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Environmental heterogeneity drives within-host diversification and evolution of Pseudomonas aeruginosa. Within-host pathogen evolution and diversification during the course of chronic infections is of importance in relation to therapeutic intervention strategies, yet our understanding of these processes is limited. Here, we investigate intraclonal population diversity in P. aeruginosa during chronic airway infections in cystic fibrosis patients. We show the evolution of a diverse population structure immediately after initial colonization, with divergence into multiple distinct sublineages that coexisted for decades and occupied distinct niches. Our results suggest that the spatial heterogeneity in CF airways plays a major role in relation to the generation and maintenance of population diversity and emphasize that a single isolate in sputum may not represent the entire pathogen population in the infected individual. A more complete understanding of the evolution of distinct clonal variants and their distribution in different niches could have positive implications for efficient therapy.

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