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Intraspecific variation and population structure of the German cockroach, *Blattella germanica*, revealed with RFLP analysis of the non-transcribed spacer region of ribosomal DNA

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Abstract. Little information is available on genetic variation within and between populations of pest cockroaches. In this study, intraspecific *Hind*III polymorphism was investigated in the German cockroach, *Blattella germanica* (Linnaeus) (Dictyoptera, Blattaria: Blattellidae), using restriction fragment length polymorphisms (RFLP) of the non-transcribed spacer (NTS) region of ribosomal DNA (rDNA). Individual male insects were collected from infestations at three different pig farms. Each population was characterized by *Hind*III restriction fragment frequencies and haplotype (a particular X-chromosome pattern) frequencies. The inheritance of the X-chromosome *Hind*III rDNA patterns over 12 generations (3 years) follows Mendelian patterns, and the stability of this polymorphic marker indicates infrequent genetic recombination of variable sites. Although pairwise genetic distance measures were uncorrelated with geographical distance, the pattern of genetic differentiation of the three cockroach populations suggests that human-mediated transport of cockroaches is an important force in shaping the population genetic structure of cockroach infestations, at least at the regional scale of 10–100 km. Sequence variation in the ribosomal NTS is a useful marker, and RFLP of rDNA is a simple, robust and reproducible technique for differentiating recently diverged cockroach populations.

Key words. *Blattella germanica*, German cockroach, non-transcribed spacer, population, RFLP, ribosomal DNA.

Introduction

The German cockroach, *Blattella germanica* (Linnaeus), is a cosmopolitan pest species that is obligately commensal with humans (associated strictly with human habitations, farms, food stores, waste areas and other anthropogenic habitats, and not known to occupy any natural habitats). In most places it is the most prominent and most important of the pest cockroaches (Schal & Hamilton, 1990; Brenner, 1995). This pest poses both direct and indirect hazards to humans and animals. Foremost among these is the recent recognition that exposure to cockroach-produced proteins causes significant allergic disease and asthma among inner-city children (Rosenstreich *et al.*, 1997). A number of studies have also implicated cockroaches as potential mechanical vectors of microbial pathogens to humans and animals (e.g. Cloarec *et al.*, 1992; Rivault *et al.*, 1993) and the vector competence of German cockroaches was recently demonstrated for one of the most important bacterial pathogens of piglets, a verotoxigenic *Escherichia coli* (Zurek & Schal, 2004). As cockroaches appear to spread readily within contiguous residential communities, and their aggregate population densities can be very large, they may play a significant role in the epidemiology of enteric disease.

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Apart from some ants and termites, little is known about the genetics of populations of any structural pest, that is, pests of homes, schools, hospitals, restaurants, farm buildings, warehouses and other buildings. *Blattella germanica* readily invades favourable environments, reproduces rapidly, and its populations can reach huge, economically and clinically significant levels. Despite worldwide distribution, feral populations of this insect have not been found, not even in Southeast Asia, the epicentre of *Blattella* diversity (Roth, 1985). Commensal insects, like *B. germanica*, can spread through active dispersal or by human-mediated 'jump' transport (e.g. Suarez *et al.*, 2001). *B. germanica* adults possess functional wings, but they do not fly. Coupled with a rather narrow permissive temperature for reproduction (~15–32 °C), this would suggest that active dispersal would be limited to short distances between contiguous habitats (i.e. diffusion). Genetic differentiation of spatially separated cockroach populations is thus expected to be high, as isolated populations experience selection, genetic drift, bottlenecks (e.g. following introduction events and eradication efforts), and founder effects. Moreover, like other commensals, including house mice, the German cockroach appears to not suffer inbreeding depression, and a single mated female can readily establish a thriving population. However, three studies that used allozyme and random amplified polymorphic DNA (RAPD) markers to differentiate cockroach populations have shown extremely low differentiation among geographically distant populations in both the U.S.A. and France (Hampson & Steiner, 1982; Cloarec *et al.*, 1999; Jobet *et al.*, 2000). These surprising results could mean that there is little genetic structure among infested buildings across large geographical areas, possibly due to rampant gene flow, or that populations are differentiated but studies using these markers failed to detect genetic structure, probably because of the low variability at these loci. Preliminary results from studies of restriction-endonuclease length variation at the nuclear ribosomal DNA (rDNA) complex (Mukha *et al.*, 2000) suggested the latter.

Analysis of restriction fragment length polymorphism (RFLP) of the rDNA repeat has been used extensively in molecular systematic and population genetic studies (Karvonen & Savolainen, 1993; Suzuki *et al.*, 1994; Fazaeli *et al.*, 2000; Mukha *et al.*, 2002a; Slippers *et al.*, 2002; Aranishi, 2005; Simoes-Barbosa *et al.*, 2005). The basic organization of the rDNA has been conserved in most eukaryotes. Eukaryotic ribosomal RNA (rRNA) genes (18S-, 5.8S- and 28S-like) are arranged in tandemly repeated clusters, separated by several spacers, namely the non-transcribed spacer (NTS) and internal transcribed spacers (ITS) 1 and 2. The NTS separates neighbouring repeat units, ITS1 is located between the 18S- and 5.8S-like coding regions, and ITS2 lies between the 5.8S- and 28S-like genes; the NTS, together with the external transcribed spacer (ETS), comprise the intergenic spacer (IGS) (Gerbi, 1985) (Fig. 1).

The nucleotide sequence and length of various structural elements of nuclear rDNA units are differentially conserved over evolutionary time, with the most stable being the rRNA genes and the most variable being the NTS (Hillis & Dixon, 1991). In many eukaryotic species the high variability of the NTS is due to short subrepeats that differ in both nucleotide composition and position within the NTS (Paule & Lofquist, 1996). Tandem localization of the evolutionarily stable 28S gene and variable NTS makes rDNA a potential candidate for investigations of intraspecific variation of NTS RFLP by blot hybridization techniques (e.g. Coen *et al.*, 1982). Indeed, *Hind*III restriction enzyme digestion of *B. germanica* rDNA revealed population variation due to structural differences within the NTS (Mukha *et al.*, 2000).

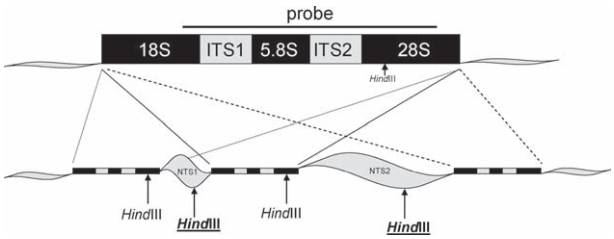

Fig. 1. Schematic representation of the eukaryotic ribosomal genes. The ribosomal genes 18S, 5.8S, 28S are separated by internal transcribed spacers ITS1 and ITS2. Hundreds of copies of these rDNA units are dispersed in tandem repeats and interspersed by non-transcribed spacers (NTS1 and NTS2) of different lengths. *Hind*III, the position of the *Hind*III site defined by sequencing; *Hind*III, variable *Hind*III sites; probe, the position of the cloned *B. germanica* rDNA fragment (Mukha *et al.*, 2000).

Within the extensive swine production industry in southeastern North Carolina (U.S.A.) there is a unique agricultural situation, where recent human-mediated dispersal of cockroaches can be investigated on a regional scale. Several features of this system make it particularly useful for studying the impact of human transport on the population genetic structure of cockroaches, and, in turn, its dramatic ecological, economic and health consequences in a human community. Firstly, inoculations and the spread of cockroaches are recent events as German cockroaches first appeared on North Carolina pig farms about 15–20 years ago. Secondly, the ~2000 farms in North Carolina are relatively isolated geographically and bordered by large fields, disallowing active dispersal of cockroaches. Thirdly, several major companies manage the majority of pig farms in North Carolina, and the farms within each company are linked by the transport of supplies from central locations. The vectors of human movement within and between farms are closely regulated because of bio-security concerns and therefore are better known than in other cockroach-infested communities. Fourthly, farms under distinct management occur at various spatial scales from distances of a few km to several thousand km over several states, and lastly, the density of cockroaches in swine barns can reach several thousand individuals per m^2 (Gore *et al.*, 2004). Although this poses risks to farm workers from inhaled allergens and potential contact with pathogenic microbes, it also offers a unique opportunity to collect large numbers of cockroaches at various geographical scales. Hence the aim of the current study was to explore this system using various molecular markers, including microsatellites and rDNA. For this, *Hind*III rDNA polymorphism was compared in individual insects from three cockroach populations collected on different pig farms.

Materials and methods

DNA extraction and blot hybridization

Total cockroach DNA was isolated from whole individuals by homogenization in extraction buffer followed by phenol-chloroform extraction and ethanol precipitation following standard protocols (Sambrook *et al.*, 1989). Restriction endonuclease digestion, agarose gel electrophoresis and Southern blot hybridization were also performed as described in Sambrook *et al.* (1989).

Polymerase chain reaction (PCR) amplifications for overlapping fragments within the *B. germanica* rDNA repeat unit, used as probe for blot hybridization, were carried out using *Taq* DNA polymerase (Promega, Madison, WI) in a PTC-100 Thermal Cycler (MJ Research, Waltham, MA). We used the primers DAMS18 (GTCCCTGCCGTTTGTACACA) and DAMS28 (CTACTAGATGGTTCGATTAGTC), based on the sequence of the *B. germanica* rDNA repeat unit (accession # AF005243). Each reaction contained 0.1 μg DNA template, 1.5 mM $MgCl_2$, 1 mM each dNTP, and 0.2 pmol of each primer in a final volume of 50 μL. The PCR regimen was as follows: initial template denaturation at 95 °C for 5 min, followed by 30 cycles of 94 °C for 1 min, 55 °C for 2 min, and 72 °C for 3 min, and a final 7-min elongation step at 72 °C. The amplified fragment was labelled with dATP-αP^{32} by Prime-a-Gene Labeling System (Promega) according to the manufacturer's recommendations.

Insect collection, maintenance and crosses

The German cockroach *B. germanica* colony was maintained in incubators at 27 ± 0.5 °C, ~50% relative humidity (RH), and 12 : 12 h light–dark photoperiod. Cockroaches were provided with Purina 5012 Rat Chow (Purina Mills, St. Louis, MO, U.S.A.) and water *ad libitum*. For crossing, newly emerged virgin females were separated from the males on the day of adult eclosion (day 0), and maintained in separate plastic cages in a separate incubator.

The *Hind*III restriction rDNA polymorphism of *B. germanica* collected from three separate pig farms was examined: Farms H1 (Sampson County, NC, U.S.A.) and Riv1 (Duplin County, NC, U.S.A.) are approximately 65 linear km apart (~100 km by roads) and managed by one company. BriJ (Duplin County, NC, U.S.A.) is ~15 km from H1 and ~115 km from Riv1, but is managed by another producer. Only fresh-collected males and oothecae (egg cases) were used for this analysis.

Data analysis

For statistical analysis GENEPOP Version 3.1 (Raymond & Rousset, 1995), GELSTATS Version 2.6 (Rogstad & Pelikan, 1996), NTSYS (Rohlf, 1997) and ARLEQUIN Version 2.0 (http://anthro.unige.ch/arlequin/) were used. Fragment and haplotype observed frequencies, average number of fragments, proportion of polymorphic loci, and average pairwise similarity were calculated according to previously described algorithms (Sneath & Sokal, 1973; Stephens *et al.*, 1992); the statistical trustworthiness of these parameters was calculated by permutation (Good, 2000) and G^2 tests. Genetic distances between populations were calculated according to Cavalli-Sforza & Edwards (1967).

Results

Hind*III restriction rDNA polymorphism in populations of* B. germanica

rDNA repeats may be located within one or a few chromosomes and are associated with the nucleolus organizer (NO); the NO of the German cockroach is located within the X-chromosome (Ross, 1988). Each male cockroach has only one X-chromosome (XO), whereas females have two X-chromosomes (XX). Therefore, polymorphic rDNA markers seen in *B. germanica* males correspond to a single X-chromosome, and each population can thus be characterized by both rDNA band (fragment) frequencies and haplotype (a particular X-chromosome pattern) frequencies.

As a first step toward using rDNA in cockroach population genetics, *Hind*III restriction fragment polymorphism was analysed in individuals randomly drawn from different laboratory strains of *B. germanica*. Individually isolated cockroach DNA samples were digested using several restriction enzymes and probed by blot hybridization using the cloned 2.7-kb *B. germanica* rDNA fragment (Fig. 1) (Mukha *et al.*, 2000). The *Hind*III restriction enzyme generated a high level of polymorphism. Several hybridization zones were revealed: Two zones, approximately 1 kb and 2 kb in length, were consistently found across all samples, and several longer zones, 5–6.5 kb in length, were polymorphic within colonies (Mukha *et al.*, 2000). Individual cockroaches could be distinguished by the number of these longer polymorphic zones. This suggests that the rDNAs of different individuals within a population are not identical, but contain diverse types of ribosomal repeats.

The internal repetitive structure of NTSs on individual X-chromosomes was examined using *Hind*III restriction of 64 adult male *B. germanica* collected from three separate pig farms. Several diagnostic *Hind*III rDNA patterns were found, represented in Fig. 2a, b and schematically shown in Table 1. In total, eight different bands were observed, numbered 1, 2, … 8 in Fig. 2a, b and Table 1. The intensity of hybridization varied across individuals, probably indicating natural variation in the quantity of specific rDNA NTS repeats among individuals within a population (Mukha *et al.*, 2000). For statistical analysis, patterns were characterized according to the number of hybridization zones within each lane (individual) without regard to hybridization intensity.

Because the rDNA cluster of *B. germanica* is located on the X-chromosome (Ross, 1988), and each male cockroach has only one X-chromosome (X0), each lane on the blot characterizes an individual X-chromosome (haplotype) (see Coen *et al.*, 1982) and each population can be characterized by both band (fragment) frequencies and haplotype frequencies. In total, 17 X-chromosome rDNA types were found, denoted A, B, … Q in Table 1; 4, 5 and 11 X-chromosome types were found on farms H1, Riv1, and BriJ, respectively.

The high level of *Hind*III restriction fragment polymorphism found in the NTS region of rDNA in *B. germanica*

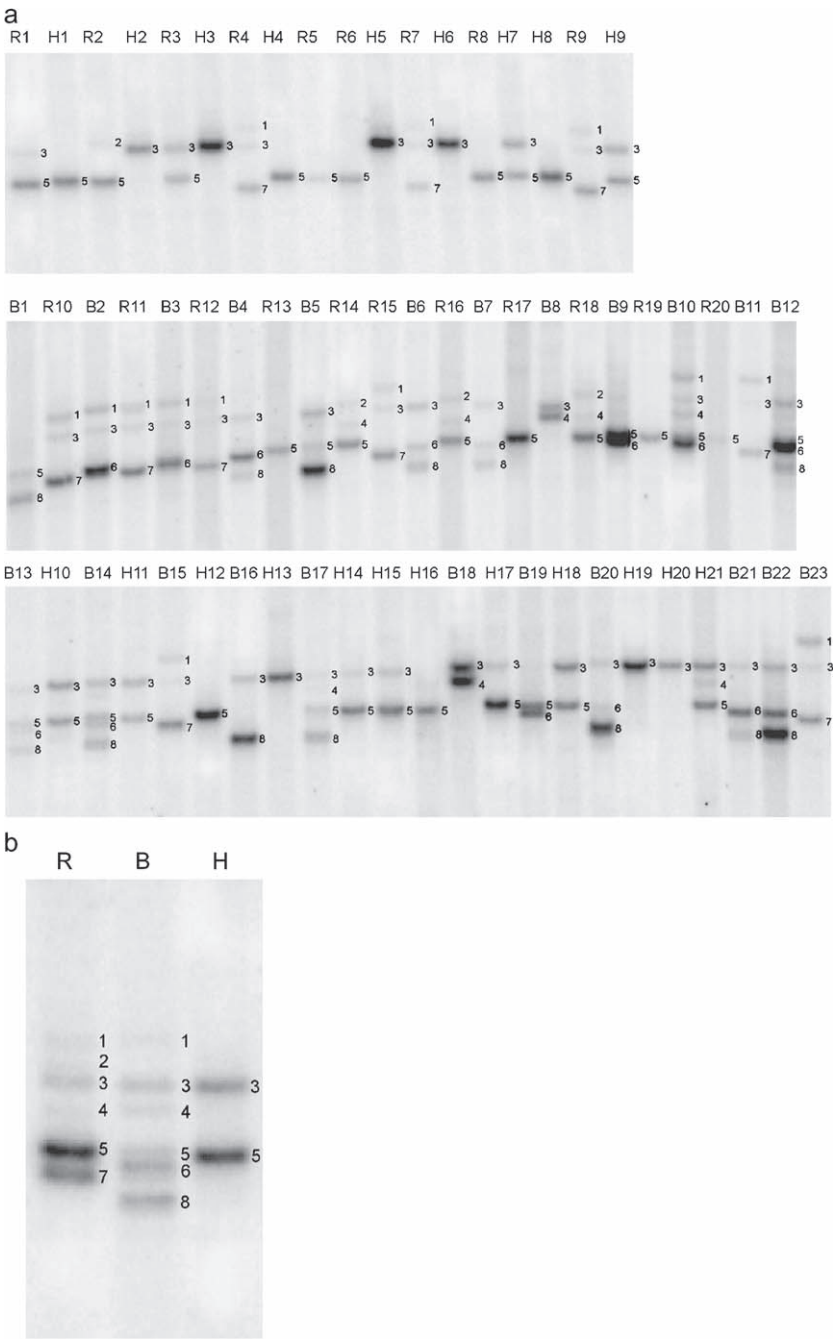

Fig. 2. Southern blot hybridization of the *B. germanica Hind*III restricted DNA with the cloned fragment of the *B. germanica* rDNA used as a probe (see Fig. 1). (a) Each lane represents an individual adult male cockroach from three swine farm populations (Riv1 farm: R1–R20; H1 farm: H1–H21; BriJ farm: B1–B23). (b) Each lane represents 50 cockroach oothecae from 50 females collected at each of the three farms Riv1 (R), BriJ (B), and H1 (H).

collected from three separate populations shows that the level of homogenization of this spacer in the German cockroach is extremely low compared with that in the rRNA genes (5.8S, 18S and 28S) and transcribed spacers (Mukha *et al.*, 2002a, b). The results also suggest that recombination within the NTS subrepeats probably played a significant role in the process of concerted evolution of members of the rDNA multigene family during the evolution of *B. germanica*. At the same time, however, recombination within the NTS subrepeats appears to generate and maintain a high level of NTS structural polymorphism, as has been shown in investigations of the dynamics of homogenization in rDNA tandem arrays of other eukaryotes (for reviews, see Ohta, 1980; Dover, 1982).

Inheritance of the X-chromosome polymorphic rDNA markers

It would be only possible to use the X-chromosome as a molecular marker for population genetics analysis if it could be demonstrated that the observed hybridization fragments form a single linkage group with low recombination rates between homologous chromosomes. Individual female and male

Table 1. Schematic representation of blot hybridization results for males (see Fig. 2a) by binary matrix.

	Population H1																				
Individual	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
fragment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1																					
2																					
3		+	+		+	+	+		+	+	+		+	+	+		+	+	+	+	+
4																					+
5	+			+			+	+	+	+	+	+		+	+	+	+	+			+
6																					
7																					
8																					
Chromosome type	A	B	B	A	B	B	C	A	C	C	C	A	B	C	C	A	C	C	B	B	D
	Population Riv1																				
Individual	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	
fragment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
1				+			+		+	+	+	+			+						
2		+												+		+		+			
3	+		+	+			+		+	+	+	+			+						
4														+		+		+			
5	+	+	+		+	+		+					+	+		+	+	+	+	+	
7				+			+		+	+	+	+			+						
8																					
Chromosome type	C	F	C	E	A	A	E	A	E	E	E	E	A	Q	E	Q	A	Q	A	A	
	Population BriJ																						
Individual	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
fragment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1		+	+							+	+				+								+
3		+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+		+	+	+	+
4								+		+							+	+					
5	+				+				+	+		+	+	+			+		+				
6		+	+	+		+	+		+	+		+	+	+					+	+	+	+	
7											+				+								+
8	+			+	+	+	+					+	+	+		+	+			+	+	+	
Chromosome type	G	I	I	H	J	H	H	K	L	M	E	N	N	N	E	O	P	K	L	H	H	H	E

+, Fragment present.

cockroaches were crossed and the inheritance of the parents' particular *Hind*III X-chromosome patterns was analysed. Each male cockroach has only one X-chromosome, whereas the female has two X-chromosomes. Both X-chromosomes of the female parent used in this crossing had the same *Hind*III pattern, whereas the male parent had a distinct X-chromosome *Hind*III pattern that differed from the female's (Fig. 3a). The inheritance of the *Hind*III pattern followed Mendelian rules: males of the first generation (F1) inherited only one of their mother's X-chromosomes, and F1 females inherited one of the mother's X-chromosomes and an additional X-chromosome from the father (Fig. 3a). In the second generation (F2), all males had one of the parents' X-chromosomes and females had two X-chromosomes representing random combinations of the initial three parental X-chromosomes (Fig. 3b).

To analyse the stability of these *Hind*III hybridization patterns, the descendants of this single-pair mating were maintained for 3 years (about 12 generations). To ensure that only a single NO cluster was being analysed, only adult males (60 individuals) of the 12th generation (F12) were examined by the same methods as F1 and F2 cockroaches. Again, all males had a *Hind*III X-chromosome pattern representing only one of the parents' X-chromosomes (Fig. 3c), indicating that no recombination had occurred among the *Hind*III fragments between homologous chromosomes, and the hybridization zones thus form a single linkage group with infrequent recombination between markers.

These results suggest that differences among individuals or populations represent rDNA polymorphisms that arose over an evolutionary time scale, and not through recent recombinations or selection for rDNA variants. Although it is difficult to clearly define the genetic distances between the parental markers and the level of recombination between them, these results clearly showed that the hybridization zones formed a single linkage group with infrequent recombination between markers. Unfortunately, all three X-chromosomes from the two parents

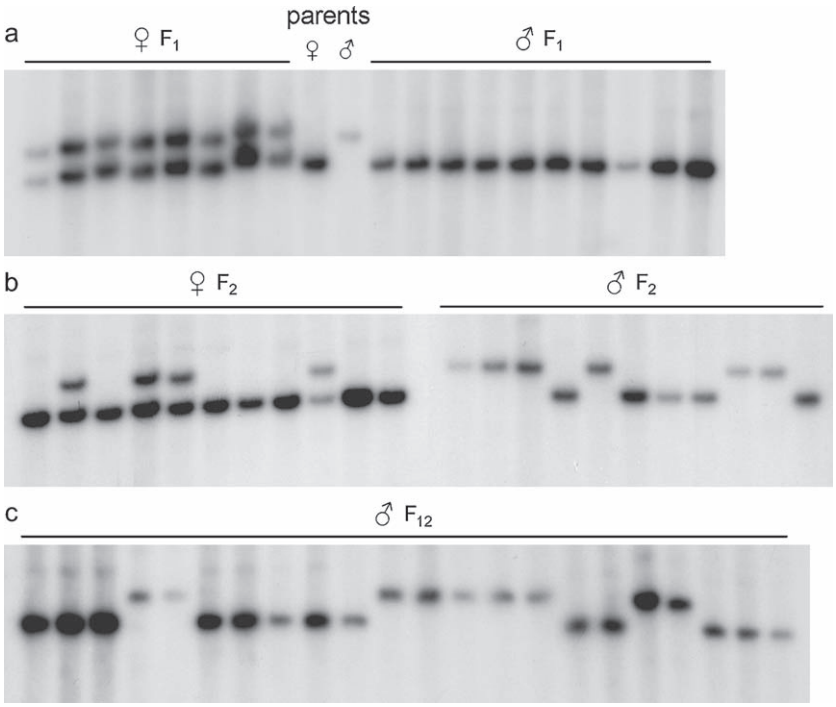

Fig. 3. Analysis of inheritance of the X-chromosome polymorphic rDNA markers. Southern blot hybridization of the *B. germanica* *Hind*III restricted DNA; each lane represents an individual adult cockroach. (a) Parents and F1 female and male offspring of the first generation. (b) F2 female and male offspring of the second generation. (c) F12 males of the 12th generation.

had the same intrachromosomal *Hind*III patterns, so these results cannot be used to gauge the level of the intrachromosomal gene conversion, which probably can change the *Hind*III patterns of individual X-chromosomes during the life of a cockroach population. Nonetheless, a similar study with *Drosophila* lines that originated from natural populations showed that intrachromosomal variability was extremely low – not more than one new rDNA variant in 3000 generations (Coen *et al.*, 1982). It is reasonable to conclude that intrachromosomal gene conversion, which plays a significant role in the evolutionary homogenization of the rDNA multigene family (Dover & Coen, 1981; Arnheim, 1983; Li, 1997; Ohta, 2000), does not constrain use of the X-chromosomes as a molecular marker for population genetics. In the current study, in particular, intrachromosomal variability is expected to be extremely low because the age of the *B. germanica* populations in infested pig farms is $<$15–20 years, or $<$80 cockroach generations.

It is also important to note, nevertheless, that because of the multicopy nature of rDNA and the homogenizing process of concerted evolution, it is possible that a new variant would have to substantially increase in frequency in the array before it could be spotted with the hybridization technique.

Genetic diversity and structure of cockroach populations

To identify all potential fragments expected to be found in individual male cockroaches, the total DNA isolated from 50 oothecae collected at each of the three pig farms was analysed. Each ootheca contains approximately 40 embryos (20 males and 20 females), or 60 X-chromosomes. Therefore, each lane in Fig. 2b corresponds to 3000 X-chromosomes and represents an overall 'genotype' of each population, with thicker bands corresponding to the most abundant variants. It is clear just by visual inspection that these farm populations are different.

Detailed analysis of these patterns showed that males within each sample were characterized by high genetic similarity, and the three populations differed from one another according to the number of hybridization zones: 3, 6 and 7 fragments in the H1, Riv1 and BriJ populations, respectively. Fragments 3 and 5 were common to all populations; the Riv1 and BriJ populations had additional common fragments 1 and 7 (Fig. 2a). The patterns of the blot hybridization results for all males (21 males from H1, 20 from Riv1, and 23 from BriJ) are summarized schematically in Table 1. Pairwise comparisons of the observed frequencies of the fragments by the G^2 test indicated that the three populations had significantly different ($P < 0.01$) frequencies of fragments: Riv1 and H1 differed in fragments 1 and 7, Riv1 and BriJ differed in fragments 2, 3, 6 and 8, and H1 and BriJ differed in fragments 1, 6 and 8 (Table 2). These results show that pairwise comparisons of the frequencies of the *Hind*III-generated fragments can be used to differentiate cockroach populations. The three cockroach populations were statistically differentiated ($P < 0.01$) based on a permutation test of the average number of fragments per individual (data not shown).

From the data in Table 1, population parameters were computed and permutation tests conducted for average pairwise similarity, for which *P*-values are shown in Table 3. The level of interpopulation differentiation was estimated by F_{ST} as calculated using the program AMOVA (ARLEQUIN Version 2.0, http://anthro.unige.ch/arlequin/), which showed a high level of among-population variability ($F_{ST} = 0.2581$, $P < 0.001$). The most differentiated populations were H1 and BriJ ($F_{ST} = 0.314$, $P < 0.001$), which were nearest to each other geographically;

Table 2. Pairwise comparisons of observed fragment frequencies in males by G^2 test.

	Pairs of populations					
	Riv1−H1		Riv1−BriJ		H1−BriJ	
Fragment	G^2	P	G^2	P	G^2	P
1	11.58	0.0007	0.40	NS	8.65	0.003
2	6.20	0.013	6.60	0.010	–	–
3	4.27	0.039	8.93	0.003	0.86	NS
4	1.27	NS	0.05	NS	1.86	NS
5	0.01	NS	2.90	NS	3.38	NS
6	–	–	23.48	10^{-6}	24.25	10^{-6}
7	11.58	0.0007	2.93	NS	4.09	0.043
8	–	–	21.21	10^{-6}	21.92	10^{-6}

NS, not significant.

a lower level of differentiation was found for populations H1 and Riv1 ($F_{ST} = 0.172$, $P < 0.001$).

Large differences were found among the three cockroach populations based upon the distributions of the rDNA X-chromosome types. Only several X-chromosome types (from a total of 17) were common in the three populations: Riv1 and H1 shared types A and C, and Riv1 and BriJ shared type E; there were no common X-chromosome types in cockroaches from H1 and BriJ. Each of the three cockroach populations also had unique X-chromosome types: types B and D in H1, F and Q in Riv1, and G, H, I, J, K, L, M, N, O and P in BriJ (Table 1). Pairwise comparisons of the observed frequencies of X-chromosome types by G^2 tests indicated that the populations were statistically different from one another (Table 4).

Finally, genetic distances between populations were computed (Cavalli-Sforza & Edwards, 1967) and principal component analysis was used to ordinate the populations in space (Fig. 4). The most closely related populations were Riv1 and H1; BriJ was significantly genetically distant from the first two populations.

Discussion

Cockroaches might well have been the first group of invasive species in the New World, as Columbus' ship, and commerce that followed, were likely to have been infested with them (Cornwell, 1968). As new global markets are developed, commensal organisms, such as cockroaches, are accidentally transported by a diverse set of vehicles into new geographical regions and habitats. However, because of their cryptic nature and nocturnal habits, new invasions and inoculations are rarely documented.

Given the worldwide pervasiveness of cockroach infestations, and a general lack of understanding of the dynamics of colonization and population genetics, this group – especially the German cockroach, *B. germanica* – is a prime candidate for detailed population structure analysis utilizing DNA sequence data. Genetic differentiation of geographically separated German cockroach populations is expected to be high, as they are likely to depend on human-aided transport for large-step ('jump') dispersal, and local populations experience strong selection (e.g. with insecticides), genetic drift, bottlenecks (e.g. following introduction events and eradication efforts), and founder effects. Yet, a study with allozymes showed low rates of genetic differentiation among urban cockroach populations in two French cities, 900 km apart, and no differentiation between the two cities (Cloarec *et al.*, 1999; see also Hampson & Steiner, 1982); only eight of 41 allozyme loci (<20%) were polymorphic and the average observed heterozygosity (H0) was only 0.059, much lower than expected (Cloarec *et al.*, 1999). A more recent investigation using RAPD markers concluded that four cockroach populations from the same two cities were genetically differentiated (Jobet *et al.*, 2000). In both studies, the levels of geographical differentiation did not differ from the level of local differentiation within each city, showing that genetic distances did not correlate with geographical distance.

Table 3. Permutation tests for average pairwise similarity for males.

	Riv1	H1	BriJ
Riv1	–	0.0022	0.0090
H1		–	0.0410
BriJ			–

The current analysis of the genetic structure of German cockroach populations based on the *Hind*III RFLP of ribosomal DNA has revealed strong genetic differentiation of three cockroach populations over relatively large geographical distances. Like Jobet *et al.* (2000), genetic isolation by distance over a scale of 10–100 km was also not found. Cockroaches collected from the BriJ farm, which is nearest geographically (~15 km) to the H1 farm, were most genetically distant from cockroaches collected from Riv1 and H1. Riv1 is ~100 km from H1 and ~115 km from BriJ. Cockroaches from Riv1 were genetically closer to cockroaches from H1. But what is most striking about these results is that H1 and Riv1 are managed by the same company, whereas BriJ is managed by another company, presumably sharing little (including cockroaches) with the other farms. Commensal cockroaches are well adapted hitchhikers and stow away in various supplies, and these preliminary data suggest that passive, human-mediated dispersal over long distances might occur along supply chains determined by co-ownership of distant farms.

At a regional scale, pig farms are found in patches within a general habitat that is inhospitable to the German cockroach. Active dispersal between unrelated farms is highly infrequent

Table 4. Pairwise comparisons of observed X-chromosome type frequencies by G^2 test.

	Pairs of samples					
	Riv1−H1		Riv1−BriJ		H1−BriJ	
Haplotype	G^2	*P*	G^2	*P*	G^2	*P*
A	0.62	NS	12.31	0.0005	8.10	0.004
B	10.74	0.001			11.82	0.0006
C	4.64	0.031	3.17	NS	13.81	0.0002
D	1.36	NS			1.50	NS
E	11.58	0.0007	2.93	NS	4.09	0.043
F	1.46	NS	1.56	NS		
G			1.27	NS	1.32	NS
H			8.35	0.004	8.65	0.003
I			2.59	NS	2.68	NS
J			1.27	NS	1.32	NS
K			2.59	NS	2.68	NS
L			2.59	NS	2.68	NS
M			1.27	NS	1.32	NS
N			3.95	0.047	4.09	0.043
O			1.27	NS	1.32	NS
P			1.27	NS	1.32	NS
Q	4.56	0.033	4.85	0.028		

NS, not significant.

therefore, and there is insufficient dispersal of cockroaches to homogenize gene frequency at a regional level. These results suggest, however, that within the supply channels of a single company, cockroaches might be readily transported to previously uninfested farms as well as to and from infested farms. Local cockroach populations would thus experience the founder effect (a small number of individuals have only a subset of the variation present in the source population) at initial colonization. However, the genetic characteristics of this population may change through bottleneck, extinction and recolonization events.

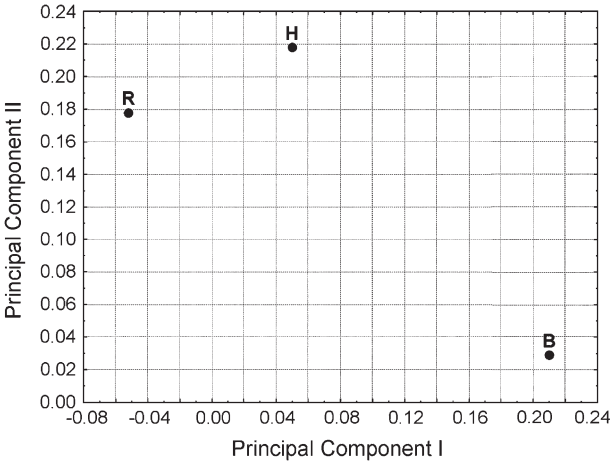

Fig. 4. Clustering of populations using principal component analysis based on the Cavalli-Sforza & Edwards (1967) chord distance, D_{CH} (genetic distance) between populations Riv1 (R), BriJ (B), and H1 (H).

Although multiple colonizations are most likely along the same channels as the initial colonization event, passive dispersal from workers' homes is also possible.

This model could have major consequences for pest control strategies in efforts to suppress populations of the German cockroach. Moreover, genetic analysis of cockroaches might provide insight into pathways of disease transmission within the swine agricultural system. To investigate this model, polymorphic microsatellite markers have been developed (Booth *et al.*, 2007) so that the structure of cockroach populations in swine farms and residences may be investigated.

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