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Genomic Plasticity in Natural Populations of *Bordetella pertussis*

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We determined the genomic organization of 14 clinical strains of *Bordetella pertussis* isolated over an 18-month period in Alberta, Canada. The maps of these 14 strains, while demonstrating general similarity of gene order, display a number of examples of genomic rearrangements in the form of large chromosomal inversions.

The current revolution taking place in the arena of bacterial genomes, through the acquisition of comprehensive genomic sequence information from a wide variety of bacteria, has given us unprecedented views into the content, structure, and diversity of these genetic structures. In addition, an increasing number of studies which have undertaken the comparative analysis of multiple representatives of the genomes of particular species are leading us away from a view of bacterial genomes as static, monolithic structures and towards the view that they are relatively fluid, plastic structures. This fluidity is manifested not only by the acquisition and loss of genetic information (5, 7, 11) but also by large-scale rearrangements leading to changes in genomic organization (3, 6, 8, 11).

We recently reported a comparative analysis of the genomes of a number of *Bordetella pertussis* strains in which we observed that the genomic organization of *B. pertussis* laboratory strains was variable (14). In the current study we have extended our analyses to include a number of *B. pertussis* isolates from a recent outbreak of whooping cough which occurred in Alberta, Canada, in 1989 to 1991 (2). The maps we derived for these strains document many examples of large-scale chromosomal rearrangements within this group of *B. pertussis* strains which are representative of natural populations.

The *B. pertussis* strains characterized in this study were isolated from a whooping cough outbreak which occurred throughout Alberta, Canada, from December 1989 to May 1991. A total of 70 strains from different locations throughout this province were previously analyzed by pulsed-field gel electrophoresis (PFGE) of chromosomal *Xba*I digests (2). By this procedure, 14 different electrophoretic patterns were distinguishable. Although 15 patterns were initially reported, digestion patterns c and f were later found to be the same (10). Strains representative of these 14 patterns were analyzed to determine their genomic organization. These strains are presented in Table 1.

The "chromosomal surveying" approach to genomic mapping has been described previously (14). This technique relies on homologous recombination to mediate insertion of two independent plasmid suicide vectors, pSS1898 and pSS1914, at chromosomal positions dictated by cloned chromosomal sequences. Both vectors contain the recognition sequence for the intron-encoded restriction enzyme I-*Sce*I, which is not found in the *B. pertussis* genome. Cleavage of chromosomal DNA prepared from a strain harboring the two suicide plasmids inserted at different genetic loci results in the liberation of a DNA fragment whose size represents the distance between the two loci. Further pairwise distance determinations yields the information required to construct a chromosomal map for the positions of the genes examined.

Each of the 14 isolates was first mapped by using a set of six chromosomal markers (primary markers) cloned into pSS1898 and pSS1914. The chromosomal loci used in this initial phase were *bvg* (virulence regulation), *cya* (adenylate cyclase toxin), *fha* (filamentous hemagglutinin), *recA* (homologue of *E. coli recA*), *prn* (pertactin), and *ptx* (pertussis toxin). This preliminary analysis allowed the construction of a framework for the placement of additional gene loci. The genetic loci *aroA* (5-enolpyruvylshikimate-3-phosphate synthase), *bfeA* (ferric enterobactin receptor), *brkAB* (complement resistance), *bpl* (lipooligosaccharide biosynthesis), *dnt* (dermonecrotic toxin), *fim2* (fimbrial subunit, serotype 2), *fim3* (fimbrial subunit, serotype 3), *fimX* (silent fimbrial subunit gene), *fur* (ferric uptake regulator), *ompR*/*envZ* (osmolarity-sensitive gene regulation), *por* (porin), *tcfA* (tracheal colonization factor), and *vrg6* (*vir*-repressed gene 6) were localized within this framework following the insertion of each respective pSS1914 derivative into a subset of the strains containing pSS1898 inserted at primary marker locations. To minimize the number of nonfruitful distance determinations (those where the distances were too great to determine by PFGE), an initial assumption of similarity of gene order with *B. pertussis* Tohama I was made. When this assumption was found to be inaccurate, additional determinations of the distance to other primary markers were made. Gene loci were always mapped with reference to primary markers flanking each side. In all cases, distance determinations between any two markers were made on the same pulsed-field gel for all 14 strains so that differences in intergenic distances were apparent.

Escherichia coli SM10 was used for transfer of all plasmids to the *B. pertussis* strains under study (12). Selection for transfer and integration of pSS1898 derivatives was done by gentamicin resistance, and counterselection against *E. coli* donors was by colicin B1 resistance (1). Selection for the subsequent integration of pSS1914 derivatives was by kanamycin resistance, with counterselection by gentamicin resistance. PFGE and I-*Sce*I digestion were performed as previously described (14).

The maps derived using a total of 19 chromosomal markers are shown in Fig. 1. It can be seen that 10 modes of gene organization were observed among the 14 strains analyzed. All the maps were similar in size, with no evidence of large dele-

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TABLE 1. Bacterial strains and plasmids used in this study[a]

Strain or plasmid	Relevant features	Source or reference
B. pertussis		
14797	*Xba*I PFGE pattern a	2
9755	*Xba*I PFGE pattern b	2
964	*Xba*I PFGE pattern c	2
9835	*Xba*I PFGE pattern d	2
6706	*Xba*I PFGE pattern e	2
12527	*Xba*I PFGE pattern g	2
14784	*Xba*I PFGE pattern h	2
2427	*Xba*I PFGE pattern i	2
8892	*Xba*I PFGE pattern j	2
3860	*Xba*I PFGE pattern k	2
11291	*Xba*I PFGE pattern l	2
12162	*Xba*I PFGE pattern m	2
12145	*Xba*I PFGE pattern n	2
8004	*Xba*I PFGE pattern o	2
Plasmid vectors		
pSS1898	Mapping vector: R6K *oriV gen oriT*	14
pSS1914	Mapping vector: ColE1 *oriV kan amp oriT*	14
pSS2070	pSS1914 with *Eco*RI site destroyed by addition of *Not*I site	This study
pSS1898 derivatives[b]		
pSS1919	10.0-kb *Eco*RI *fhaB* fragment cloned into pSS1898	14
pSS1921	17.5-kb *Eco*RI *prn* fragment cloned into pSS1898	14
pSS1923	14.0-kb *Eco*RI *cya′* and upstream fragment cloned into pSS1898	14
pSS1926	4.7-kb *Eco*RI *ptx* fragment cloned into pSS1898	14
pSS1927	17.0-kb *Eco*RI *recA* fragment cloned into pSS1898	14
pSS1930	4-kb *Eco*RI *bvg* downstream fragment cloned into pSS1898	14
pSS1914 derivatives[b]		
pSS1931	10.0-kb *Eco*RI *fhaB* fragment cloned into pSS1914	14
pSS1932	17.5-kb *Eco*RI *prn* fragment cloned into pSS1914	14
pSS1933	14.0-kb *Eco*RI *cya′* and upstream fragment cloned into pSS1914	14
pSS1934	4.5-kb *Eco*RI *fimX* fragment cloned into pSS1914	14
pSS1935	4.7-kb *Eco*RI *ptx* fragment cloned into pSS1914	14
pSS1936	17.0-kb *Eco*RI *recA* fragment cloned into pSS1914	14
pSS1937	0.83-kb *Eco*RI *vrg6* fragment cloned into pSS1914	14
pSS1938	17.5-kb *Eco*RI *fim2* fragment cloned into pSS1914	14
pSS1939	4.0-kb *Eco*RI *bvg* downstream fragment cloned into pSS1914	14
pSS1940	0.85-kb *Eco*RI *fim3* PCR fragment cloned into pSS1914	14
pSS2572	7.7-kb *Eco*RI *por* fragment cloned into pSS1914	This study
pSS2573	2.6-kb *Eco*RI *bpl* fragment cloned into pSS1914	This study
pSS2574	1.8-kb *Not*I *dnt* fragment cloned into pSS2070	This study
pSS2575	2.8-kb *Not*I *brkAB* fragment cloned into pSS2070	This study
pSS2576	1.8-kb *Eco*RI *bfeA* fragment cloned into pSS1914	This study
pSS2577	6.7-kb *Eco*RI *aroA* fragment cloned into pSS1914	This study
pSS2610	1.6-kb *Sal*I *tcfA* fragment cloned into pSS1914	This study
pSS2611	1.9-kb *Sal*I *ompR*/*envZ* fragment cloned into pSS1914	This study
pSS2612	0.84-kb *Sal*I *fur* fragment cloned into pSS1914	This study

[a] Plasmids pSS2572, pSS2574, and pSS2577 contain inserts derived from the previously described pSS1925, pSS2083, and pSS1924, respectively (14). The *bpl* fragment in pSS2573 was derived from pUCE2a, provided by D. Maskell. The *brkAB* fragment in pSS2575 was derived from pRF1003, kindly provided by A. Weiss. The *bfeA* fragment in pSS2576 was derived from pCIX7, kindly provided by B. Beals. The *tcfA* fragment in pSS2610 was derived from pTF357, kindly provided by T. Finn. The *ompR*/*envZ* fragment in pSS2611 was derived from pBP4, kindly provided by D. Maskell. The *fur* fragment in pSS2612 was derived from pRK/0.8S, kindly provided by S. Armstrong. *Sal*I fragments were first cloned into the *Sal*I site of a plasmid containing a palindromic polylinker and then excised as an *Eco*RI fragment for cloning into pSS1914.

[b] Derivatives used for mapping.

tions or insertions. The maps show hypothetical interrelationships, with arrows indicating that the maps in question could be derived one from another by inversions of large chromosomal segments. The maps of strain h and strain e could not be related by a single inversion. In this case a hypothetical intermediate is shown, such that h and e could be related by two successive inversions. It should be emphasized that the scheme presented is not the only one which is consistent with these data, and although so depicted here, it is not known that the map for strains b and o is in fact the progenitor of the others.

The maps described for these isolates differ consistently in three ways from that determined for *B. pertussis* Tohama I (13). While *dnt*, *brkAB*, and *ompR*/*envZ* have the same configuration with respect to each other, their gene order is reversed relative to that seen for Tohama I. Also, these genes are located very close to the *cya* locus, unlike Tohama I. This configuration is similar, however, to that seen with *B. pertussis* 165 and Wellcome 28, in which *dnt* and *cya* are proximal (14). An additional difference is the placement of the *fur* gene. In Tohama I this gene is located between the *brkAB* and *ompR*/

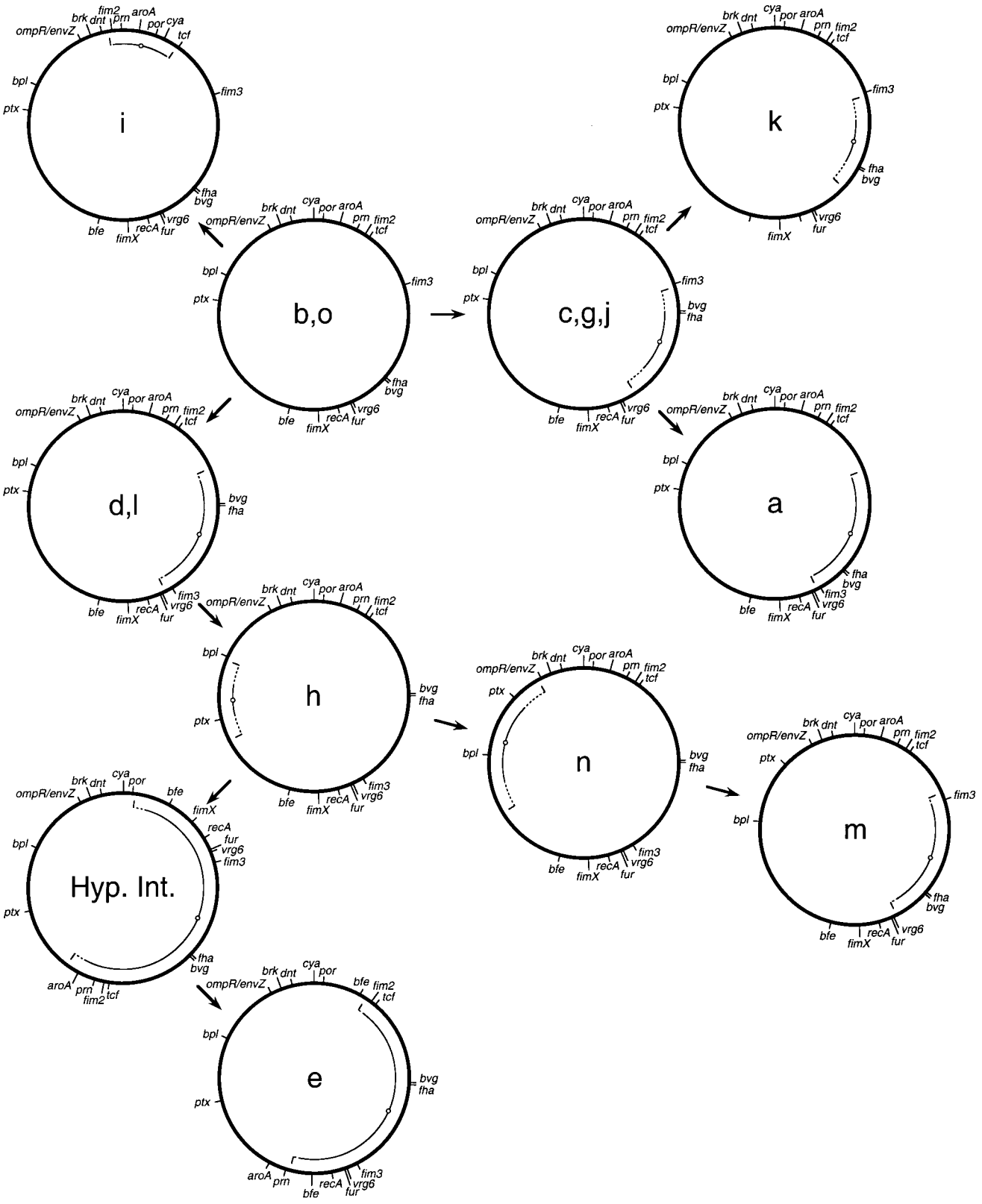

FIG. 1. Chromosomal maps of 14 clinical isolates of *B. pertussis*. Letters designate the *Xba*I digestion pattern obtained by PFGE (2). Maps show relative positions of 19 genetic loci. Maps were derived by the chromosomal surveying approach previously described and the overall strategy outlined in the text (14). Arrows are used to show hypothetical relationships between maps derived one from the other by inversions. Arcs represent the extent of inverted sequences relative to the hypothetical predecessor. Symbols: solid arc, minimum inversion which is consistent with the differences in the two maps; dotted arc, maximum inversion consistent with the observed differences; small circle on arc, center of inverted sequences. Hyp. Int., hypothetical intermediate.

envZ loci, whereas in the strains mapped here, the *fur* gene is found between *recA* and *vrg6*.

Interestingly, a number of the inversions detected in these isolates involve a region of the chromosome which includes the *fha* and *bvg* operons. An inversion of this region was also seen to relate the map of *B. pertussis* 165 and Wellcome 28 to that of Tohama I (14). Although the extents of these inversions vary, their centers are similar in these cases (a, c, d, e, g, j, k, l, m, n, and the hypothetical intermediate in Fig. 1). The inversions seen on the opposite side of the chromosome (h and n in Fig. 1) can also be viewed as inversions which have approximately the same point as their center. This accounts for all but one of the inversions seen (i in Fig. 1). Previous studies concerning inversions of the chromosome of *Salmonella typhimurium* have suggested that inversions generally include the origin or terminus of replication (9). By analogy, we would predict that the origin or terminus of replication in these *B. pertussis* strains would be found at approximately four o'clock on these maps.

The results reported here are relevant to the interpretation of PFGE analysis of bacterial isolates. Changes in PFGE patterns of chromosomal restriction digests have sometimes been assumed to be due to loss or gain of restriction sites due to mutational mechanisms (15). However, our results suggest that chromosomal rearrangements may also be a contributing factor to genome variability detected by this method.

This study provides additional evidence that genomic plasticity is an attribute not just of laboratory strains of *B. pertussis* but also of natural populations living in association with the human host. Although the molecular mechanisms underlying the genomic rearrangements observed here are unknown, we can form a hypothesis based on earlier studies which examined the nature of chromosomal inversions observed in other bacterial species. Inversions observed in *E. coli* and *S. typhimurium* have endpoints within copies of IS*5* and *rrn* loci, respectively (8, 16). In these cases the inversions have apparently been catalyzed by homologous recombination between these islands of homology. *B. pertussis* is a likely candidate for inversions catalyzed in a similar manner, as approximately 100 copies of a 1-kb insertion sequence, IS*481*, are estimated to be present in the *B. pertussis* chromosome (4). Detailed molecular characterization of defined chromosomal rearrangements captured in the laboratory will shed further light on these questions.

REFERENCES

1. **Brickman, T. J., and S. K. Armstrong.** 1996. Colicins B and Ia as novel counterselective agents in interspecies conjugal DNA transfers from colicin-sensitive *Escherichia coli* donors to other gram-negative recipient species. Gene **178:**39–42.
2. **de Moissac, Y. R., S. L. Ronald, and M. S. Peppler.** 1994. Use of pulsed-field gel electrophoresis for epidemiological study of *Bordetella pertussis* in a whooping cough outbreak. J. Clin. Microbiol. **32:**398–402.
3. **Gibbs, C. P., and T. F. Meyer.** 1996. Genome plasticity in *Neisseria gonorrhoeae*. FEMS Microbiol. Lett. **145:**173–179.
4. **Glare, E. M., J. C. Paton, R. R. Premier, A. J. Lawrence, and I. T. Nisbet.** 1990. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J. Clin. Microbiol. **28:**1982–1987.
5. **Groisman, E. A., and H. Ochman.** 1996. Pathogenicity islands: bacterial evolution in quantum leaps. Cell **87:**791–794.
6. **Jiang, Q., K. Hirakatsu, and D. E. Taylor.** 1996. Variability of gene order in different *Helicobacter pylori* strains contributes to genome diversity. Mol. Microbiol. **20:**833–842.
7. **Lawrence, J. G., and J. R. Roth.** 1996. Selfish operons: horizontal transfer may drive the evolution of gene clusters. Genetics **143:**1843–1860.
8. **Liu, S. L., and K. E. Sanderson.** 1996. Highly plastic chromosomal organization in *Salmonella typhi*. Proc. Natl. Acad. Sci. USA **93:**10303–10308.
9. **Mahan, M. J., and J. R. Roth.** 1991. Ability of a bacterial chromosome segment to invert is dictated by included material rather than flanking sequence. Genetics **129:**1021–1032.
10. **Peppler, M. S.** Personal communication.
11. **Römling, U., K. D. Schmidt, and B. Tümmler.** 1997. Large genome rearrangements discovered by the detailed analysis of 21 *Pseudomonas aeruginosa* clone C isolates found in environment and disease habitats. J. Mol. Biol. **271:**386–404.
12. **Simon, R., U. Priefer, and A. Puhler.** 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in gram negative bacteria. Bio/Technology **1:**784–789.
13. **Stibitz, S.** 1998. *Bordetella pertussis* strain Tohama I. *In* F. J. de Bruin, J. R. Lupski, and G. M. Weinstock (ed.), Bacterial genomes: physical structure and analysis. Chapman & Hall, New York, N.Y.
14. **Stibitz, S., and M.-S. Yang.** 1997. Genomic fluidity of *Bordetella pertussis* assessed by a new method for chromosomal mapping. J. Bacteriol. **179:**5820–5826.
15. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
16. **Umeda, M., and E. Ohtsubo.** 1990. Mapping of insertion element IS5 in the *Escherichia coli* K-12 chromosome: chromosomal rearrangements mediated by IS5. J. Mol. Biol. **213:**229–237.