

1 **The upper respiratory tract as a microbial source for pulmonary infections in Cystic Fibrosis:**  
2 **Parallels from Island Biogeography**

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15 "When we try to pick out anything by itself, we find it hitched to everything  
16 else in the Universe."

17 -- John Muir  
18

### 19 **Abstract**

20 A continuously mixed series of microbial communities inhabits various points of the respiratory tract,  
21 with community composition dictated by distance from colonization sources, colonization rates, and  
22 extinction rates. Ecology and evolution theory developed in the context of biogeography is relevant  
23 to clinical microbiology and could reframe the interpretation of recent studies comparing  
24 communities from lung explant samples, sputum samples and oropharyngeal swabs. We propose an  
25 Island Biogeography model of the microbial communities inhabiting different niches in human  
26 airways. Island biogeography as applied to communities separated by time and space is a useful  
27 parallel for exploring microbial colonization of healthy and diseased lungs, with the potential to  
28 inform our understanding of microbial community dynamics and the relevance of microbes detected  
29 in different sample types. In this perspective, we focus on the inter---mixed microbial communities  
30 inhabiting different regions of the airways of patients with cystic fibrosis.  
31

### 32 **Introduction**

33 Individual humans, and their organs and tissues, can be considered islands. Like the islands of the  
34 Galapagos, humans constitute spatially structured environments that offer microbes abundant  
35 ecological opportunity (Figure 1). The Human Microbiome Project (<http://www.hmpdacc.org/>) has  
36 shown that different organs and systems within the human body are inhabited by different species  
37 assemblages (1). Just as the islands of an archipelago are exposed to a reservoir of potential  
38 emigrants from the mainland, individual human organ systems are exposed to potential migrants  
39 from the surrounding milieu. Irrespective of specific habitat details, community assembly and  
40 stability is driven by dispersal rates and priority effects, with the order and timing of arrival  
41 influencing the fate of particular species (2, 3).

42

43 The diversity of species inhabiting the islands of an archipelago depends on how close each island is  
44 to a mainland (from which colonizing species are derived), and the rate that species go extinct (Figure  
45 2). The theory of island biogeography, born in the 1960s (4), includes islands of many different sorts:  
46 islands as mountain tops, lakes, trees, and in fact any set of niches separated by time, space or an  
47 environmental barrier. While studies of island biogeography initially focused on ecology, there has  
48 long been awareness that patterns of species diversity cannot be fully understood outside the  
49 evolutionary processes that fuel diversification (5). Indeed, incorporation of evolutionary thinking  
50 into ecology has underpinned advances in understanding adaptive radiation, speciation, and  
51 opportunities for ecosystem restoration.

52

53 In extreme cases, for example following a forest fire, the process of community assembly is given  
54 special prominence (6). However the same processes that lead to re---establishment of a forest on  
55 barren land may also govern the formation of host---associated microbial communities. Community  
56 assembly has important implications for health and disease (2, 3, 7). For example, the capacity for a  
57 pathogen to establish itself in a given niche is likely to depend upon the presence (or absence) of  
58 competing microbes (8---12), the timing of arrival and the history of colonization events.  
59 Establishment will also be affected by host factors including the immune system, the availability and  
60 quality of nutrients (their spatial and temporal distribution), and the physical structure and properties  
61 of tissues and surfaces.

62

63 **Island Culture: distinct communities intermingle in the oropharynx---airway---lung ecosystem.** As  
64 islands that provide ecological opportunity to microbes, the unique environment of the lung in  
65 diseases such as cystic fibrosis (CF), chronic obstructive pulmonary disorder (COPD) and other  
66 inflammatory respiratory conditions are of special interest. Microbes immigrating to the lung come  
67 from various sources including air, water and food, but also from the oral cavity and other nearby  
68 islands such as the sinuses and even the GI tract. In the model proposed here, the oral cavity might  
69 be considered as the mainland, or the largest, most diverse proximal island, and various niches in the  
70 respiratory tract as islands (Figure 2). Like modern ecologists who find exceptions to the original  
71 Island Biogeography theory of the 1960s, it is necessary to acknowledge the influence of factors  
72 beyond the size and remoteness of island reservoirs on community diversity (as indicated by species  
73 richness) (13). In CF patients, the immune system, interspecies interactions and antibiotic pressure  
74 exert profound additional influences on microbial community structure.

75

76 Microbes traditionally inhabiting the oral cavity that are also capable of colonizing the lower airways,  
77 are likely to be an important source of *Attack* community microbes in lungs, as presented in the  
78 Climax---Attack---Model (CAM) (14). CAM postulates that there are two major microbial or viral  
79 communities inhabiting the CF lung: a well---adapted, persistent *Climax* community and an *Attack*  
80 community comprised of more virulent and transient microbes and viruses. While the oral cavity  
81 hosts a large diversity of microbes, the most abundant species found deep in explanted lung samples  
82 from patients with end---stage disease are usually from a small group of known CF pathogens,  
83 including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, and  
84 *Burkholderia cenocepacia* (15). Furthermore, well---studied clinical isolates of *P. aeruginosa* often

85 harbor a subset of common mutations that reveal their adaptation to the CF lung environment (16---  
86 18). Recent evidence suggests a similar trend in *Rothia mucilaginosa*, a bacterium traditionally  
87 considered to be part of the oral microbial community (19).

88

89 Oral microbes that do not normally inhabit the lungs are likely to significantly impact lung  
90 pathophysiology through the interconnected oropharynx---airway---lung ecosystem. Even if those oral  
91 microbes remain in their endemic location (the oral cavity), they may still impact populations in the  
92 lung by producing metabolites that passively travel to the lungs (20---22), or by stimulating systemic  
93 host responses that could directly impact the deep airway microbial community. Additionally, given  
94 the potential for these oral microbes to emigrate to the lungs across the continuum of the respiratory  
95 system, these same microbes may be important competitors and thus regulators of lung---invading  
96 microbes. Bacteriophage and other agents of gene exchange may also transfer DNA encoded traits,  
97 *e.g.*, antibiotic resistance (23, 24). Microbes within the oral cavity, and their migration from that  
98 source, are therefore likely to be important contributors to lung disease. It is critical that all such  
99 sources are identified and studied so that their connection with the CF lung can be understood.

100

101 Given the potential for mainland microbes to directly and indirectly affect lung communities, we  
102 argue that to understand the evolution of polymicrobial lung communities, and their related patho---  
103 physiologies, ecological dynamics must be acknowledged both within resident lung microbial  
104 communities along with those in neighboring niches.

105

106 Some have argued that oral commensals detected in sputum samples used to interrogate lung  
107 microbial communities represent contamination from the oral cavity (15, 25, 26). However, the  
108 degree to which oral microbes may emigrate and persist in lower airways is likely to vary from one  
109 patient to the next, depending upon the presence of specific microbes as well as a variety of other  
110 environmental and host factors. Thus, tracking all airway microbes is important because oral  
111 microbes need not frequently form resident communities in the lower airways to profoundly  
112 influence polymicrobial community physiology.

113

114 **A bridge to the lung: migration across the trachea.** The physical continuum of the trachea connects  
115 the oral cavity and the lungs. Indeed, lung samples from healthy humans are not sterile, and they  
116 occasionally contain microbes traditionally associated with the oral cavity (27---30). The density of  
117 bacteria in the oral cavity is orders of magnitude greater than the healthy lung (27). The composition  
118 and even the existence of microbial communities in healthy human lungs is an active area of  
119 research. Whether the upper and lower respiratory tracts of healthy people contain “tourists” which  
120 are quickly expelled, or whether there exist resident microbial communities is an important question  
121 (27, 29, 31---33). Either way, differences in commensal and pathogenic microbial load in the airways of  
122 healthy humans and those affected by various respiratory conditions are likely to be affected by the  
123 rate at which new types enter the system and the rate at which they fail to colonize, or go extinct.

124

125 Healthy and diseased lungs are equally accessible to microbial migrants – they both contain a bridge  
126 across the trachea, and significantly, migration may not always result in persistent colonization.

127 Healthy lungs have coordinated mucociliary clearance (34) which forces microbes out, and this limits  
128 the time for adaptation and establishment. This means the extinction rates of microbes that reach  
129 the lower airways are much higher in healthy lungs; impaired mucociliary clearance will reduce  
130 extinction rates and lead to larger numbers of species colonizing the lower airways (Figure 2).  
131 Emigration of oral microbes to the lower airways due to aspiration is important in a number of  
132 respiratory conditions, where a large fraction of infections are thought to have oral microbial etiology  
133 (35---39). Respiratory conditions associated with decreased extinction of microbes that reach the lower  
134 airways include smoking (31), vaccination and probiotics (40, 41), respiratory virus infection (42---44),  
135 pneumonia (36), HIV (45, 46), COPD (30, 47---49) and CF (50). In addition, improvements in oral  
136 hygiene practices in hospitals have been shown to prevent pneumonia (51). In newly transplanted  
137 lungs of CF patients, strains of bacteria found in the sinuses are later found in the newly transplanted  
138 lungs, again suggesting that microbes in the sinuses have a route to the lower respiratory tract (52).

139

140 In CF, the epithelia lining the airways are covered by abnormally dense mucus, trapping the cilia and  
141 rendering them nonfunctional (53). In addition, lung---specific immune responses are impaired in CF,  
142 including dysfunctional alveolar macrophages and autophagy (54---56). Together these defects allow  
143 the commonly observed opportunists, such as *P. aeruginosa* and *B. cenocepacia*, to persist in CF  
144 airways. Most patients harbor unique and persistent microbial communities, suggesting differences  
145 from patient to patient in exposure to microbial immigrants, or selective pressures, or both. While  
146 the well---known opportunistic pathogens are clearly important, they do not exist in isolation;  
147 interactions with other community members have important implications (11, 57---59). Indeed, there  
148 are increasing examples of infections driven by an altered polymicrobial community, rather than a  
149 single pathogen, and these will likely influence how clinicians diagnose and treat infections (59, 60).  
150 Pneumonia, COPD, CF, chronic sinusitis, periodontitis and otitis media are examples of infections  
151 where a commensal from one human site can emigrate to become a pathogen in another context  
152 (36, 51, 61---66). Like drifting seeds from a proximal island, exposure and survival rates of microbes  
153 from nearby habitats stands to profoundly influence microbial community composition and  
154 interactions.

155

156 **Spoiled sputum?** As sampling of the lungs of a living human generally requires passage through the  
157 oral cavity, the question of how frequently and how deeply oral microbes penetrate into the lower  
158 airways is unresolved. Invasive lower airway sampling methods such as bronchoalveolar lavage (BAL)  
159 enable careful sampling of a specific region of the lung with reduced potential for contamination, but  
160 these methods cannot be performed on a regular basis because of negative impacts on patients.  
161 Sputum and BAL samples are also limited in the extent to which they provide insight into spatial  
162 distribution of microbes within the airways. Currently, when typical CF pathogens are not found in CF  
163 sputum cultures, clinical microbiology labs report them as culture negative (67), essentially  
164 disregarding oral microbes as contaminants without clinical relevance. Given the interconnected  
165 topography of the oral cavity and the airways, it would not be surprising to learn that some microbial  
166 groups are found in both the oral and lung environments. Direct evidence that organisms considered  
167 oral contaminants are present in the large airway exists from early culture studies based on trans---  
168 tracheal aspirates where mixing with oral microbiota is avoided (68). In addition, next---generation  
169 sequencing has often detected surprisingly high relative abundances of species such as *Streptococcus*  
170 spp., *R. mucilaginosa*, or *Gemella* spp. in large volumes of purulent sputum (mls to tens of mls) (11,

171 19, 69). Overall, while there are limitations associated with sputum samples, the fact that they can be  
172 obtained by non---invasive means, and that they show patterns of diversity similar to lower and upper  
173 airways (8, 15, 16, 34), suggest it is sensible to obtain as much information from sputum samples as  
174 possible.

175

176 To directly assess lung microbial communities, multiple studies have examined the microbes present  
177 in explant lung samples. These samples carry their own caveat --- they are most often obtained from  
178 patients with end---stage disease, which have been shown to contain significant regional differences in  
179 community composition, and severely reduced diversity (30, 70, 71). In an attempt to reconcile the  
180 different caveats of sputum and explant lung samples in CF, a recent study compared throat, sputum  
181 and explant lung samples (average of all lobar bronchi) from the same CF patients, close to the time  
182 of transplant surgery (15). This study concluded that next---generation sequencing of DNA in sputum  
183 samples inaccurately represents airway microbial communities, and that oral microbes detected by  
184 this method should be regarded as contaminants.

185

186 We present a complementary analysis of the Goddard et al. 2012 data. Whereas Goddard et al  
187 present the relative abundance of taxa as bar plots, we took the relative abundance data from their  
188 supplementary material and visualize an unsupervised random forest in a multidimensional scaling  
189 plot. The barplots in Goddard et al. and our analysis both show that the overall microbial community  
190 composition of sputum samples closely resembles that of the explanted lung samples in half of the six  
191 samples tested (72) (Figure 3). In the other three patients, additional microbes traditionally  
192 considered oral commensals were found in sputum samples and were not found in the explanted  
193 lung samples. However, for all samples, the dominant microbe found in sputum was the same as the  
194 dominant microbe found in the explant lung samples. The lack of additional commensal microbes in  
195 three of the sputum samples may reflect antibiotic treatment and disease state rather than a lack of  
196 oral contamination. We therefore favor the interpretation that microbes considered as contaminants  
197 might be important components of CF lung ecology. We list some issues that warrant consideration:  
198

199 **First**, the careful study of explanted lungs presented in Goddard et al. provides valuable  
200 information about the end---stage disease associated microbial community DNA found in CF  
201 explanted lungs. However, end---stage disease communities are not representative of microbial  
202 community composition and activity during earlier stages. One of the most consistent signatures  
203 of CF airway microbial community evolution from longitudinal sputum sampling is the increase in  
204 antibiotic resistant pathogen load as disease state becomes more severe (15, 50, 73---75).  
205 Furthermore, by the time a lung is surgically removed, many of the airways have undergone  
206 bronchiectasis and become clogged, no longer exchanging air with upper airways. Substantially  
207 reduced microbial diversity is expected in explant samples, and is very different from the  
208 microbial community composition in the airways of younger CF patients with less severe disease  
209 (76). Community interactions involving the more diverse repertoire of microbes found in  
210 younger, earlier---stage patients are integral to the evolution of the community towards a less  
211 diverse, more antibiotic resistant state found at the end---stage of respiratory diseases, and  
212 should be studied in greater detail.

213 **Second**, sputum may come into contact with oral microbes independent of the collection of the  
214 sputum sample. Sputum may accumulate in areas between the throat and the lobar bronchi in  
215 some patients, and oral microbes may inhabit these sites. Half of the six sputum samples  
216 reported in Goddard et al 2012 had a low abundance of oral microbes. This dearth of microbes  
217 may have been due to long-term antibiotic use and acute antibiotic treatment following an  
218 exacerbation (70, 73, 75, 77). The other three sputum samples did contain a greater abundance  
219 of oral microbiota compared to the explant samples, matching microbes identified in throat  
220 swabs. The presence of oral microbes could be significant to disease progression.

221 **Third**, the authors note that they made no attempt to separate intact microbes from the  
222 surrounding material, neither in the sputum samples nor in the explanted lung samples. This is  
223 relevant because *P. aeruginosa* is known to rely on large amounts of extracellular DNA from  
224 coordinated cell death for biofilm formation (78). This DNA could accumulate over time and  
225 exaggerate the apparent abundance of *Pseudomonas* relative to other microbes, especially in a  
226 context like the lower airways of patients with severe disease, where little disruption of the  
227 microbial communities would take place (79). In many explanted lungs, the authors did in fact  
228 detect some microbes typically considered oral commensals (such as *Streptococcus spp.*), but at  
229 much lower abundances than in sputum. If the relative abundance of *Pseudomonas* was  
230 exaggerated by the sampling method, then the low but non-zero relative abundances of oral  
231 microbes could be much higher than originally considered by the authors. However it is  
232 important to note that high abundance does not equal ecosystem importance, as some oral  
233 microbes may be able to influence lung microbial communities even at very low abundances (64,  
234 65).

235

236 **Moving forward: understanding colonization and migration events in respiratory infections.** Further  
237 study is required to understand the dynamics of airway microbial communities that are relevant to  
238 disease. Human airways are exposed to microbes from many sources. While the persistent microbial  
239 community of the adjacent oral cavity is likely to be a dominant source, additional sources include  
240 nearby humans (other archipelagos, in Figure 1), animals, the home, water, food and other  
241 environmental reservoirs capable of creating airborne particulate matter. By analogy to the island  
242 biogeography example, the oral cavity can be thought of as a mainland species reservoir; the  
243 communities that become established in these locations can have a profound effect on the  
244 prospective colonizers of more remote islands further down the chain. Understanding where  
245 reservoirs of relevant microbes exist could help avoid or delay colonization, inform therapeutic  
246 strategies and potentially improve clinical outcomes.

247

248 Fortunately, modern 'omics profiling -- next generation sequencing, proteomic, and metabolomics  
249 measurements -- now allows the identities and activities of both chronic microbial communities and  
250 drifting microbial seeds to be identified with unprecedented depth and breadth. New technologies  
251 and decreasing costs allow for measurement of taxonomic identities, genome content, and  
252 transcriptomic, proteomic, and metabolomic activity of microbes in sputum samples. Insights into  
253 how movement and changes in activity of lung and oral microbes may precipitate exacerbations and  
254 other pathophysiological changes should be possible. The next major challenge is designing careful  
255 studies so that the large amounts of new data can give a clear picture of the spatial and temporal  
256 dynamics of these communities, and lead to understanding of how these dynamics impact clinical

257 outcomes. The biological question should dictate the most appropriate sampling approach.  
258 Standardization of sample collection and processing protocols is essential to producing data that can  
259 be compared, regardless of sample type.

260

261 The island biogeography analogy can be used to develop criteria for better understanding the  
262 relationships among traditionally "oral" and traditionally "CF pathogen" microbes. There may be  
263 cases where sputum samples are contaminated, and there may also be cases where "oral" microbes  
264 become established deeper in the CF lung. Perhaps these cases could be distinguished by deep  
265 longitudinal sampling. Evidence of oral microbes becoming established in the lung could include 1)  
266 persistence over time; 2) stable abundance and 3) genetic adaptation. These three lines of evidence  
267 are all predicted by island biogeography and evolutionary theory. Additional methods may also  
268 decipher the contributions of all airway microbes to the community dynamics. So far, little change in  
269 measurable density or composition of microbiota has been observed preceding CF disease flares  
270 known as pulmonary exacerbations (73, 77, 80). However, breath gas analysis and metabolite analysis  
271 of sputum samples may capture a valuable snapshot of metabolic activity that reflects the physiology  
272 of both the human host and microbes (20, 22, 81). Transcriptomic and proteomic analyses may also  
273 give deeper insight not only into the taxonomy of microbes present, but also their activities. Finally,  
274 new imaging techniques may eventually allow a non-invasive window into the spatial distribution of  
275 sputum accumulation within the airways of patients (82).

276

277 In conclusion, understanding polymicrobial community dynamics in health and disease is a compelling  
278 challenge in 21<sup>st</sup> century medicine, with consequences for infection diagnosis and treatment.  
279 Applying Island Biogeography theory to microbial communities inhabiting niches in the human  
280 airways informs our interpretation of the role of microbes found in different human sample types.  
281 When microbes that are typical of the oral cavity are found in sputum or BAL samples from CF  
282 patients, they should be considered potential immigrants, rather than contaminants. Regardless of  
283 whether microbial seeds originate from the mainland oral cavity or an adjacent island in the airways,  
284 they have the capacity to alter the dynamics of the polymicrobial community that impacts patient's  
285 quality of life.

286

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292 the 2013 CF workshop where this project began.

293

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295

296 **Figure Legends**

297 **Figure 1.** Parallels between island biogeography and polymicrobial lung colonization. A) In Island  
298 Biogeography theory, the mainland is the greatest source of species diversity, with individual island  
299 species composition depending on the distance from the mainland. B.) Human airway microbial  
300 colonization is likely to display a similar dependence on the distance from the mainland (largely the  
301 oral cavity, shown in yellow, which is the richest and most diverse source of microbes with proximity  
302 to the lung). C.) Other people, along with the air, water and other environments, are also important  
303 sources of microbes which can immigrate to the islands in the human airways, and influence the  
304 polymicrobial community

305

306 **Figure 2.** Classic Island Biogeography. The richness of species depends on the Colonization rate (left Y--  
307 axis) and the Extinction rate (right Y---axis), adapted from (4). Migration through the trachea offers  
308 colonization opportunity to microbes from multiple sources, and impaired mucociliary clearance  
309 decreases the extinction rate. Gray circle (1) represents a small distant island (i.e. the lung) with few  
310 species, while gray circle (2) identifies the mainland or a large proximal island with high species  
311 diversity, such as the oral cavity. Diversity is comprised of both the number of species and their  
312 distribution, or evenness, and can be indicated by different measures of species richness and  
313 frequency. The number of species, or species richness, is an indicator of diversity. The term diversity  
314 is used throughout this perspective as informed by the species richness, which can be predicted in  
315 the Island Biogeography model.

316

317 **Figure 3** Multidimensional scaling (MDS) of an unsupervised Random Forest comparing the relative  
318 abundance of taxa derived from 16S sequencing of lung explant samples (red) with sputum samples  
319 (blue) and oropharyngeal swabs (green) from six CF patients (see legend), data from (15), analysis  
320 conducted in R with the package Random Forest (72). Shared community composition leads to  
321 clustering of sputum and lung samples in most cases, while some sputum and throat samples cluster  
322 together.

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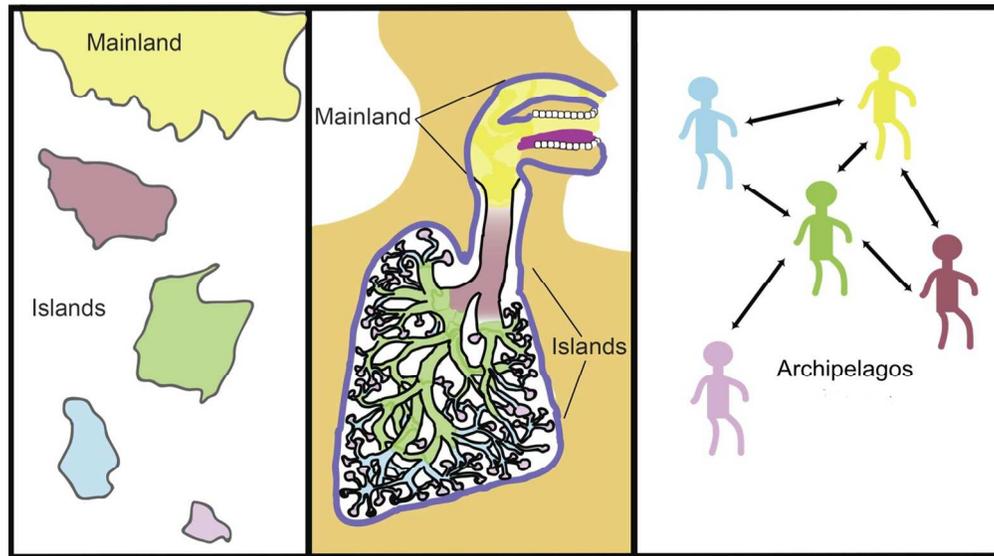


Figure 1. Parallels between island biogeography and polymicrobial lung colonization. A) In Island Biogeography theory, the mainland is the greatest source of species diversity, with individual island species composition depending on the distance from the mainland. B.) Human airway microbial colonization is likely to display a similar dependence on the distance from the mainland (largely the oral cavity, shown in yellow, which is the richest and most diverse source of microbes with proximity to the lung). C.) Other people, along with the air, water and other environments, are also important sources of microbes which can immigrate to the islands in the human airways, and influence the polymicrobial community  
267x149mm (150 x 150 DPI)

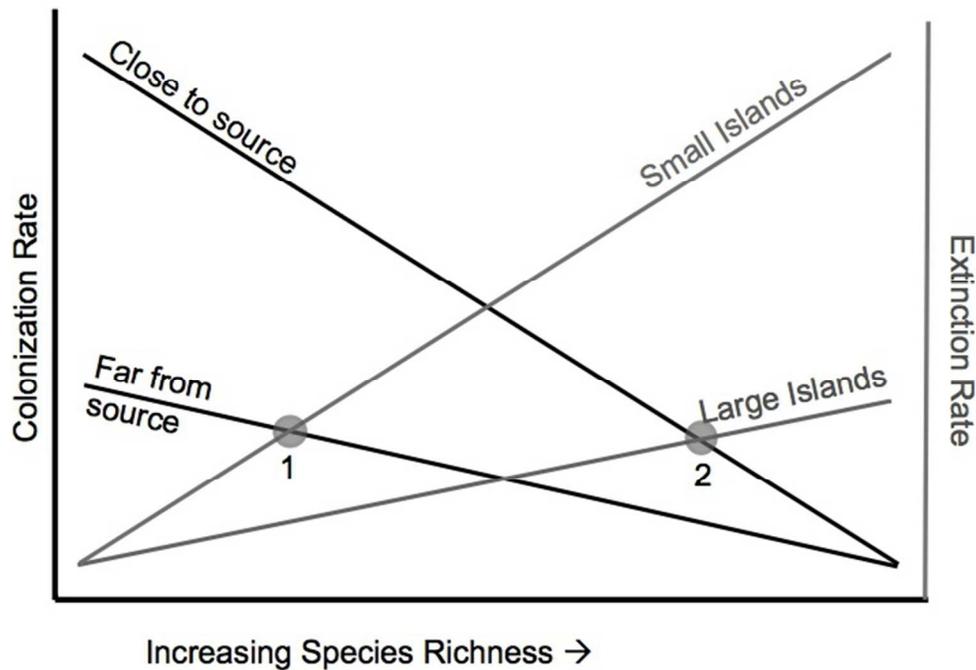


Figure 2. Classic Island Biogeography. The richness of species depends on the Colonization rate (left Y-axis) and the Extinction rate (right Y-axis), adapted from (4). Migration through the trachea offers colonization opportunity to microbes from multiple sources, and impaired mucociliary clearance decreases the extinction rate. Gray circle (1) represents a small distant island (i.e. the lung) with few species, while gray circle (2) identifies the mainland or a large proximal island with high species diversity, such as the oral cavity.

Diversity is comprised of both the number of species and their distribution, or evenness, and can be indicated by different measures of species richness and frequency. The number of species, or species richness, is an indicator of diversity. The term diversity is used throughout this perspective as informed by the species richness, which can be predicted in the Island Biogeography model.

123x86mm (150 x 150 DPI)

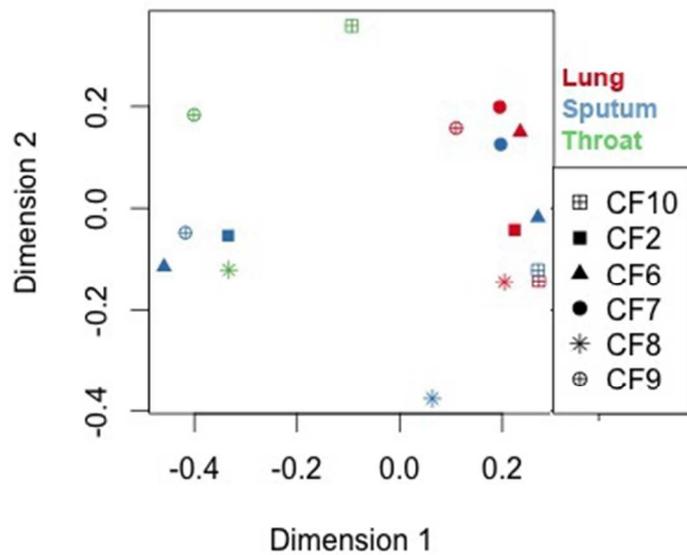


Figure 3 Multidimensional scaling (MDS) of an unsupervised Random Forest comparing the relative abundance of taxa derived from 16S sequencing of lung explant samples (red) with sputum samples (blue) and oropharyngeal swabs (green) from six CF patients (see legend), data from (15), analysis conducted in R with the package Random Forest (72). Shared community composition leads to clustering of sputum and lung samples in most cases, while some sputum and throat samples cluster together.  
190x139mm (72 x 72 DPI)