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Niche segregation and genetic structure of *Campylobacter jejuni* populations from wild and agricultural host species

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Abstract

Bacterial populations can display high levels of genetic structuring but the forces that influence this are incompletely understood. Here, by combining modelling approaches with multilocus sequence data for the zoonotic pathogen *Campylobacter*, we investigated how ecological factors such as niche (host) separation relate to population structure. We analysed seven housekeeping genes from published *C. jejuni* and *C. coli* isolate collections from a range of food and wild animal sources as well as abiotic environments. By reconstructing genetic structure and the patterns of ancestry, we quantified *C. jejuni* host association, inferred ancestral populations, investigated genetic admixture in different hosts and determined the host origin of recombinant *C. jejuni* alleles found in hybrid *C. coli* lineages. Phylogenetically distinct *C. jejuni* lineages were associated with phylogenetically distinct wild birds. However, in the farm environment, phylogenetically distant host animals shared several *C. jejuni* lineages that could not be segregated according to host origin using these analyses. Furthermore, of the introgressed *C. jejuni* alleles found in *C. coli* lineages, 73% were attributed to genotypes associated with food animals. Our results are consistent with an evolutionary scenario where distinct *Campylobacter* lineages are associated with different host species but the ecological factors that maintain this are different in domestic animals such that phylogenetically distant animals can harbour closely related strains.

Keywords

admixture; *campylobacter*; introgression; multilocus sequence typing

Introduction

Campylobacteriosis, caused principally by the species *C. jejuni* and *C. coli*, is the most common form of bacterial gastroenteritis worldwide. The sporadic nature of human disease, together with the number of potential sources of infection and high genetic diversity of *Campylobacter*, has impeded attempts to understand the epidemiology and ecology of this organism. Recently, large-scale population sampling studies combined with nucleotide

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Data accessibility: Nucleotide sequence data were amalgamated from Colles *et al.* 2008a; McCarthy *et al.* 2007; Rotariu *et al.* 2009; Sheppard *et al.* 2009b, 2008.

sequence-based typing of isolates have enabled model-based attribution of the main sources of human infection as being farmed animals, particularly poultry (Sheppard *et al.* 2009b; Strachan *et al.* 2009; Mullner *et al.* 2010; Pires *et al.* 2010). As with other zoonotic bacteria, the ability to colonize multiple hosts is a key feature of the ecology of *Campylobacter*, and a better understanding of the relationship between host niche and lineage structure is important for interventions aimed at reducing disease.

If bacterial populations become separated from a recombining ancestral gene pool, for example when colonizing a new niche, they may diversify over time giving rise to distinct niche-associated ecotypes (Cohan & Koeppel 2008). Analysis of *C. jejuni* and *C. coli* populations has shown that there is substantial genetic differentiation between isolates from different host sources (McCarthy *et al.* 2007) (Colles *et al.* 2008b; Sheppard *et al.* 2010a,b), but the importance of ecological isolation in generating and maintaining this population structure remains poorly understood. Furthermore, horizontal gene transfer (HGT), mostly involving the replacement homologous sequence from closely related donor lineages (Fraser *et al.* 2007), is frequent in *Campylobacter* both within (Suerbaum *et al.* 2001) and between microbiological species (Sheppard *et al.* 2008, 2011) and this may disrupt clonal signals of descent.

Here, we investigate niche segregation and genetic admixture among genetically distinct, but ecologically similar, *C. jejuni* that inhabit the intestinal tract of many farm and wild animal species (Rosef *et al.* 1983; Waldenstrom *et al.* 2002; Miller *et al.* 2006; Sheppard *et al.* 2009a). We use multilocus sequence typing (MLST) data that describe the genetic variation at seven housekeeping loci and a model-based technique for assigning ancestral populations, to estimate the number of host-associated lineages and the level of admixture within *C. jejuni* populations from multiple sources. The degree of lineage association with different host species is analysed, and the origin of recombinant *C. jejuni* alleles found in *C. coli* sequence types (STs) is determined. We test two hypotheses. First, that host origin is a predictor for *C. jejuni* genotype and second, that recombinant *C. jejuni* alleles in hybrid *C. coli* lineages are unevenly distributed among host origin populations.

Methods

Sequence type (ST) data sets

Nucleotide sequence data from seven housekeeping loci of 2732 *C. jejuni* isolates were amalgamated from published data sets widely sampled in England and Scotland (McCarthy *et al.* 2007; Colles *et al.* 2008a; Sheppard *et al.* 2008, 2009b; Rotariu *et al.* 2009). These isolates originated from nine source categories: chicken (1284 isolates), farm ruminants (829 isolates), passerine birds (288 isolates), ducks and geese (103 isolates), pigeons and doves (69 isolates), unidentified gulls (43 isolates), unidentified wild birds (26 isolates), pheasants (5 isolates) and sand and environmental waters (85 isolates). To investigate interspecies introgression, these *C. jejuni* isolates were designated the donor group and a group of 48 hybrid *C. coli* STs – previously shown to contain alleles from both *C. coli* and *C. jejuni* (Sheppard *et al.* 2008) – constituted the recipients.

ST genealogy

A consensus genealogy of concatenated ST sequences was constructed using CLONALFRAME (Didelot & Falush 2007). This method is designed to estimate genealogies in recombining bacteria. It identifies each tract of nucleotide polymorphisms originating from HGT as a single event. Each analysis consisted of 50 000 burn-in iterations followed by 50 000 data collection iterations. The consensus genealogy was based on three independent analyses, each with a cut-off of 75% consensus for inferring relatedness.

To test the associations between relatively recently diverged lineages in the $C_{\text{LONALFRAME}}$ genealogy, a clade was defined as a lineage containing two or more STs that share a common ancestor at 0.04 coalescent units on $C_{\text{LONALFRAME}}$ trees.

This coalescent depth was selected because model inferences of deep branches are less reliable. We therefore took the smallest cut-off that allowed definition of clusters. The cut-off was not on the basis of clade membership and as such does not bias the results. The degree of association of these multi-ST clades with host species was tested by calculating a score for the association of a clade with a particular host and then permuting the data set randomly 10 000 times to allow a comparison of the observed score to the range of scores that might arise randomly. The association score was calculated by: (i) allocating each ST to one species if only isolated from that species and equally between two species if isolated from both, (ii) making a score for each clade of the number of STs isolated from only one species minus the number isolated from any of the other species and (iii) summing the positive value of these scores across all clades. Permuted scores were obtained by randomly assigning each ST among the clades and then calculating the score as above.

Clustering and admixture

The number of ST clusters (K) and the level of admixture among clusters were estimated using BAPS 5.1, a Bayesian method for inferring admixture events (Corander & Marttinen 2006). STs were assumed to consist of linked loci arranged linearly, K was assumed to lie in the range 1-20 and estimates were based on the combined results from 50 analyses. Clusters in *C. jejuni* were characterized according to the percentages of each cluster that originated from seven of the nine sources of isolates (Table 1). The other two sources were excluded: pheasants because of the small sample size and unidentified wild birds because of ambiguous source identification. Clusters were categorized as host source-associated when a source accounted for more than 60% of the isolates in the cluster.

Source attribution of interspecific recombination

The source attributions of the hybrid *C. coli* STs containing alleles from *C. jejuni* (Sheppard *et al.* 2008) were estimated by analysing the BAPS 5.1 output files using BRAT, a Bayesian program for the detection of recombination events in bacterial populations (Marttinen *et al.* 2008). Hybrid *C. coli* STs were analysed individually, and 48 alleles, previously identified as having originated from transfer from *C. jejuni* to *C. coli* (Sheppard *et al.* 2008), were assigned to the BAPS-defined clusters. The mean probability of this population representing each of two putative populations (farm and non-farm or poultry and ruminant) was then calculated to determine the overall population assignment probability for each recombinant allele. These values were used to determine the putative origin of the recombinant allele. The BRAT model does the assignment on the basis of marginalization of sitewise posterior probabilities from Bayes' formula such that it takes into account both the level of molecular variation and uncertainty about cluster parameters. Therefore, large BAPS clusters are not automatically favoured by their sheer size, because they can be more heterogeneous and thus fail to give conclusive assignment probabilities (Marttinen *et al.* 2008). Statistical testing of association with farm or non-farm niches and between chicken and ruminant hosts was undertaken using the binomial confidence interval in Stata with a significant association when the whole confidence interval was on one or other side of 0.5.

Results and discussion

In the genealogy estimated using $C_{\text{LONALFRAME}}$, there was evidence for genetic structuring of STs by host source (Fig. 1A). The 2732 *C. jejuni* isolates contained 715 STs. In total, 39 (5%) STs of 545 that occurred in farm sources occurred in both chicken and ruminant and

the remainder occurred in only one of these species. A total of 132 STs occurred in wild birds, of which 21 (3%) STs also occurred in farm sources. Only eight (1%) of the 132 STs occurred in more than one species of wild bird. Although lineages of closely related STs sometimes included isolates from both farm (chicken and ruminant) and wild bird sources (Fig. 1a), results of the permutation test confirmed that different STs in the same lineage were far more likely to originate within the same host species than would be predicted by chance alone. Although individual lineages showed host association, these host-associated lineages were not clustered at a deeper phylogenetic level (Fig. 1b).

B_{APS} analysis of all 2732 *C. jejuni* isolates (715 STs) yielded 18 most likely clusters. Eleven of which were source-associated (Table 1): three (1, 3 and 16) with chicken, three (5, 9 and 11) with ruminants, two (7 and 14) with duck and geese and one each with passerines (4), pigeons and doves (13), and gulls (15). Four further clusters (6, 8, 10 and 17) were associated with farm sources, *i.e.* the chicken and ruminant sources combined. Likewise, 14 of the 17 clusters from the second group of isolates from chicken and ruminants were source-associated: eight with chicken and six with ruminants, and the remainder with both sources.

Host differentiation by genotype can be obscured because of genetic admixture and the migration of bacteria between host species. The B_{APS} software was originally designed for analysis of historical admixture indicative of ancestral interdependence and not recent introgression in bacteria. It has been used to investigate gene flow in bacteria (Tang *et al.* 2009), and here, we demonstrate that the B_{APS} approach was effective in describing the population structure and recent evolutionary events within *C. jejuni*, giving results consistent with independent methods for inferring relatedness among STs including clonal complex designations and the C_{LONALFRAME} genealogy. For example, 87% of isolates in B_{APS} cluster 11 are from ruminants (Table 1). This cluster corresponds to a ruminant-dominated lineage on the C_{LONALFRAME} tree (Fig. 1a) and the ST-61 clonal complex which has previously been shown to be ruminant associated (Sheppard *et al.* 2010a).

Genotypes from ducks and geese, pigeons and doves and passerine birds all had distinct genetic separation with relatively low levels of admixture. This was not the case for wild birds of unknown origin and genotypes from environmental samples, which had no coherent genetic structure and were generally assigned to other wild bird groups and unknown-farm origin. The results suggest that *C. jejuni* genotypes sampled from the abiotic environmental (water and sand) were effectively faecal samples derived from a mixture of origins, some of which are unknown. While *Campylobacter* can survive in the environment, it is not generally considered to proliferate outside of the animal host (Rollins & Colwell 1986; Evans *et al.* 2003; Richardson *et al.* 2007) and, therefore, isolates most likely represent recent contamination from other sources. Host-associated lineages were also evident among chicken and ruminant isolates. However, in these sources, there were also large numbers of isolates of chicken or ruminant origin (Fig. 2). This could be explained by: (i) HGT between isolates associated with chickens and ruminants, (ii) movement of lineages between these host niches or (iii) recent shared ancestry of these lineages, resulting in insufficient genetic variation among housekeeping loci to allow segregation by host using B_{APS}.

The finding that host or niche (farm) origin is a predictor for *C. jejuni* genotype provided a framework for determining the source of alleles from *C. jejuni* that were introgressing into *C. coli* populations. Of the 48 recombinant alleles, 22 were assigned with probability >0.75 to a farm niche and eight to non-farm, with the remainder (18) not assignable to either source. This demonstrates a significant association of recombinant *C. coli* alleles with this niche (0.73, 95% CI 0.57–0.91). Considering different farm animals, there was a weak evidence for an association with chicken, which does not reach statistical significance.

Overall, 19 alleles were chicken associated and 10 were more strongly associated with ruminants (0.66, 95% CI 0.47–0.84) using the same 0.75 cut-off. If considering host association restricted to those isolates assessed as being farm associated, only 12 show evidence of association with either chicken (nine) or ruminant (three) source (0.75, 95% CI 0.46–1). Therefore, where assignment was possible, a significant number (73%) of interspecies recombinant alleles originated in farm-associated (chicken or ruminant) *Campylobacter* genotypes (Fig. 3). This supports the idea that recombinant alleles in hybrid *Campylobacter* lineages are unevenly distributed among host origin populations and concurs with existing observations (Sheppard *et al.* 2008).

The less pronounced separation by host species and greater admixture among *Campylobacter* species in farm animals, as compared to wild birds, is consistent with the agricultural niche being a potential site for horizontal gene transfer. The dominance of genetically similar or identical ‘farm-type’ *C. jejuni*, present in organisms as distantly related as cattle and chickens, may suggest that selection for these types transcends host species in the farm environment. Compared to their wild predecessors, livestock animals represent a very different host niche for *Campylobacter*, i.e. potentially able to promote increased recombination, in some cases across species boundaries. An example of this difference is seen in modern intensive poultry farming – the origin of many of the farm-type interspecies recombinant alleles. During the year 2000, an estimated 20×10^9 birds were in production, the majority of which were reared at high stocking densities in intensive systems and slaughtered after 36–50 days (Scientific Committee on Animal Health and Welfare 2000). This is a dramatically different niche to the wild jungle fowl ancestors.

In conclusion, large-scale genotyping and statistical genetics techniques have enabled an improved understanding of the link between the ecology and evolution of *Campylobacter*. The data presented here are consistent with anthropogenic manipulation of the host niche having potentially affected lineage associations seen in wild hosts. This may have contributed to genetically distant domestic animals (chickens and cattle) sharing similar genotypes. Changing adaptive landscapes or removing ecological barriers to recombination may be important factors in the emergence of pathogenic genotypes, and understanding these processes is an important step towards ameliorating some of the harmful consequences of environmental change.

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Fig. 1. Genealogical tree of *C. jejuni* and lineage association with host.

(a) $C_{LONALFRAME}$ genealogy of *C. jejuni* STs of isolates from published data sets. The source of isolates is indicated by colour for STs from chickens (red), ruminants (blue), chicken and ruminants together (grey), ducks and geese (yellow), gulls (light blue), pigeons and doves (brown) and passerines (purple). Isolates from pheasants, unidentified birds and the environment were not included on the tree. Open circles indicate STs found in both a farm animal and wild bird host. The dominant clonal complex and B_{APS} cluster (in brackets) associated with each clade is indicated. The scale bar gives the genetic distance in coalescent units. (b) Comparison of a measure of association between lineages (clades) and isolate source species observed with that expected by chance in *C. jejuni* from chickens, ruminants, ducks and geese, pigeons and doves, gulls, and passerines. Arrows indicate the observed measure of association, and histograms are the range of results from 10 000 random permutations of the data set.

Fig. 2. Genetic structure of *C. jejuni* genotypes from different host groups.

Each bacterial isolate is represented by a vertical bar, showing the probability that it originates from each of the putative group defined using BAPS. The sources associated with each BAPS cluster are indicated by different colours. If each host/niche group had a genetically distinct population with no admixture between sources, then the charts would be uniform in colour for chicken (red), ruminants (blue), the environment (green), ducks and geese (yellow), gulls (light blue), pigeons and doves (brown) and passerine birds (purple). Isolates coloured grey represent farm types that are associated with both chicken and ruminant sources. Pheasants and unidentified birds were not included.

Fig. 3. Origin of interspecies recombinant alleles.

Alleles from hybrid STs ($n = 48$) were assigned to host-associated B_{APS} cluster origin populations using B_{RAT} . Each isolate is represented by a vertical bar, showing the estimated probability that it originates from each of the putative sources indicated by different colours. Two independent analyses were carried out assigning hybrid alleles to (a) farm (■) and non-farm (□), and (b) chicken (■) and ruminant (■). An equal area of each colour would be expected in the absence of genetic differentiation by host association.

Table 1
Proportions of isolates (%) in each Baps-defined cluster that were collected from the seven sources

Source	Cluster [*]																	
	1 (230)	2 (44)	3 (51)	4 (265)	5 (38)	6 (147)	7 (70)	8 (279)	9 (53)	10 (595)	11 (172)	12 (131)	13 (73)	14 (111)	15 (53)	16 (20)	17 (15)	18 (385)
Farm	83	6	69	0	65	61	1	63	97	85	94	49	0	17	0	100	73	39
Chicken	73	3	67	0	2	31	1	30	1	38	7	10	0	15	0	67	33	34
Ruminants	10	3	2	0	62	30	0	33	96	47	87	39	0	2	0	33	40	5
Non-farm	17	94	31	100	36	39	99	37	3	15	6	51	100	83	100	0	27	61
Environment	4	27	5	18	0	24	1	33	3	10	6	9	13	6	13	0	27	25
Ducks and geese	0	9	24	0	1	8	95	1	0	2	0	0	1	63	5	0	0	10
Gulls	3	13	0	2	35	6	3	0	0	0	0	36	6	11	76	0	0	8
Pigeons and doves	6	16	0	3	0	1	0	0	0	2	0	3	79	3	4	0	0	14
Passerine birds	4	29	2	77	0	0	0	3	0	1	0	3	1	0	2	0	0	4
Dominant clonal complex(es)	257	952	661	177,682	403	22,48,353	702	206,354,443	21,52,574,607	61	42	179	573,692,1034,1287	1275	45	45	283	45,283

The sum of isolates (%) from farm and non-farm subcategories is given in bold.

* The number in each cluster (sum of probabilities) is in brackets below to the cluster number.