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Diversity, Geographic Distribution, and Habitat-Specific Variations of Microbiota in Natural Populations of the Chicken Mite, *Dermanyssus gallinae*

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ABSTRACT *Dermanyssus gallinae* is considered to be the most economically significant ectoparasite to affect egg-laying poultry in Europe. This mite can also act as a vector for a number of pathogens. The array of bacteria associated with *D. gallinae* mites could provide insight into the biology and population dynamics of arthropods, but at the present time little information is available. To understand the intra- and interpopulation diversity of its associated microbiota, we analyzed the whole internal bacterial community of natural populations of *D. gallinae* originating from two types of poultry farm habitats (standard and free-range) in two regions of France (Brittany and the Rhone-Alpes). Total DNA was extracted from individual or pooled mites, and polymerase chain reaction temporal temperature gradient gel electrophoresis of 16S rRNA was then done to separate bacterial DNA fragments associated with the host arthropod. A large diversity of bacteria was detected, but principally firmicutes and γ-Proteobacteria. Between-group analyses of temporal temperature gradient gel electrophoresis-banding patterns revealed that bacterial populations clustered into categories according to their geographic origin and the habitat specifics of the farms. Some degree of stability of bacterial populations was observed within a specific time scale. These results suggest that environmental factors either recent (e.g., poultry farming practices) or long-standing (e.g., geographic isolation) may affect the bacterial communities present in *D. gallinae.* Further knowledge of the microbiota associated with *D. gallinae* and its variation would indeed offer new perspectives for biological control methods to prevent the establishment, proliferation, and transmission of pathogenic bacteria.

KEY WORDS *D. gallinae*, microbiota, diversity, biological control

The chicken mite, *Dermanyssus gallinae*, is probably the most widespread mite species found in birds in Europe (Chauve 1998, Roy et al. 2009). In poultry farms and particularly in egg-laying hens, it is the cause of anemia and a decrease in egg production resulting in a decline of poultry welfare. It can also cause dermatitis in humans (Bellanger et al. 2008). It has also been associated, in varying degrees, to various pathogens of medical and veterinary importance such as viruses (equine encephalitis viruses, fowl pox virus) or bacteria (*Pasteurella multocida*, *Coxiella burnetii*, and more recently *Salmonella* Enteritidis) (Valiente Moro et al. 2005, 2007, 2009). It is difficult to eradicate from poultry farms because of its particular biological characteristics, such as its short life cycle, its high resistance to starvation, and its suspected resistance to acaricides. Despite the damage and economic losses it causes, there are currently no efficient control methods capable of eradicating the pest (Chauve 1998).

In recent years, evidence has been gathered as to the importance of bacterial populations living inside arthropods (Dillon and Dillon 2004). For instance, in *Psoroptes ovis*, the mite that causes sheep scab, gut bacteria may be crucial to the mite as a food source and it is also possible that these bacteria are a source of protective antigens for a potential vaccine (Hogg and Lehane 1999). Moreover, bacteria present in the invertebrate gut have been shown to be important for mineralization, nitrogen fixation, vitamin synthesis, and the degradation of pesticides (Cruden and Markovetz 1987, Breznak and Brune 1994, Nardi et al. 2002). Studying microbial communities could therefore provide insight into the biology and population dynamics of arthropods, which are essential for a better understanding of pathogen transmission.

Little data are available concerning the bacterial communities associated with *D. gallinae.* Recently published studies include an overview of the bacterial composition in *D. gallinae* obtained from standard

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Table 1. List of poultry farms according to their habitat specific and geographic location

	Brittany	Rhone-Alpes	
	Standard	Standard	Free-range
Farms (Department)	BS1 (Côtes d'Armor) BS2 (Côtes d'Armor) BS3 (Côtes d'Armor) BS4 (Côtes d'Armor) BS5 (Côtes d'Armor) BS6 (Côtes d'Armor) BS7 (Côtes d'Armor) BS8 (Côtes d'Armor) BS9 (Côtes d'Armor) BS10 (Côtes d'Armor) BS11 (Côtes d'Armor) BS12 (Côtes d'Armor) BS13 (Côtes d'Armor) BS14 (Finistère) BS15 (Finistère) BS16 (Ille et Vilaine)	RS1 (Drome) RS2 (Drome) RS3 (Isère) RS4 (Isère) RS5 (Rhône)	RFR1 (Ain) RFR2 (Drome) RFR3 (Bresse) RFR4 (Rhône) RFR5 (Bresse) RFR6 (Rhône) RFR7 (Rhône) RFR8 (Rhône) RFR9 (Rhône) RFR10 (Ain) RFR11 (Ain)
No. individuals mites analyzed	240	75	165
No. pools of 10 mites analyzed	80	25	55
Total no. mites analyzed	1,040	325	715

poultry farms in the Brittany region using molecular methods (Valiente Moro et al. 2009) and preliminary data on endosymbiotic bacteria living inside the chicken mite (De Luna et al. 2009). These studies highlighted the presence of a cryptic community in the gut of *D. gallinae;* these include endosymbionts (*Spiroplasma* sp. Anisosticta, *Candidatus* cardinium Hertigii, and *Ricketsiella* sp.) and potentially pathogenic agents (*Erysipelothrix rhusiopathiae*, *P. multocida*). Contrary to other arthropods such as flies, termites, or ticks, little is known about the composition of the bacterial communities associated with the chicken mite. One could imagine that the diversity of the bacterial population could be strongly influenced by specific environmental characteristics such as the geographical location and habitat of poultry farms.

This work describes the differences in the bacterial communities associated with *D. gallinae* taken from two areas of France (Brittany and the Rhone-Alpes) and two specific farm habitats (for standard and free-range layer hens). Changes in bacterial populations over time were also examined. To do this, polymerase chain reaction (PCR) profiles of bacterial communities found in chicken mites were separated by temporal temperature gradient gel electrophoresis (TTGE), to allow identification of single base changes in a segment of DNA. In this technique, double-stranded DNA is subjected to an increasing temperature gradient and melts depending on the sequence loaded in the acrylamide gel. Partial melting of the DNA reduces its mobility, allowing differentiation of the various sequences from their positions in the gel. The PCR gene fragments corresponding to ≈180 bp of the bacterial conserved V3 region of 16S rRNA had been sequenced previously (Valiente Moro et al. 2009).

Materials and Methods

Collection of Mites. The populations of *D. gallinae* were collected from two different types of laying hen-breeding facilities. The first consisted of standard poultry farms with several tens of thousands of battery-caged birds in which standard poultry operations are similar from one poultry unit to another. The second type corresponded to free-range farms, including several thousand birds comingling as large flocks in a single room or outdoor pen. In addition to the poultry habitat, two geographical localities were also selected in France: the Brittany and the Rhone-Alpes regions. To assess the temporal variation of microbiota, two farms were monitored over a long period of time; one was analyzed for consecutive 3 yr (RFR7, RFR8, and RFR9) and the other at the beginning and end of a 3-yr period (RFR10 and RFR11). The characteristics of these farms are given in Table 1. Veterinarians controlled all the farms and there was no indication of infection or disease in the flocks during the sampling. Mites were collected using traps placed in nests or perches or simply by picking up samples of dried droppings from the duckboards (Zenner et al. 2009). The samples were placed in hermetically sealed bags for transport to the laboratory, where they were manipulated with a paintbrush and a suction pump attached to a collector. Only nymph and unengorged adult mites were selected for the analysis.

DNA Extraction, PCR-TTGE Amplification, and Analysis of TTGE-Banding Patterns. All the mites were alive at the time of processing for DNA extraction. Samples of *D. gallinae* consisted of 15 individual mites and five groups of 10 mites from each farm. Methods used for DNA extraction and universal PCR-TTGE were performed according to Valiente Moro et al. (2009). A fragment of ≈180 bp of the bacterial conserved V3 region of 16S rRNA was amplified with a broad-range bacterial primer set 350 F-GC (50-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCCGGGGGGCTCCTACGGGAGGCAGCAG-T-30) and PC535 (50-GTATTACCGCGGCTGCTGGCA-30).

Briefly, the external cuticles of the mites were first disinfected to ensure subsequent detection only of their

internal bacterial populations (Valiente Moro et al. 2007). To allow gel to gel comparison after migration of the PCR samples, a reference marker containing 16S rDNA fragments of eight known bacteria (*Borrelia garinii*, *Pasteurella multocida*, *Erysipelothrix rhusiopathiae*, *Campylobacter jejuni*, *Escherichia coli*, *Salmonella enterica* subsp. enterica serovar Enteritidis, and *Mycobacterium avium*) was run in parallel to the samples. TTGE-banding patterns were analyzed using Quantity One software (Bio-Rad, Marnes-la-Coquette, France). Bands were identified from the similarity of their positions in different lanes from different gels. The "front rate" parameter (Rf value), corresponding to band identity, was used to generate a dissimilarity matrix table corresponding to the presence (1) or absence (0) of each band in a sample and was performed for each individual farm (Fromin et al. 2002). The band identity referred to in a previously established identification list and bacterial taxa were classified according to biological categories and taxonomic affiliations (Valiente Moro et al. 2009). Bacterial categories were defined as follows. "Saprophytic bacteria" are those for which no species in the genus have been described as being pathogens other than opportunistic pathogens. The term "strict pathogen" is applied to bacteria for which most of the species belonging to the genus are pathogens for animals. Bacteria for which most of the species in the genus cause a disease in a compromised host, but which would not usually cause disease in a healthy host, are considered "opportunistic pathogens." The last category used was that of arthropod "symbionts," i.e., bacteria that cannot survive outside the arthropod host.

Statistical Analyses. The analysis of variance test was used to compare the bacterial composition of the mites according to their taxonomic and biological affiliations. Multivariate analysis methods were used to explore bacterial community fingerprints and the influence of habitat-specific characteristics of the farms, their geographical situation, and chronological monitoring. Principal component analysis (PCA) and between-group analysis (BGA) were used to display and test the differences between farm types (standard versus free-range) and between geographical regions (Brittany versus Rhone-Alpes) (Benzécri 1983, Dolédec and Chessel 1987). The variables (columns of the data table) considered in this study were the TTGE bands (binary response 0/1), and the samples (rows) were the mite pools taken from the poultry farms (five pools of 10 mites for each farm).

Several data tables were built and analyzed, but only three are presented in *Results.* The data table used to test the geographical effect had 87 rows (18 farms × 5 pools, with three missing pools from two farms) and 48 columns (TTGE bands). The data table used to test the habitat-specific effect had 65 rows (13 farms × 5 pools) and 45 columns (TTGE bands); that used for interregion and interhabitat comparisons had 127 rows (26 farms × 5 pools, with three missing pools from two farms) and 56 columns (TTGE bands).

PCA is the simplest of all multivariate analysis methods; its aim is to summarize a large data table into a few simple parameters. It operates by transforming a large number of potentially correlated variables into a smaller set of uncorrelated "principal components" while keeping as much as possible of the information (i.e., inertia, the sum of variances) contained in the initial data table.

BGA is a multivariate analysis method comparable to discriminant analysis; its aim is to evidence differences between groups in a data table. It is simply the PCA of the table of group means, with groups being defined in this study either as farm types or geographical regions. The advantage of BGA compared with discriminant analysis is that is can be used even when the number of samples is low (even lower than the number of variables), or when variables are correlated. Row scores of the data table can be computed by projecting the rows of the initial table on the principal components of the BGA. The statistical significance of the differences between groups can be tested with a permutation test (Monte-Carlo test). Computations were carried out using R statistical software (R Development Core Team 2009), using the *ade4* package (Chessel et al. 2004).

Multivariate analyses and graphical displays can be redone interactively online, using this reproducibility page: http://pbil.univ-lyon1.fr/TTGE2/.

Results

Diversity of *D. gallinae*-Associated Microbiota. To explore the bacterial diversity associated with *D. gallinae*, individual and pooled mites collected from different poultry farms underwent PCR-TTGE analysis. In total, 2,080 mites were examined: 480 individual mites and 160 pools of 10 mites.

Bacterial diversity was studied by considering both the taxonomic (Fig. 1) and biological diversity (Fig. 2). Two analyses were performed; the first corresponded to the overall distribution of bacterial taxa by regrouping all farms from the same category, and the second also corresponded to the overall distribution by taking into account each farm of a specific habitat. As shown in Figs. 1A and 2A, a large proportion of clones (approximately one-third of the bands) was unidentified, suggesting that part of the bacterial community remained unexplored. The overall analyses did not show significant differences between the habitat specifics of the farms ($P < 0.5$; Figs. 1A and 2A). In terms of abundance, Firmicutes were the predominant taxon (with >30%), followed by the γ-Proteobacteria phylum (~20%; Fig. 1A). The phylum of β-Proteobacteria was very rare (only one or two farms per specific habitat) or even totally absent, as was the case from the standard farms of the Rhone-Alpes region. The percentage of different phyla was similar between the two types of poultry farm, but intrafarm variability was observed with bacteria only present in one or two farms, as confirmed by BGA analysis. Concerning the biological categories, saprophytes were predominant (~30%), followed by the opportunistic pathogens (~20%; Fig. 2A). Both the strict pathogens and symbionts represented <10% of bacteria per habitat specific.

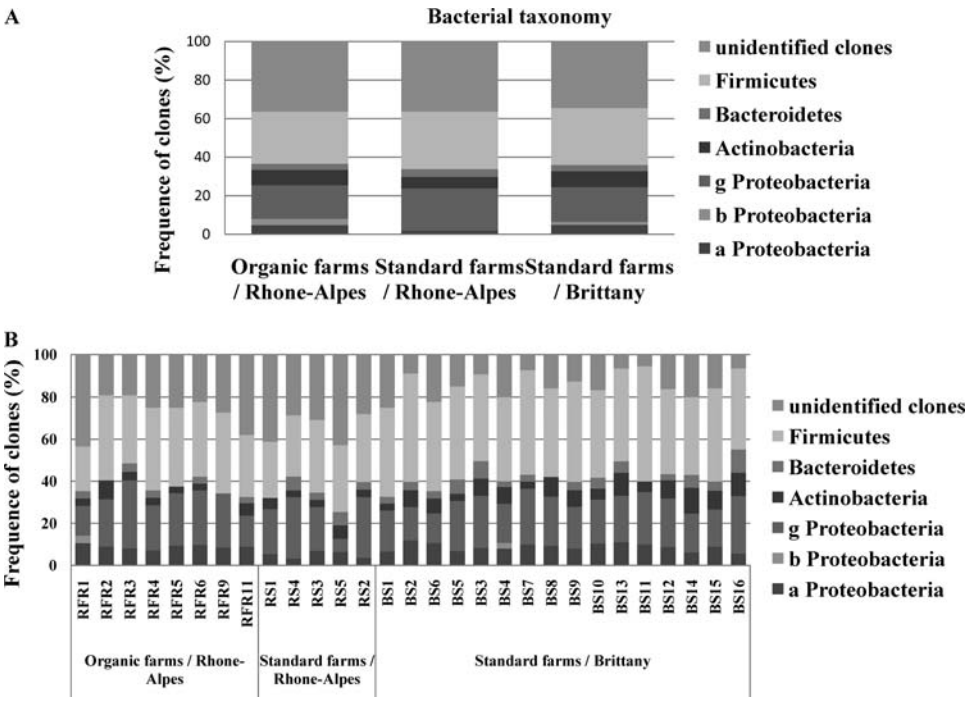

Fig. 1. Analysis of bacterial community according to their taxonomic affiliation. Bacterial taxa identification was obtained from the sequencing of separate bacterial 16S rRNA fragments after PCR-TTGE migration. The data for *D. gallinae* from standard poultry farms in Brittany were obtained from a previous study (Valiente Moro et al. 2009). (A) Overall analysis by regrouping all farms of a same category. (B) Detailed analysis by taking into account each farm with a specific habitat.

Bacterial diversity was also analyzed by considering individually each farm of a specific habitat (Figs. 1B and 2B). Statistical analyses did not show significant differences between farms ($P < 0.5$). Contrary to the previous results obtained, the analysis performed highlighted the fact that some farms did not contain either strict pathogens (RFR1 and RS1) or symbionts (RFR10 and BS10). The proportion of unidentified clones for mites originating from the standard farms in Brittany was lower than that previously obtained from the overall analyses.

Between-Group Analysis of *D. gallinae*-Associated Microbiota. Variations in the associated bacterial communities between the different farms were observed in the PCR-TTGE profiles according to the origin of the mite samples. Whereas some bands were widespread, occurring in approximately half or more of the profiles, some bands were restricted to only a few individuals. TTGE profiles were described by assigning a relative position (Rf) to each detected band with respect to the external marker. Several tables containing TTGE profiles from selected farms were then created and analyzed using BGA to compare the banding patterns under different sampling conditions.

BGA on Geographical Effect. Bacterial community composition was first analyzed using the data set corresponding to the TTGE-banding patterns obtained from standard poultry farms in Brittany and the Rhone-Alpes regions. Between-farms BGA revealed that banding patterns clustered into two main groups, corresponding to the two regions (Fig. 3). This structure was extremely strong and confirmed by the Monte Carlo permutation test ($P < 0.001$). The first two axes of BGA explained 24 and 12% of the total variation. These values were very high for presence-absence variables, and confirmed the strength of the structures. On the first axis, one group was composed of farms from the Rhone-Alpes (on the right), and a second group was composed of farms from Brittany (on the left). Farm BS16 was the only exception: although it is in Brittany, it is located on the right among the farms in the Rhone-Alpes. The second axis showed that there was another effect, independent of the geographical position of the farms, which opposed farm BS1 to the group of farms (BS15/BS4/BS9/BS8) in Brittany, and farms (RS5/RS3) to farms (RS1/RS4) in the Rhone-Alpes. This effect still remains unclear and merits further investigation.

BGA on Habitat-Specific Effect. Bacterial community composition was also analyzed with BGA to test the habitat effect (standard versus free-range). The data table contains the TTGE-banding patterns from free-range and standard poultry farms from the same geographic area (Rhone-Alpes region). Bacterial communities of *D. gallinae* appeared to be very different from one habitat to another: the permutation test (standard versus free-range) was highly significant ($P < 0.001$). As shown in Fig. 4, standard poultry farms were grouped together on the left part of the graph, whereas free-range farms were spread on the

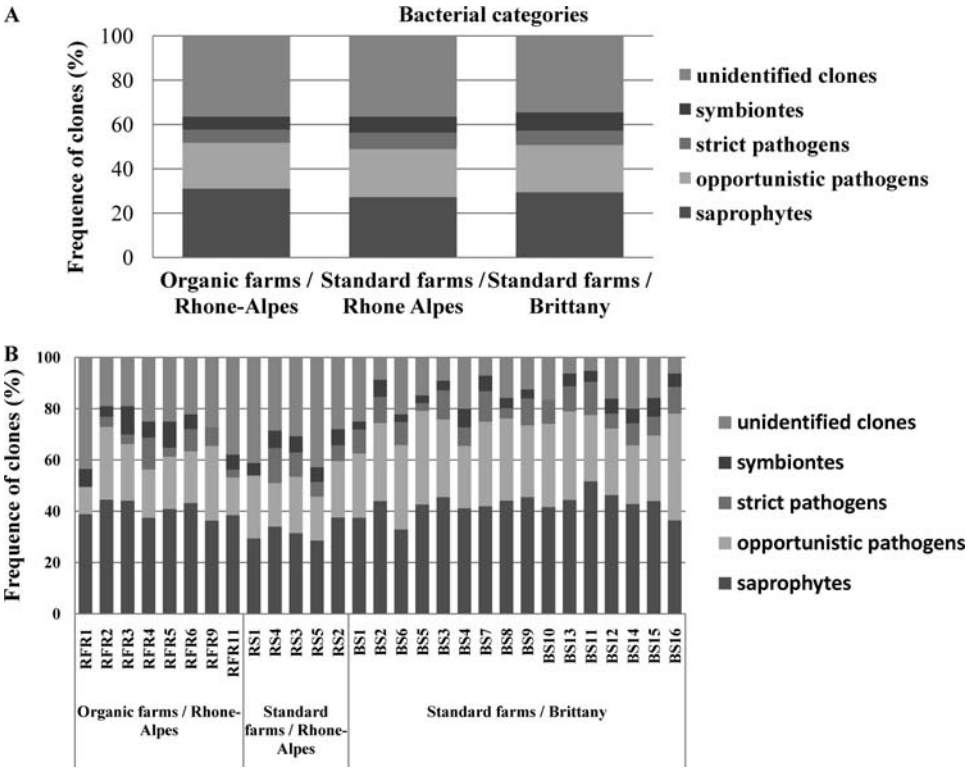

Fig. 2. Analysis of bacterial community according to their biological category. Bacterial taxa identification was obtained from the sequencing of separate bacterial 16S rRNA fragments after PCR-TTGE migration. The data for *D. gallinae* from standard poultry farms in Brittany were obtained from a previous study (Valiente Moro et al. 2009). (A) Overall analysis by regrouping all farms of a same category. (B) Detailed analysis by taking into account each farm of a specific habitat.

right. Moreover, the diversity (projected variance) of the five samples from each farm was clearly higher for free-range than for standard farms.

BGA on Temporal Variations. Statistical analyses were also used to test for a year effect on bacterial community composition. Two specific farms were observed over a period of consecutive 3 yr (RFR7, RFR8, and RFR9) or at a 3-yr interval (RFR10 and RFR11). BGA showed no difference for these two farms compared with the others, and no particular structure for the corresponding chronological successions.

Interregion and Interhabitat Comparisons. Another BGA was used to compare the TTGE profile variability for different geographical regions and habitat type simultaneously. All the molecular community profiles were used, taking into account all the mite samples independently of their origin (Fig. 5). All the samples belonging to the Rhone-Alpes standard farms were grouped together in the top left part of the graph. Those corresponding to the free-range farms in the Rhone-Alpes are clustered into another group in the bottom left of the graph. Those from the standard farms in Brittany were located in the right part of the graph. Only one farm (BS16) did not cluster with its original group (standard farm in Brittany) and was found close to standard farms in the Rhone-Alpes. The global permutation test was also extremely significant ($P < 0.001$). BGA showed that the interregion variability was comparable to the interhabitat variability, but revealed pronounced differences in bacterial contents according to the type of habitat (battery versus free-range) and geographical location (Brittany versus Rhone-Alpes).

Discussion

In light of recently published data on the bacterial community associated with the chicken mite in standard poultry farms, it seemed interesting to study the stability or variation of this community according to external factors such as geographic origin or type of poultry facility (Valiente Moro et al. 2009). Microbiota comparison between mites originating from standard and free-range farms revealed significant clustering with respect to poultry habitat. These habitat differences may reflect major ecological differences between sites leading to a variation in the distribution of mite populations, as already observed (Sparagano et al. 2009). In standard poultry farms, birds are bred in overlaid cages, with intensive production and submitted to an alternating, intensive lighting program. Conversely, the free-range method of farming husbandry is a method in which the animals are allowed to roam freely instead of being confined in any way. So, one of

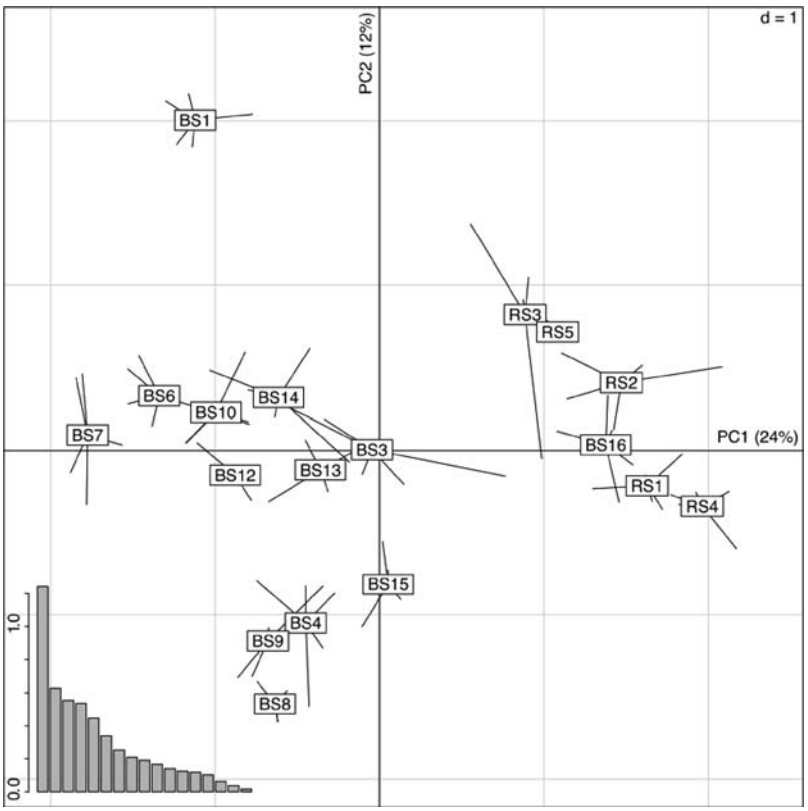

Fig. 3. BGA on TTGE profiles of *D. gallinae*-associated microbiota from farms in Brittany and the Rhone-Alpes. BS = Brittany-standard farms. RS = Rhone-Alpes-standard farms. The percentage of explained variance for each principal component is given along the corresponding axis. The eigenvalues bar chart of the between-farms BGA is given in the lower left corner of the graph. The value *d* in the upper right corner is the size of the background grid and gives the scale of the graph. The five samples from each farm are grouped and linked to the farm label to form a five-pointed irregular star. Farms in Brittany are located on the left of the graph, and are in opposition to farms in the Rhone-Alpes, which are on the right. The only exception is farm BS16, which is located among the farms in the Rhone-Alpes.

the main differences between the two systems is the potential for the introduction of bacteria from outside by wild animals or even mites from wild birds. With few exceptions, the microbial colonization of most arthropod species has not been studied and the terminology still remains unclear. The assumption is that many species initially derive their microbiota from the surrounding environment, such as the skin of the animal host, but the persistence of strains of the ingested species is unknown. Dillon and Dillon (2004) used the term "locally indigenous microbiota" to describe the microorganisms acquired by individual insects, which multiply within the gut, but are not necessarily present in all members of a single community. This term implies that a range of microbial species acquired from the external environment may occupy the same niche, but allows that the microbial species involved may interact positively with the insect host. Other factors such as heredity or diet may also influence its composition. It is well known that chicken mites parasitize chickens only at night to suck their blood and then return to cracks, crevices, and droppings in the chicken house before day break (Kirkwood 1963). As a result of the hematophagous character of *D. gallinae*, transient microbes such as pathogens could be ingested with the blood meal. All these observations on the behavior of mites in their surrounding habitat lead to the question of how they are affected by external factors (introduction of new population of mites from wild birds, contact with external source of bacteria . . .). Studies on the population genetics of chicken mites in different buildings could improve understanding of these points. A clustering according to the location of the farms was also obtained from mite samples originating from Brittany and the Rhone-Alpes, confirming the role of the geographic area in microbiota composition. Comparison between both variability levels obtained showed a high influence of the habitat effect (standard versus free-range), comparable to the geographical location effect (Rhone-Alp versus Brittany). From the temporal analysis of these microbiota, no tendency was noted, suggesting some degree of stability of bacterial populations over time and under these breeding conditions. Despite the fact that the laying hens are renewed each

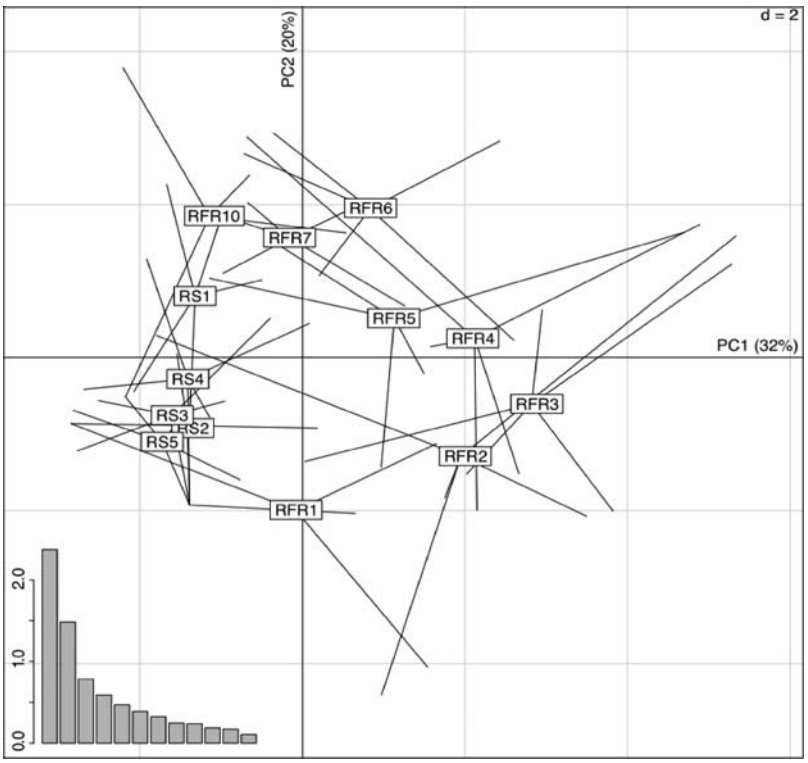

Fig. 4. BGA on TTGE profiles of *D. gallinae*-associated microbiota from standard and free-range farms in the Rhone-Alpes. RFR = Rhone-Alpes-free-range farms. RS = Rhone-Alpes-standard farms. The percentage of explained variance for each principal component is given along the corresponding axis. The eigenvalues bar chart of the between-farms BGA is given in the lower left corner of the graph. The value *d* in the upper right corner is the size of the background grid and gives the scale of the graph. The five samples from each farm are grouped and linked to the farm label to form a five-pointed irregular star. Standard farms are located on the left of the graph, and are in opposition to free-range farms, which are on the right. The within-farm diversity for free-range farms is higher than for standard farms.

year, *D. gallinae* microbiota does not follow any particular ecological succession related to hen populations, farm age, or season. It showed that these mites persist in buildings even between flocks of poultry. In line with previous comments on the differences between systems, it suggests that external factors could influence their composition, but not their stability. Here again, data regarding the population genetics of mites over several years should improve our understanding. In addition to between-region and between-farm variability, there were minor differences between mite samples taken from the same farm, suggesting that bacterial patterns are specific to individuals rather than to poultry farms. The substantial heterogeneity in microbiota distribution suggests that strong ecological factors operate that maintain a habitat-specific diversity of the bacteria. The difference in composition in bacterial species between farms may not indicate absolute change in some community members, but may be indicative of a shift in the dominant species in relation to the environmental context.

Most arthropod species are inhabited by large and, diverse microbial communities in their digestive system, with the host-microbe relationship ranging from pathogenic to mutualistic (Casadevall and Pirofski 2000, Dillon and Dillon 2004). The most intriguing aspects of the gut bacteria-arthropod association are how the multispecies bacterial communities are structured, the function of each group or species, and how these functional groups interact. The only information available on this is from studies on the functions performed by the termite microbiota and how the different metabolic groups coexist and complement one another (Warnecke et al. 2007). In mites and particularly oribatid mites, bacterial communities have been shown to vary depending on the species, the age of the specimens, the habitats from which they were isolated, and the substrates on which they fed (Seniczak and Stafaniak 1978, Wolf and Rockett 1984). In our study, no beneficial characteristics were attributed to bacterial categories identified in *D. gallinae,* and their widespread presence in soil, plants, and water suggests that these bacteria are more likely to be transient microbes rather than a permanent flora of mites. This could be the case for *P. multocida* and *E. rhusiopathiae*, which have been detected in *D. gallinae.* The distribution of these pathogens in mites originating from farms in the same region has been observed to be relatively stable. Conversely, when analyzing spatial

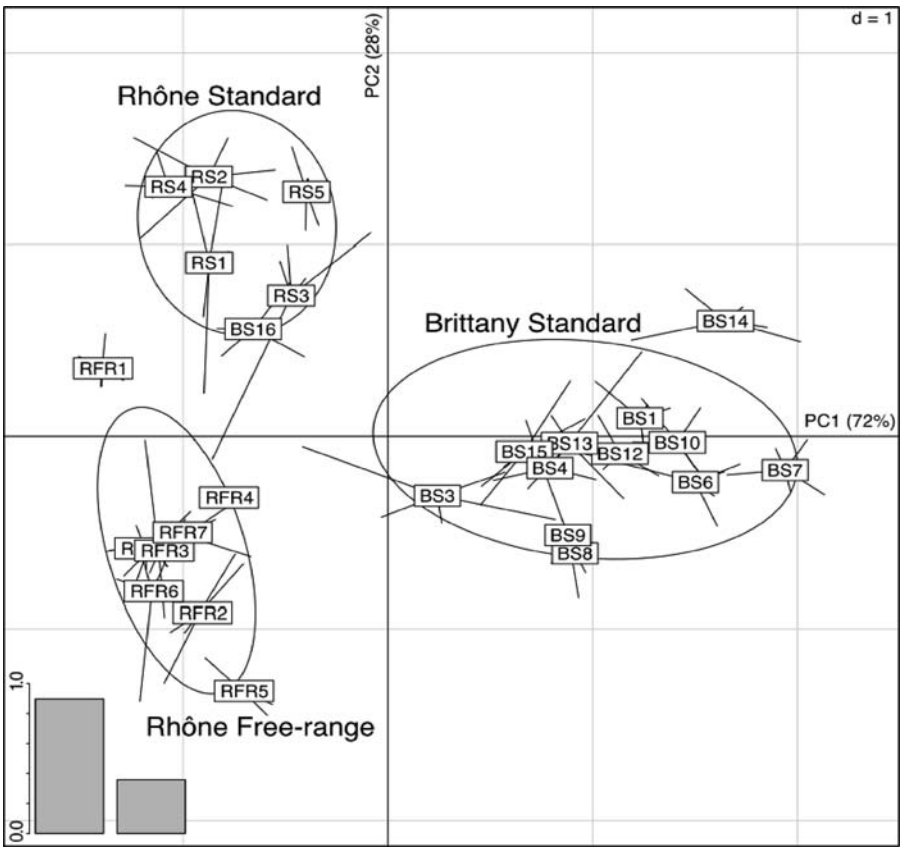

Fig. 5. BGA on TTGE profiles of *D. gallinae*-associated microbiota for all the farms. RFR = Rhone-Alpes-free-range farms. RS = Rhone-Alpes-standard farms. BS = Brittany-standard farms. The percentage of explained variance for each principal component is given along the corresponding axis. The eigenvalues bar chart of the between-farms BGA is given in the lower left corner of the graph. The value *d* in the upper right corner is the size of the background grid and gives the scale of the graph. The five samples from each farm are grouped and linked to the farm label to form a five-pointed irregular star. The three ellipses are the inertia ellipses corresponding to the three groups of farms. Farms in the Rhone-Alpes are located on the left of the graph, and are in opposition to farms in Brittany, which are on the right. Free-range farms in the Rhone-Alpes are in the lower left part of the graph, and are in opposition to standard farms, which are in the upper part. These three groups of farms are almost completely separated, with the exception of farm BS16, which is located among the standard farms in the Rhone-Alpes. The within-farm diversity is comparable for the three groups.

variation in the distribution of *Borrelia burgdorferi* sensu lato genospecies in questing ticks in a region of East Europe, Etti et al. (2003) observed a high degree of local variation in the distribution of these bacteria. Therefore, it would be noted that because of the limited sampling and bias of the method used, the diversity of cryptic microbiota should usually be underestimated. In effect, our results only show the bacteria that had been successfully amplified with PCR-TTGE. Between 20 and 80% of bacteria still remain to be identified if we are to have an idea of the real bacterial diversity associated with the chicken mite. Mites may harbor additional bacterial taxa that yield little or no 16S rRNA gene PCR amplification products using this method, as a result of PCR primer bias, low template abundance, or other factors, as already shown (Muyzer and Smalla 1998).

Conclusions

To date, although mite populations represent a wide and rich group of species, studies carried out on their associated microbial populations have been poorly documented. These new findings on an ectoparasite model will lead to further investigations as to whether such microbiota impacts the host by assessing their effect on host longevity, physiology and metabolism, development and copulatory success, or transmission of mite-borne diseases, as has already been suggested for other important medical and veterinary ectoparasites.

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