Pervasive positive linkage between bacterial social traits suggests the predominance of competitive interactions in natural *Pseudomonas* communities

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ABSTRACT

Bacteria frequently cooperate by sharing secreted metabolites such as enzymes and siderophores. Many of these 'public good' traits are linked at the regulatory level, and studies on laboratory systems have shown that trait linkage is a key determinant of strain dynamics and community composition. Here, we test to what extent regulatory linkage among social traits occurs in natural *Pseudomonas* communities by investigating investment levels and correlations between five public goods: biosurfactants, biofilm matrix components, proteases, pyoverdines, and toxic compounds. Our analyses involving 315 isolates from soil and freshwater communities revealed that their investment into social traits varied dramatically, and that correlations between traits were frequent, exclusively positive, and sometimes habitat-specific. Our results indicate that *Pseudomonas* communities are dominated by strains lying on a continuum between a 'social' type producing multiple public goods, and an 'asocial' type showing low investment into social traits. This pattern suggest that competitive interactions, where asocial types act as 'supercheaters' and exploit social types, are more common than the mutualistic exchange of different public goods among strains with specialized trait repertoires. Overall, our work sheds light on the complex interdependencies among social traits, and indicates that expression constraints shape the evolution of microbial sociality in nature.

Keywords: public goods; bacterial communities; competition; mutualism; regulatory linkage

INTRODUCTION

Bacteria are social organisms. They form multicellular structures such as biofilms, communicate with each other via chemical signals, and engage in group-coordinated motility, resource acquisition and 'chemical warfare' against predators or competitors (West et al. 2007; Foster 2010). Many of these social behaviours involve the cooperative release of costly secondary metabolites. For instance, biofilm formation requires the production of structural polysaccharides, group-coordinated motility often involves the production of biosurfactants, and resource acquisition is based on the production of exo-enzymes and metal-chelating siderophores (Griffin et al. 2004; Nadell et al. 2009; Kearns 2010; Jousset et al. 2013). Once released, these compounds are – in principle – publicly accessible to all neighbouring cells. Such 'public goods' do therefore not only benefit (clonemates of) the producer, but can also affect other community members. The resulting interactions cover all aspects of bacterial life, ranging from 'cheating' (where strains deficient for the production of a public good free-ride on the common pool of this resource) (West et al. 2006; Özkaya et al. 2017) over inter-strain competition (Cordero et al. 2012; Bruce et al. 2017; Leinweber et al. 2018) to division of labour between bacterial strains exchanging different types of public good (van Gestel et al. 2015; Dragoš et al. 2018). There is increasing evidence that such social interactions play a crucial role during hosts infection (Rumbaugh et al. 2009; Pollitt et al. 2014; Andersen et al. 2015; Granato et al. 2018), in various biotechnological and environmental processes (Bachmann et al. 2016; Hesse et al. 2018) and within the microbiome (Rakoff-Nahoum et al. 2016).

While many aspects of bacterial social interactions are well understood for individual traits, it is important to consider that different traits are often linked at the regulatory level.

48 Regulatory linkage leads to an additional level of complexity, as selection on one trait can have
49 pleiotropic consequences for other traits (Jousset et al. 2009; Wilder et al. 2011; Dandekar et al.
50 2012; Wang et al. 2015). For example, for *Pseudomonas* bacteria it was shown that a reduction
51 of exo-protease production can increase sensitivity to predation (Jousset et al. 2009), and reduce
52 metabolic abilities and toxin resistance (Dandekar et al. 2012; Wang et al. 2015), thereby
53 compromising the spread of cheaters. Especially when regulatory linkage occurs between two or
54 more public good traits, the resulting pleiotropic effects can have far-reaching consequences.
55 Positive linkage – where mutations reducing the production of one public good also reduce the
56 production of a second public good – can accelerate the loss of cooperation, and could hence
57 favour the emergence of 'supercheaters' that simultaneously free-ride on multiple public goods
58 (Granato et al. 2018). Conversely, negative linkage – where a reduced production of one public
59 good leads to an increase in the production of another public good – can promote the emergence
60 of 'mosaic producers' with a limited public good repertoire, which can potentially complement
61 each other (Driscoll et al. 2011; Ross-Gillespie et al. 2015). In addition to these two scenarios,
62 both positive and negative linkage can stabilize cooperation, namely if changes in the expression
63 of linked traits have negative net fitness effects on the individual (Harrison and Buckling 2009).

64 While these studies on laboratory systems show that regulatory linkage and the resulting
65 constraints are key determinants of bacterial social interactions and strain co-existence, we know
66 little on whether trait linkage occurs in natural bacterial communities and whether it has
67 disruptive or stabilising effects on communities. Here, we tackle this question and investigate
68 whether and how the production of five different public goods is correlated across 315
69 *Pseudomonas* strains isolated from eight soil and eight freshwater communities (18-20 strains

70 per community). In a first step, we screened each strain for the production of: (i) pyoverdines, a
71 highly variable class of siderophores used to scavenge iron (Griffin et al. 2004); (ii) proteases used
72 to digest extracellular proteins (Diggle et al. 2007); (iii) biosurfactants used during group-motility
73 (Xavier et al. 2011); (iv) structural components used to form biofilms (Nadell et al. 2009); and (v)
74 toxic compounds used to fight predators and competitors (Jousset 2012). We then used our
75 phenotypic data set to ask whether: (a) strains vary in the extent to which they invest in the public
76 goods; (b) there is a phylogenetic signal for trait expression, where more closely related strains
77 produce similar amounts of a particular public good; (c) production levels of different public
78 goods correlate with one another, suggesting positive or negative regulatory linkage; and (d)
79 there are differences in linkage patterns between habitats.

80 MATERIALS AND METHODS

81 **Sampling, isolation and identification of Pseudomonads.** We sampled 16 *Pseudomonas*
82 communities from soil and pond habitats (n = 8 communities per habitat) located on the campus
83 of the University of Zürich (47.40° N, 8.54° E), Switzerland. We isolated 320 strains (n = 20 strains
84 per community) on a medium selective for fluorescent pseudomonads, and then verified their
85 affiliation to this group by amplifying and sequencing part of the housekeeping gene *rpoD* (a
86 commonly used phylogenetic marker for pseudomonads) (Mulet et al. 2009). The 315 isolates
87 that could be unequivocally identified as pseudomonads were preserved as freezer stocks until
88 their use in our experiments (see (Butaitė et al. 2017) for details on the sampling, isolation and
89 identification procedure).

90 **Phenotypic screening of natural isolates.** We used standard phenotypic assays to screen all
91 isolates for the production of the five public goods (see supplementary methods for details).
92 Before each assay, we grew our isolates from freezer stocks for 24h in rich medium, either in 50
93 ml Falcon tubes under shaking conditions (190 rpm) (for the protease assay), or in 96-well plates
94 under static conditions (for all other assays). We quantified biosurfactant production as the
95 extent of the biosurfactant-induced collapse of droplets of culture supernatant on a hydrophobic
96 surface (Bodour and Miller-Maier 1998). Specifically, we grew isolates for 24h in a glucose-
97 supplemented minimal medium, harvested the supernatant, spotted 5 µl supernatant-droplets
98 on an oil-coated lid of a 96-well plate, and then measured the droplet surface area after one
99 minute using the software ImageJ (Schneider et al. 2012). We used the crystal violet assay to
100 quantify the production of biofilm matrix components (Savoia and Zucca 2007). In particular, we
101 first grew isolates in Lysogeny Broth (LB) for 24h in 96-well plates, and then measured the amount
102 of crystal violet retained by the newly formed biofilm matrix at 570nm using an Infinite M200 Pro
103 microplate reader (Tecan Trading AG, Switzerland). To quantify protease production, we spotted
104 1 µL aliquots of bacterial culture onto skim milk agar in 24-well plates, and then used ImageJ to
105 measure the size of the proteolytic halo around colonies after 24h of growth (Jousset et al. 2013).
106 To quantify pyoverdine production, we grew isolates in iron-limited casamino acids medium for
107 24h, and measured the natural fluorescence of pyoverdines in the culture supernatants
108 (emission|excitation at 400|460 nm, respectively) (Kümmerli et al. 2009). Finally, for the
109 production of toxic compounds we developed a supernatant feeding assay to assess to which
110 extent isolates affect the growth of six reference strains (*P. aureofaciens* ATCC13985, *P.*
111 *entomophila*, *P. putida* IsoF, *P.syringae* B728a, *P.protegens* CHA0, and *P. aeruginosa* PA01). We

112 first generated supernatant from each isolate after 24h growth in LB, then exposed the reference
113 strains to each supernatant, and finally measured their growth in LB during 24h. For each
114 screening assay, we grew isolates under static conditions at 28°C in fourfold replication, and
115 measured bacterial growth of the public-goods producing cultures (as optical density at 600 nm,
116 or – in the case of protease production – as colony size).

117 **Phylogenetic signal in public goods production.** To explore the relationship between the
118 phylogeny of our *Pseudomonas* isolates and their public goods production, we constructed
119 maximum-likelihood phylogenetic trees based on partial rpoD gene sequences. We constructed
120 separate trees for soil and pond habitats, based on, respectively, 149 and 148 (of the 158 and
121 157) strains for which we obtained sequence lengths ≥ 600 bp. We used *P. aeruginosa* PAO1 as
122 an outgroup, and included 20 characterised fluorescent pseudomonads as references to cover
123 the broad phylogenetic diversity of our natural strains (see (Butaitė et al. 2017) for details on tree
124 construction). Next, we mapped the production profiles of strains onto the trees (using the web
125 tool iTOL (Letunic and Bork 2016)), and then calculated Blomberg's K for each trait and habitat,
126 to quantify the extent to which phylogeny predicts public good production profiles. K-values close
127 to one reflect strong phylogenetic signals, suggesting that phylogenetic history is the main factor
128 driving the trait covariance among strains). Conversely, K-values close to zero reflect weak
129 signals, suggesting that factors apart from phylogeny affect trait evolution (Blomberg et al. 2003).
130 Note that we ignored branch lengths for the calculation of the phylogenetic signal (cf. (Butaitė et
131 al. 2017, 2018)), because the high variability of branch lengths observed among our isolates led
132 to biased and underestimated phylogenetic signals.

133 **Statistical analysis.** All statistical analyses were conducted using R version 3.5.0 (www.r-
134 project.org). We used a five-step procedure to investigate the production of – and correlation
135 among – different public goods in our *Pseudomonas* communities. To preclude that differences
136 in growth lead to spurious correlations among social traits, we first checked for associations
137 between growth and public goods production. As growth was positively correlated with the
138 production levels of biofilm matrix components (Spearman's rank correlation: $\rho = 0.341$, $p <$
139 0.001), proteases ($\rho = 0.238$, $p < 0.001$) and pyoverdines ($\rho = 0.750$, $p < 0.001$), we scaled the
140 production levels of these traits relative to growth. Growth was independent of the production
141 levels of biosurfactants ($\rho = -0.058$, $p = 0.301$) and toxic compounds ($\rho = 0.094$, $p = 0.097$). Next,
142 we scaled the measurements of public goods production either relative to the corresponding
143 measurements of the laboratory reference strain that had been grown alongside the natural
144 isolates, or – in the case of toxin production – relative to the growth of the reference strains in
145 the absence of our isolates' supernatants. Note that we transformed the supernatant effect (by
146 calculating [supernatant effect - 1]*[-1]) so that values above and below zero indicate,
147 respectively, an inhibition (i.e. toxicity) and stimulation of growth. In the third step of our
148 analyses, we transformed the measurements of all five social traits using a rank-based inverse
149 normal (RIN) transformation (Bishara and Hittner 2012). This allowed us to investigate the
150 production of and correlations among public good traits using parametric analyses. The
151 transformation was necessary, as preliminary analyses based on the untransformed
152 measurements unequivocally yielded heavily-skewed residual distributions. In the fourth step,
153 we used a nested-design permutational multivariate analysis of variance (PERMANOVA) to test
154 whether the social profiles, including all five social traits, differed between soil and pond isolates.

155 Finally, we used linear mixed models to test for (habitat-specific) links between the social traits.
156 We used the 'false discovery rate' (Benjamini and Hochberg 1995) to correct the p-values of
157 correlations between pairs of social traits for multiple testing. Where appropriate, we included
158 'community' as a random effect to account for our nested sampling design. Further details on
159 the statistical analyses are provided in the Supplementary Material.

160 RESULTS

161 ***Natural Pseudomonas isolates greatly vary in their social profiles***

162 The social profile of our 315 *Pseudomonas* isolates differed between habitats, and varied
163 substantially both among and within communities (figure 1). The difference between habitats
164 (PERMANOVA: n_{perm} = 1000; habitat: F_1 = 2.545, p = 0.045, figure 1a) was mainly driven by a
165 higher production of pyoverdines and marginally higher biofilm matrix component production in
166 freshwater as compared to soil isolates (table 1). By contrast, variation among communities (F_{14}
167 = 3.907, p = 0.001, figure 1b-f) was high for all five social traits (table 1). Within-community
168 variation followed trait-specific patterns (table S1). In particular, communities were dominated
169 by strains producing no or low amounts of biosurfactants, while harbouring only few members
170 producing high amounts of this public good (figure 1b). A similar albeit less pronounced pattern
171 governed variation in biofilm production, with most strains producing low to intermediate
172 amounts of matrix components, and only few strains investing more substantially in this social
173 trait (figure 1c). Within-community variation in the production of proteases was characterized by
174 a bimodal pattern, whereby 35.9 % of all isolates produced no proteases at all, while the
175 remaining isolates produced considerable amounts of this public good (figure 1d). By contrast,
176 variation in pyoverdine production spanned the entire continuum from zero to extremely high

177 production levels (figure 1e). Similarly, the extent to which isolates had toxic effects on our
178 references strains covered the entire continuum from being atoxic to strongly inhibitory (figure
179 1f). In summary, the high variation in the production of all five types of public good leaves ample
180 scope for the occurrence of both negative and positive trait linkage.

181 ***Phylogenetic signal for public goods production is higher in soil compared to pond***

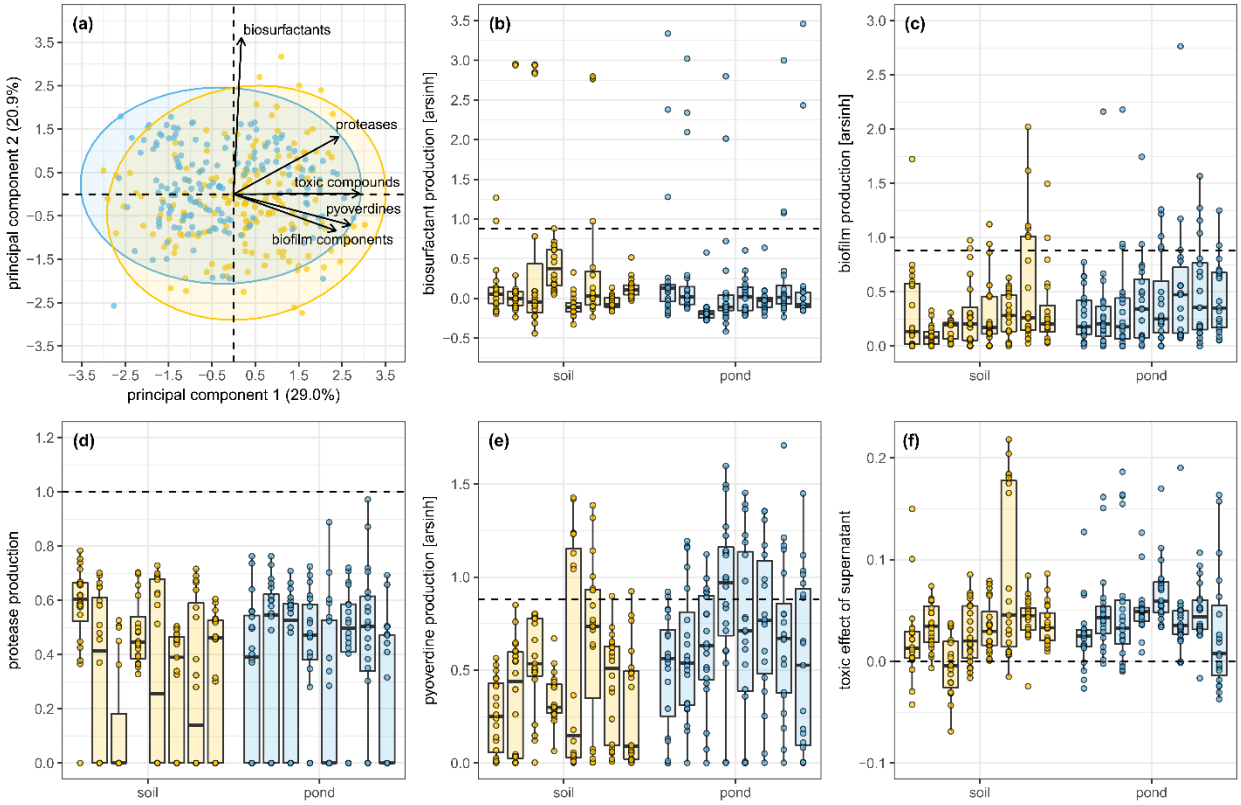
182 The production levels of all five public goods showed a phylogenetic signal (table 2, figure 2a-b).
183 Overall, phylogenetic signals were higher among soil than among freshwater isolates (paired t-
184 test; $t_4 = 3.790$, $p = 0.0193$). In both habitats, the signals were significantly higher than expected
185 from the null model for all but one trait. Specifically, biofilm production yielded a significant signal
186 only among soil but not among freshwater isolates (table 2). Signals were generally of
187 intermediate strength, indicating that both common ancestry and frequent changes in trait
188 expression profiles within clades of related strains shape public good production profiles.

189 Table 1 | Among-community variation in the expression of public good traits. Given are the grand median as
190 well as the minimal and maximal median of the production of biosurfactants, biofilm matrix components,
191 proteases, pyoverdines and toxic compounds in soil and freshwater communities, as well as the results of
192 tests for habitat- and community-specific differences in the production levels of these traits.

	grand [min|max] median		Δ habitat		Δ community	
	soil	pond	F_1	p	F_{14}	p
biosurfactants	0.017 [-0.111|0.388]	-0.005 [-0.194|0.132]	1.387	0.282	6.439	**0.001**
biofilm	0.200 [0.080|0.285]	0.301 [0.178|0.492]	3.795	0.065	1.971	**0.017**
proteases	0.402 [0.000|0.605]	0.485 [0.000|0.546]	0.152	0.705	3.278	**0.001**
pyoverdines	0.380 [0.090|0.803]	0.698 [0.551|1.127]	9.344	**0.009**	2.379	**0.004**
toxins	0.031 [-0.004|0.045]	0.039 [0.008|0.059]	1.505	0.245	5.855	**0.001**

Significant p-values (here and below) in bold.

193 **Figure 1 | Social profile of *Pseudomonas* isolates.** Depicted are (A) an overall representation of the social
194 profile of 315 natural *Pseudomonas* strains from eight soil and eight pond communities (in yellow and blue,
195 respectively), as well as the variation in the production of (B) biosurfactants, (C) biofilm components, (D)
196 proteases, (E) pyoverdines and (F) toxic compounds within and across communities. The depiction of the
197 social profile in (A) is based on the first two components extracted from a principal component analysis
198 based on the RIN-transformed values. Shaded areas are 95% confidence ellipses. The values of (B)-(E) are
199 relative to the respective value of a laboratory control (*P. aeruginosa* PAO1, indicated by the dashed line).
200 Values in (F) are the average toxic effect of the strains' supernatant on the growth of six reference strains.



201 ***Positive correlations between public good traits predominate***

202 The production levels of the five examined public goods were positively correlated in seven out
203 of ten possible pairwise comparisons (table 3, figure 3). While four of these positive correlations

204 showed congruent patterns in soil and pond communities, the remaining three were habitat-
205 specific (table 3). In particular, the production of proteases and biosurfactants correlated only
206 among freshwater (slope ± se 0.183 ± 0.073, $t_{124.7}$ = 2.488, p = 0.021) but not soil isolates (-0.034
207 ± 0.075, $t_{155.9}$ = -0.459, p = 0.700), whereas the production of pyoverdine and biofilm matrix
208 components was positively correlated among soil (0.348 ± 0.067, $t_{155.7}$ = 5.220, p < 0.001) but not
209 freshwater isolates (0.112 ± 0.085, $t_{154.3}$ = 1.319, p = 0.271). Moreover, pyoverdine production
210 increased with toxicity in both habitats, but this increase was more pronounced among soil
211 (0.456 ± 0.083, $t_{153.3}$ = 5.498, p < 0.001) as compared to freshwater isolates (0.167 ± 0.067, $t_{153.2}$
212 = 2.493, p = 0.021). When focusing on specific trait combinations, we found that biosurfactant
213 production was poorly linked to any of the other four traits, with only one positive correlation
214 occurring with protease production in ponds (table 3, figure 3). In stark contrast, we observed
215 pervasive positive correlations between the production of biofilm matrix components, proteases,
216 pyoverdines, and toxic compounds (table 3, figure 3).

217 **Table 2 | Phylogenetic signal in the production of public goods.** Given are the phylogenetic signal (Blomberg's
218 K) and the results of a randomization test (under the null hypothesis of the absence of a phylogenetic signal)
219 for the production of biosurfactants, biofilm matrix components, proteases, pyoverdines, and toxic
220 compounds among soil and freshwater isolates, respectively.

	soil		freshwater	
	Blomberg's K	p	Blomberg's K	p
biosurfactants	0.499	**0.001**	0.206	**0.023**
biofilm	0.349	**0.001**	0.181	0.063
proteases	0.516	**0.001**	0.357	**0.001**
pyoverdines	0.325	**0.001**	0.251	**0.001**
toxins	0.283	**0.001**	0.211	**0.004**

221 **Figure 2 | Maximum-likelihood cladograms for soil (A) and pond (B) isolates based on partial rpoD sequences.**

222 For both habitats, published *rpoD* sequences of 20 well-characterized fluorescent pseudomonads and *P.*

223 *aeruginosa* PAO1 were integrated into the cladograms to demonstrate taxonomic affiliation and diversity

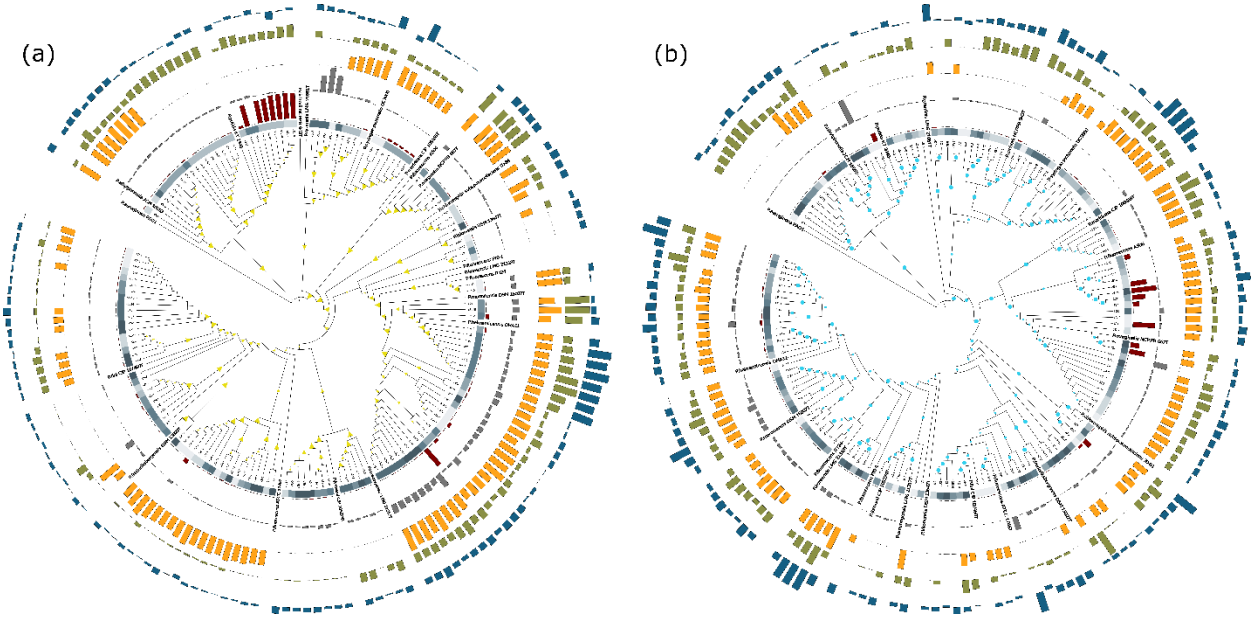
224 of our environmental isolates. Yellow triangles and blue circles indicate bootstrap values (50-100%) for

225 branches in the soil and the pond cladogram, respectively. From the inside outwards, bars depict the

226 relative production levels of biosurfactants (red), biofilm components (grey), proteases (orange),

227 pyoverdine (green), and toxic compounds (blue), respectively. Colour strips around cladograms represent

228 the communities (in shades of grey) from which isolates originated.



229 **Table 3 | Pairwise correlations among public good traits.** Shown are the (habitat-specific) pairwise
230 correlations of the production of biofilm matrix components, proteases, pyoverdine and toxic compounds
231 with the respective other social traits.

	biofilm		proteases		pyoverdines		toxins	
	χ_1^2	p	χ_1^2	p	χ_1^2	p	χ_1^2	p
biosurfactants (bis)	0.013	0.908	7.531	**0.006**	0.212	0.700	1.226	0.349
habitat	3.840	0.050	0.291	0.590	9.096	**0.003**	1.629	0.202
bis : habitat	0.551[§]	0.458[§]	4.374	**0.037**	0.898[§]	0.343[§]	3.284[§]	0.070[§]
biofilm (bif)			7.222	**0.016**	2.190	0.139	26.245	**< 0.001**
habitat			0.032	0.859	7.108	**0.008**	0.829	0.363
bif : habitat			1.479[§]	0.224[§]	4.666	**0.031**	3.559[§]	0.059[§]
proteases (pro)					27.686	**< 0.001**	40.363	**< 0.001**
habitat					6.709	**0.010**	1.194	0.275
pro : habitat					1.225[§]	0.268[§]	0.126[§]	0.722[§]
pyoverdines (pvd)							6.040	**0.014**
habitat							0.375	0.541
pvd : habitat							7.484	**0.006**

232 [§] values prior to the removal of the interaction from the model. Note that we obtain the same results when
233 the respective 'response' and the 'explanatory' social traits are swapped (Table S2).

234 Discussion

235 Although regulatory linkage between bacterial social traits occurs frequently and has important
236 consequences for strain interactions, pathogenicity and community functioning in laboratory
237 systems (Harrison and Buckling 2009; Driscoll et al. 2011; Ross-Gillespie et al. 2015; Granato et
238 al. 2018), we know surprisingly little about the prevalence and direction of social trait linkage in
239 natural bacterial communities. Here we addressed this knowledge gap by examining and
240 comparing the production levels of five social traits across 315 *Pseudomonas* isolates originating

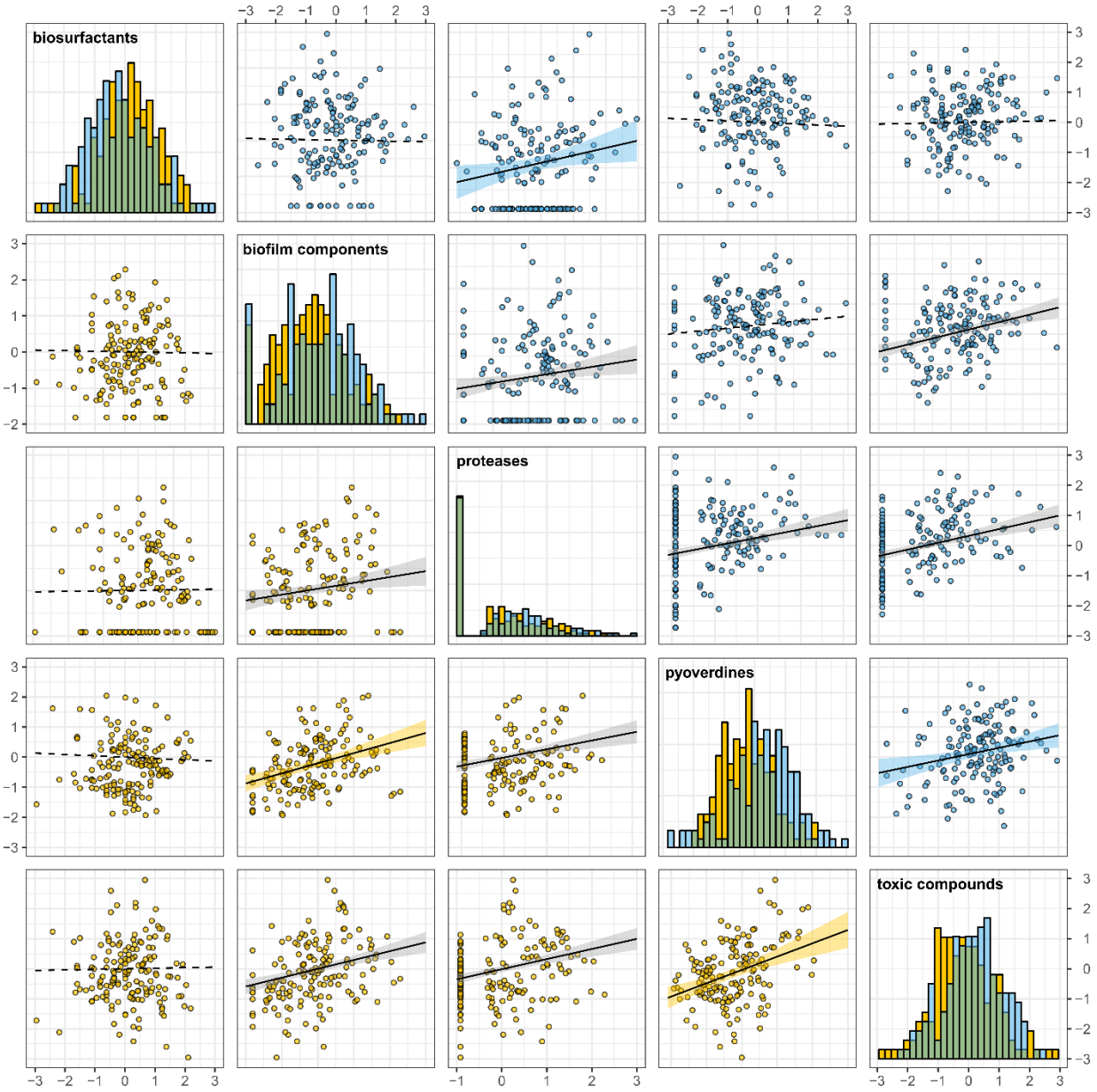
241 from eight soil and eight freshwater communities. The social traits we focused on included the
242 production of biosurfactants used during group motility; matrix components required for biofilm
243 formation; proteases used for extra-cellular protein digestion; pyoverdines required for iron
244 scavenging; and toxic compounds used to fight competitors and predators. We found that (i)
245 natural isolates varied dramatically in the extent to which they invest in social traits, and showed
246 that (ii) correlations between social traits occurred frequently, (iii) were exclusively positive, and
247 (iv) in some cases habitat-specific. A comparison across traits suggest that natural *Pseudomonas*
248 communities are dominated by strains that lie on a continuum between two main strain types –
249 a 'social' type that is proficient in expressing multiple social traits, and an 'asocial' type that
250 generally shows low investment levels into any of the social traits. Conversely, mosaic producers
251 with a specialized trait repertoire characterized by high investment in only some (but low
252 investment in other) social traits were rare. These patterns suggest that communities of natural
253 pseudomonads are dominated by competitive interactions with 'asocial' types potentially acting
254 as supercheaters that simultaneously exploit multiple social traits. In contrast, mutualistic
255 interactions based on the reciprocal exchange of different public goods seem less common.

256 At the mechanistic level, correlated trait expression could arise because public goods are
257 linked at the regulatory level. Well-known and wide-spread co-regulatory systems in
258 pseudomonads include quorum sensing systems, the GacS/GacA two-component system, and
259 systems where public goods themselves serve as signals to co-regulate other social traits (Lamont
260 et al. 2002; Jimenez et al. 2012; Dumas et al. 2013). Alternatively, trait expression could be
261 environmentally induced, which would mean that strains classified as non-producers for a
262 particular social trait might actually express this trait under different conditions. For instance,

263 some strains might grow poorly in our artificial culture media and thereby remain below the
264 quorum-sensing threshold required for social trait expression. Such environmental effects might
265 explain the generally low levels of biosurfactant production (and thus the scarcity of correlations
266 between the production of biosurfactants and the other public goods). But overall, we believe
267 that environmental trait induction plays a relatively minor role in our data set. This is because
268 the production of public goods (except for pyoverdines) was assessed in nutrient-rich media,
269 where strains grew well. Moreover, we corrected for differences in bacterial densities whenever
270 the production level of a public good correlated with growth. In the scenarios discussed below,
271 we therefore assume that the observed correlations at least partly reflect regulatory linkage.

272 At the evolutionary level, correlated trait expression could arise because high and low
273 public good producers occupy – and are locally adapted to – different niches. In this scenario, the
274 'asocial' type would occur in microhabitats where social traits are not required or where
275 coordinated actions among cells are difficult due to low bacterial densities (Kümmerli et al. 2014;
276 Butaitė et al. 2018). Conversely, the 'social' type should mainly occur in microhabitats supporting
277 higher bacterial densities and/or featuring conditions under which cooperation is beneficial.
278 However, we believe that such a clear-cut segregation of social profiles according to niches is
279 unlikely because pseudomonads are typically motile (Raaijmakers et al. 2010), and because
280 bacteria can occur at high density in both terrestrial (Whitman et al. 1998) and aquatic habitats
281 (where they aggregate locally on particles (Cordero et al. 2012)). Alternatively, correlated trait
282 expression could be the result of evolution driven by social interactions involving the exploitation
283 and/or cross-use of public goods among strains (Diggle et al. 2007; Nadell et al. 2009; Van Gestel
284 et al. 2014; Butaitė et al. 2017).

Figure 3 | Correlation patterns between social traits. Shown are the pairwise correlations between social traits of 315 natural *Pseudomonas* strains from eight soil (in yellow) and eight pond (in blue) communities. Solid and dashed lines depict significant and non-significant relationships, respectively. Bands in habitat-specific coloration as well as grey bands are 95 % confidence intervals derived from linear models and represent, respectively, habitat-specific correlations and correlations that are consistent across habitats.



290 The evolution of exploitation and cross-use of public goods at the community level can be
291 conceptualized within the framework of the so-called Black Queen Hypothesis (BQH) (Morris et
292 al. 2012). This hypothesis has been coined to explain the evolution of metabolic dependencies
293 among free-living microbes. It predicts that the loss of a metabolic function is selectively favoured
294 at the individual level as long as the function is costly, still accessible via other community
295 members, and available in sufficient amounts to support growth. Such conditions should
296 promote the evolution of two distinct phenotypes: so-called 'beneficiaries' that are deficient for
297 the production of one or multiple public goods, and so-called 'helpers' that produce these goods
298 for everyone in the community. The BQH predicts that the likelihood to lose a function
299 accelerates for strains that have already lost a function before, thereby passively locking
300 producing strains into their role as helpers (Morris et al. 2012). The observed co-existence of the
301 'social' and 'asocial' types in our *Pseudomonas* communities is well in line with this scenario.
302 Moreover, differences in linkage patterns among community members could explain why certain
303 strains become 'helpers', whereas others become 'beneficiaries'. As discussed above, positive
304 linkage of traits could lead to the simultaneous loss of multiple traits, and thus increase the
305 chances of becoming a 'beneficiary'. Conversely, no or negative linkage, but also positive linkage
306 involving fewer traits could increase the likelihood of strains being locked into the 'helper'-
307 function, as the simultaneous loss of multiple functions is impeded.

308 If social interactions drive the evolution of the 'social' and 'asocial' strain types, their co-
309 existence can be explained in multiple ways. In the case of competitive interactions, where
310 'asocial' types exploit the 'social' types, public goods cooperation and strain co-occurrence could
311 be maintained by negative frequency-dependent selection (Ross-Gillespie et al. 2007). Similar to

312 the BQH, the concept of frequency-dependent selection applies if the social traits are essential
313 and a certain amount of public goods is required for community survival. In this case cheaters
314 might only experience high relative fitness when rare, but not when common, leading to an
315 equilibrium frequency at which the two strain types can co-exist. Alternatively, antagonistic co-
316 evolution between the two types could explain the co-existence of asocial and social types
317 (Kümmerli et al. 2015; O'Brien et al. 2017). For instance, increased biofilm formation in strains
318 that highly invest in proteases and pyoverdine could be a direct response to cheating. This is
319 because biofilm formation induces spatial segregation between cell types (Van Gestel et al. 2014;
320 Irie et al. 2017) and can thus prevent cheaters from efficiently exploiting other public goods
321 (Drescher et al. 2014). Similarly, the increased toxicity of strains producing high amounts of
322 proteases and pyoverdine could represent an adaptation to selectively target asocial types, which
323 show lower toxin production and might thus be more susceptible to toxins (Jousset et al. 2009;
324 Wang et al. 2015). Finally, cheating 'asocial' bacteria might be constrained by pleiotropic effects
325 resulting from trait linkage. For example, it was shown that cheating on pyoverdine or proteases
326 can compromise the ability of *Pseudomonas* bacteria to, respectively, form biofilms or defend
327 themselves against predation (Harrison and Buckling 2009; Jousset et al. 2009).

328 In the case of commensalistic or mutualistic interactions, strain co-existence could be
329 favoured if a certain amount of public goods is required to support the community (Morris et al.
330 2012), or if strain-specific public-good specialization promotes division of labour between strains
331 via the exchange of public goods (Dragoš et al. 2018). Such interactions seem, however, relatively
332 rare in our communities. Commensalistic interactions require that the utilization of public goods
333 by the 'asocial' type does not affect the 'social' type's fitness (Morris et al. 2012) – an assumption

334 that is unlikely to be met because our isolates are generally closely related and therefore likely
335 have overlapping resource requirements. Mutualistic interactions involving the exchange of
336 different public goods require strains with specialised trait repertoires. However, the absence of
337 negative correlations among public good traits suggests that such specialized mosaic producers
338 are uncommon in our communities, such that cross-feeding scenarios, similar to those described
339 in laboratory systems (Driscoll et al. 2011; Pande et al. 2014; Kim et al. 2016; Dragoš et al. 2018),
340 might be rare among our natural isolates. Our findings are in line with other studies showing that
341 competitive interactions prevail in natural bacterial communities (Foster and Bell 2012), and that
342 mutualistic interactions only evolve under limited conditions (Oliveira et al. 2014).

343 Our observation that there are habitat-specific correlations suggest that trait linkage can
344 change in response to different environmental selection pressures. Changes in the local
345 environment, for instance when a soil strain is washed into a pond, could thus rapidly promote
346 the breaking of existing but superfluous linkage patterns or select for new ones that are beneficial
347 in the new habitat. The moderate phlyogenetic signals we detected are in line with this notion,
348 as they suggest that the expression levels of social traits can evolve relatively flexibly. In this
349 context, the overall higher phylogenetic signal in soil as compared to freshwater communities
350 could indicate faster and more flexible evolution of public good repertoires in ponds.

351 In conclusion, our findings indicate that social trait linkage (i) is pervasive in natural
352 *Pseudomonas* communities, (ii) commonly promotes competitive rather than commensalistic or
353 mutualistic interactions, and (iii) underlies the predominance of strains lying on a continuum
354 between two main strain types – a 'social' type that is proficient in expressing multiple social
355 traits, and an 'asocial' type that generally shows low investment levels into most of the social

traits. While our study solely focuses on pseudomonads, it would be interesting to see whether the same patterns of trait linkage occur beyond the genus boundary, especially for traits such as proteases and toxins, which have more broad-spectrum effects than pyoverdine, which is a characteristic trait of the fluorescent pseudomonads. Moreover, while our use of standardized laboratory assays allowed us to assess the strains' genetic potential to produce specific public goods, we do not know to which extent they would do so under natural conditions, and whether strains can plastically adjust public goods production in response to changes in environmental or social conditions. Our work therefore only provide a first glimpse of the complexity of microbial social life in natural communities. This glimpse, however, suggests that social trait linkage could play an important role in explaining community composition and functioning.

Data Accessability. The dataset supporting this article has been uploaded as part of the supplementary material, and will be made available in the Dryad Repository upon acceptance.

Competing interests. We have no competing interests.

Authors' contributions. JK and RK designed the experiments; JK and MALC conducted the experiments; JK performed the statistical analyses; JK, MALC and RK wrote the paper. All authors gave final approval for publication.

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