Phase variation mediated niche adaptation during prolonged experimental murine infection with Helicobacter pylori

Laurence Salaùn,¹ Sarah Ayraud² and Nigel J. Saunders¹

¹Bacterial Pathogenesis and Functional Genomics Group, The Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK
²Laboratoire de Microbiologie A, CHU La Milètrie, Université de Poitiers, France

Changes in the repeats associated with the recently redefined repertoire of 31 phase-variable genes in Helicobacter pylori were investigated following murine gastric colonization for up to one year in three unrelated H. pylori strains. Between the beginning and end of the experimental period, changes were seen in ten genes (32%), which would alter gene expression in one or more of the three strains studied. For those genes that showed repeat length changes at the longest time points, intermediate time points showed differences between the rates of change for different functional groups of genes. Genes most likely to be associated with immediate niche fitting changed most rapidly, including phospholipase A (pldA) and LPS biosynthetic genes. Other surface proteins, which may be under adaptive immune selection, changed more slowly. Restriction-modification genes showed no particular temporal pattern. The number of genes that phase varied during adaptation to the murine gastric environment correlated inversely with their relative fitness as previously determined in this murine model of colonization. This suggests a role for these genes in determining initial fitness for colonization as well as in subsequent niche adaptation. In addition, a coding tandem repeat within a phase-variable gene which does not control actual gene expression was also investigated. This repeat was found to vary in copy number during colonization. This suggests that changes in the structures encoded by tandem repeats may also play a role in altered protein functions and/or immune evasion during H. pylori colonization.

INTRODUCTION

Phase variation is a mechanism of reversible ON/OFF gene switching normally mediated by DNA mutation or modification. It is strongly associated with genes involved in adaptation to different environmental conditions, such as adaptation to different sites of infection, different steps of host interaction and immune evasion (reviewed by Salaùn et al., 2003; Saunders, 2003; van der Woude & Baumler, 2004). This process is frequently associated with virulence and an organism’s capacity to establish and maintain colonization and cause disease.

Helicobacter pylori is known to phase vary many of its genes. Initial studies of the complete phase-variable gene repertoire were performed based upon analysis of the two completed genome sequences (Tomb et al., 1997; Saunders et al., 1998; Alm et al., 1999). Using a previously established comparative approach (Snyder et al., 2001), this repertoire was recently reassessed and extended, based upon the two completed genome sequences. These candidate genes and their associated repeat polymorphisms have been investigated in a representative sample of unrelated strains (Salaùn et al., 2004), based upon the multilocus sequence typing (MLST)-based population structure associated with human migration (Falush et al., 2003), on which basis a group of 30 genes has been established as phase variable, or very likely to be phase variable, in this species. An additional phase-variable gene, described by De Vries et al. (2002), has been included in the study reported here.
flagella expression and motility (Josenhans et al., 2000), adhesion properties (Ilver et al., 1998; Peck et al., 1999; Yamaoka et al., 2000; Mahdavi et al., 2002) and adaptation to acidic environments (Tannaes et al., 2001).

To date, no study has addressed the behaviour of a complete phase-variable gene repertoire in adaptation to a complex infection model system in any species. This study was performed to gain an overall picture of the rapidity of phenotypic change, and to determine the relative importance of the different phase-variable genes, either singly or in combination, in adaptation to this colonization niche. We have assessed the repeat-associated changes in the 31 repeat-associated phase-variable genes in the mouse model of gastric colonization, using three unrelated H. pylori strains. We find that all the functional classes of phase-variable genes show changes over the course of the infection, that the time-course of changes differs between different classes of genes, and that the number of changes observed can be related to the relative fitness of the strains within the model system. We also report variations in a coding tandem repeat structure within one of the phase-variable genes that also shows variation indicative of functional selection.

METHODS

The bacterially derived materials used in this study were derived from the previously described study of Ayraud et al. (2003). It should be noted that the samples used differ in one important respect from those described in the former publication. For the purposes of this study, as described below, samples were deliberately prepared from multiple colonies. This approach avoids sampling single colonies, which might be unrepresentative of the larger colonizing population, and allows the presence of mixed populations to be observed, thereby providing an indication of the degree and time-course of changes.

H. pylori strains. Three H. pylori strains were used for this study: the Sydney strain (SS1), previously adapted to the mouse stomach (Lee et al., 1997), and two strains (Hp141 and Hp145) freshly isolated from patients (Ayraud et al., 2003). Growth conditions prior to inoculation were as described by Ayraud et al. (2003). The strains Hp141 and Hp145 were isolated from human stomach biopsies onto Skirrow medium (BioMérieux), and the strain SS1 was grown on blood agar. The three strains were subcultured once in Brucella broth (BioMérieux) for 2 days to prepare the infecting inocula.

Experimental infections. Experimental infections were conducted with 6-week-old female C57BL/6 inbred mice, as previously described (Ayraud et al., 2003). Animals were serially killed (two mice for each sacrifice time point), and their stomachs entirely removed after sacrifice and homogenized in 1 ml Brucella broth. The resulting suspension was serially diluted and cultured on Skirrow medium. Ten to fifteen colonies from the stomach culture of each of the two mice were randomly selected from each sacrificial day for each infecting strain. The colonies obtained from the two mice were pooled together and DNA was extracted for further experiments. Genomic DNA of the infecting strains and the emerging strains was extracted with purification columns (QIAmp DNA minikit, Qiagen) according to the manufacturer’s instructions.

Amplification and sequencing of the repeat-containing regions. At first, only the infecting strain and the strain obtained at day 150 for SS1 (the latest time point available for this strain) and at day 360 for Hp141 and Hp145 were analysed; then, if a change in phenotype was observed at this extended time point, the strains emerging at day 3 and at day 21 were also analysed. PCRs were performed to amplify the regions of the putative phase-variable genes containing the potentially variable repeats. Primers used in this study for the amplification of phase-variable genes were described previously (Salau¨n et al., 2004). In order to investigate variation in the number of tandem repeats, primers were designed to amplify the two full z-1,3-fucosyltransferase genes (HP0651 and HP379): HP0651WF (5’-caacgcttgatgtagctagc-3’) and HP651WR (5’-ccttgctgcttgctctctct-3’); HP379WF (5’-ggctgctggctgctgctgct-3’) and HP379WR (5’-ggctgctggctgctgctgct-3’). The product amplified by the existing primers for HP0619 already included the tandem repeat. PCRs were carried out using Taq DNA polymerase (Invitrogen) according to the manufacturer’s instructions. PCR products were sequenced using the PCR primers. Sequencing was performed using ABI Prism BigDye Terminator cycle sequencing, version 3.0 (Applied Biosystems) and was resolved on an ABI Prism 3100 DNA sequencer (Applied Biosystems). Sequences were read, edited and aligned using Seqlab, from the Wisconsin Package, version 10.2 (Genetics Computer Group) through the Oxford University Bioinformatics Centre. The ON/OFF status of the phase-variable genes was determined on the basis of the repeat lengths.

The amplification and sequencing methods were exactly the same as those previously extensively tested and validated for working and interpreting repeat lengths (Salau¨n et al., 2004), in which genomic templates and cloned templates were compared, and example electropherograms are presented.

RESULTS AND DISCUSSION

Phase variation is a process of stochastic reversible ON and OFF switching of genes that typically provide adaptive advantages to different environmental conditions. Adaptation mediated by this mechanism provides competitive advantages for colonizing subpopulations that are more adapted to their environmental conditions than their co-colonizing neighbours, so that they rise to dominance through a process of clonal replacement. The rate of change of the population composition is primarily determined by the relative fitness differences between the alternate phenotypes (Saunders et al., 2003). We have used a redefined list of phase-variable genes identified in H. pylori (Salau¨n et al., 2004) to perform the first study of the changes within a complete repertoire of phase-variable genes in adaptation to a model infection system.

Although the murine experimental model of infection has its limitations with respect to the presence of human host-specific ligands, it is nevertheless one of the most established and widely used tools for the investigation of H. pylori infection. It has been shown that strains are stable with regard to major chromosomal physical features, and within this model system, using the same strains and mice used for this study, the only change previously identified was within the ppk gene [Ayraud et al., 2003; L.S., unpublished]. Random amplified polymorphic DNA (RAPD) and multilocus enzyme electrophoresis (MLEE) data, Prolonged colonization of the gastric environment, as well as exposure to immunological responses, are common features of both
the model system and natural infection, and this and similar model systems are used extensively in investigations of colonization (Moran et al., 2000) and vaccine studies for this pathogen (Nedrud, 1999; Raghavan et al., 2002; Sommer et al., 2004).

The number of genes with altered repeat lengths that would affect expression

Prolonged colonization is associated with alterations in several phase-variable genes. Between the time of initial colonization and the longest time point (150 or 360 days), changes in the length of the repeats were seen in 15 of the 31 genes studied in one or more strains. Changes in five of these repeats would not alter the expression of the associated gene, for example, changing between two lengths associated with an OFF phenotype. Ten genes were associated with repeat length changes that resulted in alterations to in-frame shifting of the encoded proteins which would alter their expression status in one or more strains (Table 1). These included genes from each of the functional classes previously described (Saunders et al., 1998; Salaün et al., 2004), with the exception of frxA (HP0642), which is the only phase-varied gene associated with metabolic responses. This gene was maintained as ON in strains SS1 and Hp145, and OFF in strain Hp141, indicating an absence of a strong selective pressure for either phenotype in this model system.

Differences in the time course of altering different phenotypes

Differences in the rate of change indicate that different levels of selective pressure act on different genes during the course of infection. For each gene that showed altered expression at the end of the study period, intermediate genomic samples were investigated to determine the point at which changes occurred during colonization. The time points selected were at 3 days, to reflect adaptation to immediately acting selective environmental conditions, and at 21 days, to allow for the development of an adaptive immune response, although changes at this time point might also reflect weaker selection pressures acting over a longer time.

The phospholipase A gene (pldA) was among the most rapidly changing genes, with changes occurring within the first 3 days of colonization (Table 2). The change in pldA showed a phenotypic selection from an initial inoculum, which comprised a mixture of ON and OFF phenotypes, to an exclusively ON population. This is consistent with the application of a purifying selection on the population by the transition from in vitro conditions to conditions in the stomach, and also with the involvement of pldA in the adaptation to acid conditions that is necessary for residence in the stomach, as previously proposed (Tannaes et al., 2001). For what niche the alternate phenotype is adaptive is currently unknown.

Early variations in the status of the LPS biosynthetic genes (HP0379 and HP0217) were also observed. These changes may reflect an early role for LPS structures in colonization and acid resistance (Logan et al., 2000; Moran et al., 2002). In contrast to the results of our study, Webb & Blaser (2002) reported slower changes in the expression of these genes in their mouse-model infection study of LPS biosynthesis genes, in which the population reached its stable Lewis phenotypes distribution in about 20 weeks. However, these studies were done in C3H/HeJ mice, a lineage in which B cells and macrophages do not respond significantly to LPS (Wong et al., 1999), and in which the stomach ligands available for adhesion may differ. Such differences might account for the observed differences between these model infections.

In contrast, genes encoding outer-membrane proteins (babB and hopZ) changed status later in the course of infection (following 21 days infection), a time frame consistent with a role for phase variation in adaptation to avoid specific immune responses mounted against these exposed surface proteins. Notably, across all the genes showing alterations that would affect expression, the changes that were observed at earlier time points are consistent with those observed at one year. This suggests a relatively constant selective pressure on these phenotypes over the duration of the experiment and between the different individual mice, which contrasts with the progressive changes seen in some other model systems of LPS phase variation (for example, Inzana et al., 1992). This may be more consistent with a model in which some or all of these changes are associated with other interactions, although less strongly selected for than the most rapid changes, such as altered adhesion properties, as has recently been suggested by others (Solnick et al., 2004).

The status of the genes encoding enzymes of the restriction-modification system (e.g. HP1353-4 in Hp145) showed no particular pattern of changes over time, and did not show consistent changes in independently colonized mice. This suggests that there may be changes related to the relative fitness of the phase variants, but that they are not necessarily adaptive to specific constant environmental conditions present in this model system. This would be consistent with a model in which the alternate phenotypes are either associated with competition between subpopulations of colonizing bacteria, or are randomly co-selected within bacteria in which other genes that are under direct selection have phase varied.

It is impossible to determine the rate of phase variation or, therefore, to measure the actual selective pressure within a model infection system such as this, because there is no means by which to measure the number of bacterial divisions during colonization or accurately determine the exact composition of the initial inocula. Equally, the nature of this model requires serial sacrifice at the sampled time points, which precludes performing studies over time in individual mice. However, if some basic assumptions are
made, it is possible, on the basis of our published model system (Saunders et al., 2003), to determine the selective pressures or fitness differences between the strains that would account for changes in the time frames studied. If one assumes that the bacteria can divide once per hour and that there is a typical phase variation mutation rate of 1/10 000 per generation per cell, then 3 days of culture represents approximately 75 generations. A difference in fitness of 10% or greater between the alternate and original phenotype would normally result in complete replacement of the original phenotype in this time frame. This may well be an overestimate of the replication rate and if, for example, the rate of division were only once in 4 h, then a fitness difference of 50% or greater would be required to effect a similar change. By 3 weeks, fitness differences of only 2% or 6%, for 1 h and 4 h division rates, respectively, would bring about a complete replacement of one phenotype by another. This indicates that changes seen in the population may reflect quite subtle niche-selective pressures, and that even rapidly changing phenotypes, such as for pldA, may reflect moderate differences in the fitness of the associated phenotypes. It should be noted that these models and this experimental system do not distinguish between the positive selection for an adaptive change and the selection against a

Table 1. Repertoire of the 31 phase-variable genes of H. pylori and their status in the three mouse-adapted strains

<table>
<thead>
<tr>
<th>Function encoded by the phase-variable gene</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>LPS biosynthesis</strong></td>
<td></td>
</tr>
<tr>
<td>z-1,3-Fucosyltransferase</td>
<td>HP0651</td>
</tr>
<tr>
<td>z-1,2-Fucosyltransferase</td>
<td>HP0379</td>
</tr>
<tr>
<td>z-1,2-Fucosyltransferase</td>
<td>HP0093-4</td>
</tr>
<tr>
<td>Lex2B</td>
<td>HP0619</td>
</tr>
<tr>
<td>RfaJ (z-1,2-glycosyltransferase)</td>
<td>HP0208</td>
</tr>
<tr>
<td>RfaJ (z-1,2-glycosyltransferase)</td>
<td>No homologue</td>
</tr>
<tr>
<td>β-1,4-N-Acetylgalactoamyl transferase</td>
<td>HP0217</td>
</tr>
<tr>
<td><strong>Cell-surface-associated proteins</strong></td>
<td></td>
</tr>
<tr>
<td>FljB (flagellar protein)</td>
<td>HP0684-5</td>
</tr>
<tr>
<td>OM adherence associated protein</td>
<td>HP1417</td>
</tr>
<tr>
<td>Streptococcal M protein</td>
<td>HP0058</td>
</tr>
<tr>
<td>OipA (proinflamatory outer-membrane protein)</td>
<td>HP0638</td>
</tr>
<tr>
<td>TlpB (methyl-accepting chemotaxis protein)</td>
<td>HP0103</td>
</tr>
<tr>
<td>BabB (Lewis b antigen-binding adhesin)</td>
<td>HP0896</td>
</tr>
<tr>
<td>SábB (sialic acid-binding adhesin)</td>
<td>HP0722</td>
</tr>
<tr>
<td>SábA (sialic acid-binding adhesin)</td>
<td>HP0725</td>
</tr>
<tr>
<td>HopZ (adhesin)</td>
<td>HP0009</td>
</tr>
<tr>
<td>Oxaloacetate/malate translocator</td>
<td>HP0143</td>
</tr>
<tr>
<td>PldA (phospholipase A)</td>
<td>HP0499</td>
</tr>
<tr>
<td>HcpB (cysteine-rich protein B with β-lactamase activity)</td>
<td>HP0335</td>
</tr>
<tr>
<td><strong>DNA restriction/modification system</strong></td>
<td></td>
</tr>
<tr>
<td>Adenine-specific methyltransferase</td>
<td>HP1353-4</td>
</tr>
<tr>
<td>Type III restriction enzyme M protein</td>
<td>HP1369-70</td>
</tr>
<tr>
<td>Type II R/M enzyme β-subunit</td>
<td>HP1471</td>
</tr>
<tr>
<td>Type III R/M modification enzyme</td>
<td>HP1522</td>
</tr>
<tr>
<td>HsdR (type I restriction enzyme R protein)</td>
<td>HP0464</td>
</tr>
<tr>
<td>MboIIIR (type II restriction enzyme R protein)</td>
<td>HP1366</td>
</tr>
<tr>
<td>Type III restriction enzyme M protein</td>
<td>No homologue</td>
</tr>
<tr>
<td><strong>Metabolic proteins/other proteins</strong></td>
<td></td>
</tr>
<tr>
<td>FrxA (NAD(P)H flavin oxidoreductase)</td>
<td>HP0642</td>
</tr>
<tr>
<td><strong>Hypothetical ORFs without identified homologies</strong></td>
<td></td>
</tr>
<tr>
<td>Hypothetical protein</td>
<td>HP0744</td>
</tr>
<tr>
<td>Hypothetical protein</td>
<td>HP1433</td>
</tr>
<tr>
<td>Hypothetical protein</td>
<td>HP0767</td>
</tr>
<tr>
<td>Hypothetical protein</td>
<td>No homologue</td>
</tr>
</tbody>
</table>
Table 2. Follow-up of the changes in status of phase-variable genes of *H. pylori* during the course of infection in the mouse model

<table>
<thead>
<tr>
<th>HP no.</th>
<th>Repeat</th>
<th>Gene [time point (day)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP0379*</td>
<td>A</td>
<td>ON ON ON ON ON ON</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>OFF OFF ON OFF ON ON ON OFF OFF ON</td>
</tr>
<tr>
<td>HP0217*</td>
<td>G</td>
<td>OFF ON ON OFF ON OFF ON OFF ON ON</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>OFF OFF OFF ON OFF ON ON ON ON</td>
</tr>
<tr>
<td>HP0103</td>
<td>G-pro</td>
<td>(pro) (pro) (pro) (pro)</td>
</tr>
<tr>
<td></td>
<td>[TC]</td>
<td>OFF OFF OFF ON OFF OFF OFF OFF</td>
</tr>
<tr>
<td></td>
<td>[CT]</td>
<td>ON ON ON OFF ON ON OFF OFF OFF ON</td>
</tr>
<tr>
<td>HP0499</td>
<td>G</td>
<td>ON—OFF ON ON ON ON ON ON—OFF ON ON ON</td>
</tr>
<tr>
<td>HP1353-4*</td>
<td>G</td>
<td>G OFF Absent Absent</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>OFF OFF OFF OFF OFF OFF OFF OFF</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>OFF OFF OFF OFF OFF OFF OFF OFF</td>
</tr>
<tr>
<td>HP1522</td>
<td>G</td>
<td>OFF OFF OFF OFF OFF OFF OFF OFF</td>
</tr>
<tr>
<td></td>
<td>jhp1297 C</td>
<td>ON ON ON ON ON</td>
</tr>
<tr>
<td>HP0744</td>
<td>[AG]</td>
<td>ON OFF OFF OFF OFF ND ND ON</td>
</tr>
<tr>
<td>HP1433</td>
<td>C</td>
<td>Absent Absent OFF OFF OFF OFF OFF OFF ON</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The ON/OFF status of genes HP0379, HP0217 and HP1353-4 is directed by two repeats.

phenotype that is inherently less fit under the particular conditions investigated. In essence, it does not specifically address adaptation to a new niche, although this is one possible basis for change; rather, it measures the relative fitness of the available alternative phenotypes to the selective pressures exerted upon the population.

### Lack of change in core LPS biosynthesis

Observed alterations in LPS biosynthetic gene expression only involved those affecting terminal LPS structures (HP0379 and HP0217). The phase-variable *rfaJ* homologue (HP0208; jhp0820), encoding a family 8 glycosyltransferase predicted to affect an inner-core alpha-linked glucose to either glucose or galactose structure, remained in the OFF configuration in all three strains during colonization. This is the most usual observed phenotype for this gene (Salauîn et al., 2004) and probably reflects the fact that the conditions selective for the alternate structure were not a feature of this model system.

### An association between the numbers of phase-varied genes and initial strain fitness for the model system

The number of phase-varied genes decreases with the degree of original strain adaptation for mouse colonization. Previous studies have compared the relative fitness of the strains used in this study in mouse colonization competition experiments, in both co-infections and superinfections (Ayraud et al., 2003). On the basis of competition for colonization, strain SS1 is the most fit in the murine gastric environment, followed by strain Hp141 and then strain Hp145. The number of genes that were phase varied in each strain was inversely proportional to the previously established degree of adaptation to mouse colonization (using the same strains and the same mouse system). Strain SS1 exhibited only one phenotypic change, strain Hp141 underwent four changes, while strain Hp145 had eight phase variation changes in phenotype. Notably, in the colonization comparison studies (Ayraud et al., 2003), strain SS1 produced a much higher initial colonization load of the stomach than the other two strains, while the other strains achieved their peak colonization densities later, which might also be related to the initial proximity of SS1 to a fit phenotypic gene configuration for this niche. Perhaps only a small subpopulation of the other strains was able to survive initially. The association between phase-varied gene phenotypes and mouse or other animal colonization potential raises interesting questions with respect to the basis of different strain properties.

### The final combination of expressed genes differs between the strains tested

The final phenotypes of the varied phenotypes were not the same for all genes across all three strains at the end of the colonization period (Table 2). One explanation for this could be that the different *H. pylori* strains used different strategies to adapt to the mouse environment, and that the
different strategies can be equally successful for colonization. The gene complements of each strain will inevitably differ between these strains, which may also contribute to their different behaviours. For example, interactions between LPS and bacterially encoded LPS-binding proteins might also be expected to influence population behaviour. Similarly, since LPS structures are the product of interacting phase-variable genes, the order of gene switching and existing phenotypes may influence the final phenotype selected. However, the consistency between early changes and final phenotypes for each individual strain strongly suggests that for a given strain, with a common starting expressed gene configuration, there is a particular phenotype which is most readily achieved and adaptive for a given colonization niche.

**Alterations in a coding tandem repeat**

The tandem repeat within the *lexA2B* homologue (HP0619) in strain Hp145 showed length variation during colonization. Three of the LPS biosynthetic genes (HP0651, HP0379 and HP0619) contain a 21 nucleotide coding tandem repeat (which encodes a repeated 7 amino acid motif) towards the 3′ end of the gene, for which the copy number differs between strains. Based upon the behaviour of proteins in other species, coding tandem repeats have the potential to generate changes in the protein through alterations in the number of repeated copies, which might lead to alterations in protein function and/or immunogenicity. A recent survey in *Neisseria* has shown that repeats like these can show several differences between strains, but their observed differences have not yet been extended to studies of their stability during infection (Jordan et al., 2003). The repeats within the phase-variable LPS biosynthetic genes were sequenced at each time point in all three strains (see Supplementary Table S1, available with the online version of this paper at http://mic.sgmjournals.org, for details of repeat numbers for each strain and time point). During the course of infection, the number of coding tandem repeats located at the 3′ end of the genes encoding the two fucosyltransferases (HP0651 and HP0379) remained unchanged. In contrast, changes occurred in the number of tandem repeats within the gene encoding the glycosyltransferase (HP0619) in strain Hp145, but not in the other two strains. The changes occurred in two different colonizing populations, as detected at days 3 and 360. The rapid change detected at day 3 may reflect either a functional advantage or, potentially, co-selection with another altered phenotype, such as *pldA*. This type of variation might also be involved in immune evasion strategies. It is premature to invoke a mechanism for selection in this regard, but the observed changes strongly suggest that this mechanism is an additional source of functional flexibility in this species. The different changes observed between the two altered populations indicate that this is not simply the product of a founder effect from a second population in the inoculum.

This study addressed the whole repertoire of known phase-variable genes simultaneously in a model infection, and reveals that many of these genes are phase varied in the process of adaptation to colonization of the murine gastric environment. It shows that these genes display different temporal changes reflecting the relative importance of the different associated selective pressures (Saunders et al., 2003) or, alternatively, in some cases the time necessary to mount an immune response. The correlation between the adapted nature of the strains to the mouse environment and the number of genes that change during colonization suggests that the expression state of phase-varied genes in the population may significantly influence the capacity for initial colonization and hence exchange between hosts, and suggests that diversity within the initial inoculum is likely to influence colonization potential. This indicates that phase variation, and the associated population diversity, affects the population fitness in both initial colonization and subsequent adaptation.

**ACKNOWLEDGEMENTS**

A Wellcome Trust Advanced Research Fellowship awarded to N.J.S. supported N.J.S. and L.S.

**REFERENCES**


