (Roth 1985). As a result, within the agricultural landscape, farms may essentially act as island populations. Average genetic diversity is often lower within island populations when compared with their mainland equivalents for species with poor dispersal ability (Frankham 1997); thus, the detection of reduced allelic diversity in agricultural German cockroach populations (5.47 in this study), compared with urban residential populations (7.68 in Crissman et al. 2010), is not unexpected. Although, one may also consider buildings within residential communities as island populations, the potential for the movement of individuals between islands within this environment, due to the absence of bio-security practices and established conduits for movement (e.g., plumbing including sewer lines, electrical conduits, elevators, shared laundry, and trash facilities), is potentially greater. Significant genetic differentiation between all pairs of farms, regardless of geographic location, and lower allelic diversity on farms than in residential settings suggest that cockroaches are only infrequently introduced into farms. Nevertheless, loci exhibiting greater than 4 alleles were detected in all populations. Therefore, multiple introductions over time, albeit rare, are likely. Alternatively, but much less likely, population foundation by single, large, and genetically diverse propagules is possible. We initially hypothesized the latter, with warehouses and feed mills as potential sources of such propagules. However, careful inspections of such facilities fail to uncover any cockroach infestations, suggesting that new introductions are likely not from a single or just a few supplies routes.

Within farms, cockroach populations are subjected to demographic bottlenecks through both insecticide applications and periodic room cleansing. Demographic bottlenecks resulting from room cleansings are likely to be ephemeral, experienced in all farms regardless of insecticide application regime, and may result in genetic homogenization within the farm building as a consequence of the forced movement of surviving individuals to adjacent rooms. Insecticide-based control, in contrast, has the potential to reduce genetic diversity as treatments are farm wide and the residual effect can be long lasting (Gore et al. 2004). Although results from other species subjected to insecticidal treatment vary greatly (Street et al. 1998; Pérez de Rosas et al. 2007; 2008), genetic diversity was significantly reduced within populations of *B. germanica* under strict insecticide control. *Blattella germanica* populations are characterized by their exponential growth rate, which within 3 months may undergo a 24- to 28-fold increase in population size (Ross 1976; Ross et al. 1984). Bottlenecks are therefore likely to be ephemeral, and the genetic signature lost rapidly (Cornuet and Luikart 1996); however, the long-term effect on evolutionary potential may be detrimental (Frankham et al. 1999). Populations that rebound after a genetic bottleneck may exhibit blurred patterns of coancestry such that historical connections are subsequently difficult to trace.

Conclusions

Although a number of species live synanthropically with humans, few rely entirely on humans for their survival and distribution. Unlike other cosmopolitan human-commensal organisms, such as the house mouse, *Mus musculus domesticus*, the rats, *Rattus rattus* and *R. norvegicus*, and the house sparrow, *Passer domesticus*, which are capable of active dispersal (Parkin and Cole 1985; Dallas et al. 1995; Pocock et al. 2004; Brouat et al. 2007), or parasitic arthropods (e.g., fleas) that can disperse on humans or pets, dispersal of *B. germanica* outside of human dwellings is unlikely (Roth 1985; Rivault 1989; Rivault and Cloarec 1991; Jobet et al. 2000) and is unlikely to play a significant role in shaping local population genetic structure (Crissman et al. 2010). Patterns of genetic association are therefore highly constrained and not necessarily associated with distance. Thus, analogies with other common human-commensal species are impossible to draw with any degree of accuracy. This study therefore broadens our understanding of the impact of human-mediated dispersal

on the genetic structure of agricultural, economically important insect pests within a geographic framework where dispersal is highly constrained, thus providing valuable information for the development and/or modification of targeted strategies for insect control within this unique environment.

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