**Life history variation, competition and co-existence in a bacterial phyllosphere community**

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**ABSTRACT**

Competition is rife within bacterial communities, but we poorly understand the traits that underlie various types of competitive fitness, the correlations among them, and their distributions in natural bacterial populations. The short-term fitness of a particular genotype within populations and communities rests on whether or not competitive abilities are non-hierarchical (i.e. intransitive) or context-dependent. Using 39 *Pseudomonas* spp. strains isolated from the phyllosphere of a native plant, we (1) characterized a suite of life history trait variances and co-variances, (2) examined how trait variation affected competitive fitness in spatially explicit microcosms, and (3) estimated how intransitive and positive indirect interactions among strains might promote co-existence. Overall, competitive fitness differed between two major clades, *P. fluorescens* and *P. syringae*. Exploitative competitiveness was best explained by shorter lag time in *P. fluorescens* relative to *P. syringae* and increased maximum growth rate in *P. syringae* relative to *P. fluorescens*. Interference (i.e. inhibition) positively correlated with exploitative ability. The lack of trade-offs, and the distinct trait correlations for each clade, illustrates the evolutionary flexibility of the relationships among these dimensions of life history and fitness. A modest but potentially important fraction of intransitive interactions reversed competitive outcomes wherein inferior competitors would have been excluded. Thus, standing variation in life history and competitiveness potentially stabilizes the co-existence of strains through the fitness-equalizing force of inhibition-mediated facilitation**.**

**INTRODUCTION**

Ecologists have long recognized that distinct forms of competition operate simultaneously within communities: competition to exploit shared resources, and competition to interfere with another species’ ability to do so. Competition of both sorts is a potent source of natural selection operating within microbial communities (Hibbing et al. 2009; Cornforth and Foster 2013; Mitri and Richard Foster 2013). When estimated systematically, competitive (net negative) interactions vastly outnumber cooperative (net positive) interactions when diverse mixtures of bacterial genotypes are brought together (Foster and Bell 2012). In bacteria, interference traits can take the form of lethal toxins (e.g. bacteriocins) or other factors that crowd out or smother competing bacterial cells (Mitri and Richard Foster 2013), while exploitative competitive ability is often explained in terms of some combination of growth-related traits, especially maximum growth rate (Lenski et al. 1998; Hibbing et al. 2009). The traits of organisms involved in each form of competitiveness likely evolve in tandem; but what are these underlying traits, and what are the correlations among them? Describing the life history correlates of competitiveness is essential for predicting how organisms might evolve in response to diverse and overlapping forms of natural selection arising from the diverse competitive regimes that predominate in natural microbial communities (Warringer et al. 2011; Bergstrom et al. 2014).

Theoretical studies assume that interference competition trades offs with aspects of life history involved in exploitative competition such as maximum growth rate (Kerr et al. 2002; Neumann and Jetschke 2010). Investment in exploitative vs. interference competitive ability thus should be approximately zero-sum. But empirical evidence for such trade-offs is scant, and recent attempts to quantify the costs of interference competition traits have failed to detect them (Garbeva et al. 2011). Furthermore, a trade-off between maximum growth rate and growth efficiency is predicted on metabolic (Pfeiffer et al. 2001) and theoretical grounds (Frank 2010). Selection to increase exploitative ability therefore is assumed to involve increasing maximum growth rate at the expense of efficiency (Mitri and Richard Foster 2013). This prediction is supported in some cases (Luckinbill 1978; MacLean 2007; Jasmin and Zeyl 2012) but not in most others (Luckinbill 1979; Ostrowski et al. 2005; Novak et al. 2006), and may arise from environmental rather than genetic correlations (Jasmin and Zeyl 2012). If such trade-offs exist and impose constraints on the evolution of competitiveness, a pattern indicating as much should be evident in the extant variation present within a community. The ways in which the life history traits underlying exploitative and interference competition are themselves correlated may help determine the balance between the levels of competitiveness achieved by genotypes as well as how diverse microbial communities assemble.

A separate but related issue is to understand how diversity within communities influences the outcome of competitive interactions between genotypes and affects the strength or direction of selection on phenotypic traits (Ohgushi et al. 2012). Because bacterial communities in nature exhibit high species diversity, the outcome of any focal interaction may be reversed by nearby species (Rojas-Echenique and Allesina 2011). This effect is captured by the concept of intransitivity, which is the non-hierarchical arrangement of competitive abilities among diverse genotypes or species. The net effect of intransitivity is that competitiveness is highly context-dependent: one genotype’s ability to dominate another depends on the frequency of any number of other genotypes. Empirical intransitive interaction networks have been discovered both within (Sinervo and Lively 1996) and between species (Lankau and Strauss 2007), and theoretical studies have shown that introducing even modest intransitivity into competitive interaction networks can provide a buffer against species (or genotype) extinction (Laird and Schamp 2006; Laird and Schamp 2008; Rojas-Echenique and Allesina 2011; Laird 2014). Perhaps the most famous type of intransitivity takes the form of the rock–paper–scissors (R–P–S) game, where A invades B, B invades C, while C invades A. However, other forms of intransitivity such as facilitation may be common in nature (Brooker et al. 2008; Bronstein 2009; McIntire and Fajardo 2013). One potentially avenue of facilitation in bacterial communities is through differential sensitivities of the two focal competing species to interference competition (e.g. a secreted toxin) from a third player. This scenario lies at the intersection of exploitative and interference competition, and its occurrence is likely maximized by intermediate frequencies of both the production of, and resistance, to secreted inhibitors. Teasing apart how exploitative and interference competition interact in a community context remains a long-standing challenge (DeLong and Vasseur 2013). Very little empirical work has attempted as much for microbial communities, and has instead focused on toxin-mediated interference competition in pair-wise and three-way comparisons (Kerr et al. 2002; Lenski and Riley 2002; Kirkup and Riley 2004). Thus, studies are needed that directly measure the frequency of different exploitative and interference competitive abilities and to systematically assess the potential for facilitation and other forms of intransitivity to maintain life history diversity within natural communities.

The principal goals of this study were to (1) characterize the distribution of life history trait variances and co-variances among a diverse set of isolates from a wild bacterial meta-community, (2) examine how this life history variation relates to a network of competitive interactions, and (3) estimate how frequently intransitive competitive asymmetries among strains might promote co-existence. To address these goals, we build upon prior work using a natural community of endophytic *Pseudomonas* spp. bacteria isolated from a systematic survey of leaves of a perennial forb, bittercress (Brassicaceae; *Cardamine cordifolia* A. Gray) in the Rocky Mountains of North America. Bacterial strains were isolated from the internal leaf (endophytic) compartment of bittercress and include a broad range of strains from the cosmopolitan phytopathogen *P. syringae* (Morris et al. 2008) and *P.* fluorescen, a member of the rhizosphere that was found here to be a common endophytic species. Competitive interactions between functionally diverse *P. syringae* isolates can alter the success of leaf colonization by other strains (Kinkel and Lindow 1993; Wilson and Lindow 1994a; Wilson and Lindow 1994b). Although the types of traits that contribute to these competitive interaction outcomes are unknown, both phenotype-modifying compounds and toxins are secreted by phyllosphere-dwelling bacteria, especially among *Pseudomonas* spp. (Lindow and Brandl 2003; Quiñones et al. 2005; Dulla and Lindow 2009; Dulla et al. 2010; Ma et al. 2013). Phyllosphere bacteria co-localize the surface and interior of leaves (Monier and Lindow 2005). Thus, there is potential for direct interactions between competing bacteria to affect the patterns of assembly within phyllosphere communities.

We obtained 39 phyllosphere *Pseudomonas* spp. isolates from a bittercress population and a well-characterized laboratory reference strain (*P. syringae* pv. maculicola str. ES4326). We measured the outcome of pairwise competitive interactions among strains in this set, wherein strains competed for shared resources in spatially explicit microcosms. We quantified each strain’s ability to invade and defend against invasion and derived a composite measure of competitiveness that incorporated both invasive and defensive ability. We simultaneously assessed each strain’s capacity to inhibit surrounding competitors and their ability to resist strains with inhibitory capacities. Using independent measurements of maximum rate of increase, lag phase, and maximum yield *in vitro*, we then determined the underlying correlates of both exploitative and interference competitive abilities as well as the phylogenetic structure of such correlations.

Our analyses revealed major differences in both exploitative and interference competitiveness between the two major clades comprising the *Pseudomonas* spp. phyllosphere community of bittercress, as well as distinctions in how such competitiveness relates to underlying life history traits. Instead of the predicted trade-offs discussed above, we uncovered distinct correlates of competitiveness that involved shorter lag phase duration for *P. fluorescens*, and higher maximum growth rates for *P. syringae*. Canonical trade-offs between rate and yield were not observed across either bacterial clade; instead, we found a novel trade-off between lag phase and growth rate present only within *P. fluorescens*. Inhibition ability (i.e. interference competitive ability) did not trade-off with exploitative ability but was positively correlated with it. Such patterns suggest that the evolution of competitiveness may involve different components of life history for these bacterial groups.

Examining the community context of these interactions revealed that a modest but potentially important fraction of all three-way interactions result in the reversal of competitive outcomes that would otherwise lead to competitive exclusion of an inferior competitor. A small set of R–P–S interactions was discovered, which may lead to cyclical invasion dynamics and thus net neutrality of genotypes; but the intransitivity in our interaction network generally took the form of facilitation, whereby a toxic strain displaced a superior competitor thereby facilitating a resistant but weaker recipient. The community context of interference competition is important for predicting the exploitative capacity of resistant strains by buffering them from competitive exclusion, which alters the premium on exploitative capacity if a strain is also resistant. Such a dataset allows dissection of several dimensions of *in vitro* fitness exhibited by a natural community of phyllosphere *Pseudomonas* spp., and provides a platform for testing hypotheses about the mechanistic bases of competitive traits (e.g. toxin production and resistance). Together, this work helps build an understanding of how competitive traits might evolve in tandem with other life history traits in representatives from real communities that interact in nature.

**METHODS**

**Bacterial strain selection**

Bacterial strains were selected from among the 51 described in Humphrey et al. (2014). A total of 39 *Pseudomonas* spp., including 26 *P. syringae* and 14 *P. fluorescens* strains, were selected that represent the full range of phylogenetic diversity observed within the plant population sampled. To serve as a reference for these natural *Pseudomonas* spp. isolates, we included the laboratory reference strain *P. syringae* pv. maculicola str. ES4326 (hereafter 4326) owing to its phylogenetic similarity to strains isolated from bittercress and its extensive characterization in the laboratory as a pathogen of *Arabidopsis thaliana* (Cui et al. 2002; e.g. Cui et al. 2005; Groen et al. 2013).

***In vitro g*rowth assays**

All strains were re-streaked from –80 °C stocks in 50% glycerol onto King’s B (KB) plates containing 10 mM MgSO4 and incubated at 28 °C for 3 days. All strains had undergone only one prior cycle of isolation–growth–freezing since initial isolate on KB plates from surface-sterilized leaf homogenates from bittercress (Humphrey et al. 2014). Single colonies were picked and inoculated into 1 mL minimal media (MM) and grown overnight in a shaking incubator (250 rpm) at 28 °C. MM was prepared by combining filter-sterilized stock solutions to yield 10 mM fructose, 10 mM mannitol, 50 mM KPO4 at pH 5.6, 7.6 mM (NH4)2SO4, and 1.7 mM MgCl2­ (Mudgett and Staskawicz 1999; Barrett et al. 2011). MM at pH 5.6 has been shown to induce the expression of the type-III secretion system (T3SS) in a diversity of *Pseudomonas* spp. (Huynh et al. 1989), in contrast to KB, which results in negligible T3SS expression. T3SS expression was important for maximizing the potential relevance of our *in vitro* assay environments to those of plants, in which T3SS expression is expected. All 1 mL of each overnight culture was spun down for 3m at 3,000 x g and the supernatant was replaced with 500 µL fresh MM. The density of each culture was adjusted to OD600 = 0.2 prior to 1:100 dilution into a total of 180 µL MM inside the wells of sterile polystyrene 96-well plates (Falcon). Each 96-well plate was covered with BreathEasy® optically clear, gas-permeable plastic tape and incubated for 60 h in a BioTek 600 plate reader in which OD600 measurements were taken every 5 min with continuous orbital shaking. Identical growth assays were performed on separate days in duplicate using KB instead of MM.

**Life history trait estimation**

The R package *grofit* was used to fit smoothed functions to the bacterial growth data. Curve fits generated using logistique, Richards, Gompertz, or modified Gompertz equations failed to produce estimates with *r* ≥ 0.5 and we therefore used a non-parametric locally-weighted smoothing (LOWESS) function to estimate the following growth parameters: maximum growth rate *rm*, lag phase *L*, and maximum yield *K*. Lag phase represents the period in units of time (minutes) prior to initiation of exponential growth, while *K* is the maximum OD600 attained during the 60 h of growth. The growth curve of a few long lag-phased strains never leveled off (Fig. S1, e.g. strain 17A), and in these cases the final OD600 measurementwas taken as the value of *K.* In cases where the growth trajectory of strains exhibited multiple humps, indicative of intermediate lags between exponential phases, we captured the *rm* corresponding to the initial phase of such sustained growth (e.g. strain 20A; Fig. S1).

**Competition assays**

Pairwise high-density competition assays were conducted on plates (100 mm diameter petri dishes) with a 1x MM and 1% agar substrate, covered with 4 ml of 1x MM in 0.5% agar overlays containing a bacterial suspension of each resident strain inoculated at an initial density of 5x105•ml-1. Soft agar was cooled to approximately 42 °C prior to inoculation with each resident strain, and all soft agar pours were allowed to dry for 20 minutes. Suspensions of each of the 40 invader strains were then spotted at the same concentration in 4 µL aliquots spaced every 0.5 cm in parallel rows using an 8-channel pipettor. Plates were incubated face up for 12 h, followed by face down incubation at 28 °C for 10 days. Megacolony spots were scored for growth on days 1, 3, 5, 8, and 10. Data used for the following analyses are from day 10, by which time all interactions dynamics had leveled off.

Growth was scored in three discrete categories: ‘0’ for no visible growth of the invader above a negative control spot containing MM alone; ‘0.5’ for a largely translucent megacolony which reflected a definite presence of growth but which was relatively suppressed and confined to the megacolony margin; and ‘1’ for obvious and robust megacolony growth. Examples of each can be seen in Fig. S3.

Although interference competition can occur via direct cell-to-cell toxin injection (e.g. using type IV secretion system), we scored inhibition as the presence of a zone of clearance (halo) ≥ 1 mm surrounding the extent of the invading megacolony (Fig. S3). Inhibition interactions were ultimately scored as ‘0’ or ‘1’ regardless of the spatial extent of the halo, although variation in halo width was recorded.

Detailed inspection of plates also allowed us to score various interaction phenotypes presented in Fig. S3. This included multi-stage growth inhibition involving spreading waves of growth enhancement preceding a concentric spreading wave of inhibition (Fig. S3A); variation in the megacolony morphology resulting from particular strain pairings (Fig S3A–C); and the emergence of physiological resistance to secreted inhibitors (Fig. S3C).

**Estimating the components of competitiveness**

Each strain was assayed under 40 different conditions both as resident strain and as invader, comprising a total of 1600 interactions (including self vs. self). Three metrics of competitiveness were derived from the interaction patterns, representing offense (i.e. invasion) capacity (*Co*), defense capacity (i.e. territoriality) (*Cd*), overall competitiveness (*Cw*), inhibitory capacity (*Ct*), and resistance (*Cr*). *Co* and *Cd* were calculated based on the interaction matrix for resource competition. *Co* for each strain *i* was calculated as , where and is the total number of scored interactions for each strain as the invader with all non-self resident strains. *Co*  is the expected value of growth attained by each strain as the invader across the population of residents. *Cd* was calculated similarly except the focal strain *j* is in the resident state, is as before but has a subscript reversal and indicates the degree to which the resident prevented the growth of each invader *i*, and totals the number of interactions occurring between each focal resident and its non-self invaders. *Cd* can thus be interpreted as the expected value of growth each resident strain can preventamong the population of invaders assayed.

A composite measure of competitive ability (i.e. competitive fitness) in the context of growth, *Cw*, was calculated to reflect the net combination of offense and defense. *Cw* for each strain was defined as , and takes on any value from –1 to 1. These extremes represent absolute competitive inferiority (–1), where a strain failed to prevent any growth of any invader and similarly failed to invade any other strain, to absolute competitive dominance (1), where a strain fully invaded all residents and fully prevented growth of all invaders.

*Ct* and *Cr* were calculated based on the interaction matrix for interference competition. *Ct* for each strain was calculated as the proportion of invasions by the focal strain that resulted in halo formation (i.e. inhibition of the resident). *Cr* was calculated as the proportion of pairings as resident with each of the strains with *Ct* > 0 (i.e. toxin producers) that failed to result in halo formation. *Cr* is conditional on being exposed to an invader observed to produce toxins within the interaction network under study.

**Life history trait analyses**

Pearson’s *r* (correlation coefficients) were calculated between all pairs of growth and competitive trait measurement, and statistical significance was assessed as *p*<0.05after Benjamini–Hochberg false discovery rate correction implemented in R package *psych* (Revelle 2012). Euclidean distances between each growth trait for all pairs of strains were measured as for each trait. Genetic distance (*DG*) was calculated as the pairwise percent dissimilarity between 2690 bp of sequence comprised of four partial housekeeping gene sequences previously generated for each strain from Humphrey et al. (2014). Orthologous sequences from the genome of Psm4326 were derived from its published genome sequence (Baltrus et al. 2011 RefSeq ID NZ\_AEAK00000000.1). Mantel tests between pairs of trait and genetic distance matrixes were conducted in R using package *vegan,* and observed values were compared with those generated from 1000 matrix permutations (Oksanen et al. 2012). Permutation analysis of variance (perMANOVA) was carried out using the *adonis* function of the vegan package.

**Interaction network analyses**

*Genetic and trait distances vs. interaction outcome*

We calculated the outcome of interactions between all pairs of strains by determining when the outcomes of reciprocal interactions between strains *i* and *j* took following form: reciprocal invasibility (RI), where strains *i* and *j* invade each other; reciprocal non-invasibility (RNI), where strains *i* and *j* cannot invade each other; and asymmetric (Asym), where strain *i* invades strain *j* but *j* cannot invade *i*. We used binomial generalized linear models (GLMs) with a logit link to estimate the relationship between genetic distance on the probability of RI, RNI, or Asym outcomes. The models estimating the log odds of each interaction outcome between strains *i* and *j* () took form , where estimates the log odds ratio (LOR) between increasing values of , which is the genetic distance between interacting strains *i* and *j* (). LORs were estimated using the base *glm* function in R.

Next we estimated the probability of the three interactions types as functions of genetic distance as well as the distances between the independently measured life history traits *rm*, *L*, and *K*, described above. GLMs including trait distances took the form , where the interpretation of the model coefficients and variables are the same as in the model above.

Intransitive interactions were estimated across all trios of strains in the growth as well as the inhibition interactions using custom scripts in R (available upon request). We targeted two interaction patterns that reflect types of non-transitivity relevant to shaping the likelihood of co-existence among strains in a spatial context: rock–paper–scissors (R–P–S), and indirect positive interactions (i.e. facilitation).

In the context of competition without toxins, R–P–S describes when three pairs of asymmetric interactions combine in such a way as to make each strain superior and inferior to precisely one of the other two strains: *A* invades *B*, *B* invades *C*, and *C* invades *A*, and each of the reverse interactions results in a failure to invade. The interpretation of an R–P–S trio in the context of exploitative competition is that relative competitive fitness is equalized among strains in a trio, and the outcome of three-way competition may be transient co-existence tempered by stochastic extinction, or stable co-existence through frequency-dependent strain cycling—each as opposed to deterministic competitive exclusion.

We also examined the interference competition matrix for evidence of R–P–S trios. R–P–S in the context of interference competition resembles trios wherein the asymmetric invasion patterns described above are replaced by susceptibility and resistance to inhibitors. R–P–S trios in this case represent strains that are likely to co-exist given the right spatial context. R–P–S are stable when spatial structure allows producer–susceptible strain pairings to be progressively eliminated, leading ultimately to each strain predominantly experiencing an environment without inhibitors (i.e. are resistant to their nearest neighbor, e.g. Kerr *et al.* (2002)).

Facilitation describes a separate combination of competitive asymmetries that combine to allow a focal strain *C* to perform better vs. competitor *B* in the presence of a facilitator strain *A*. In other words, strain *A* increases the number of conditions under which strain *C* can invade. Using this general condition, we evaluated whether facilitation occurs in trios using the interference competition network on its own, as well as by jointly considering interference competition and resource competition networks.

Facilitation via interference competition results when a focal strain *C* is released from interference competition from strain *B* because of inhibition of *B* by an inhibitor-producing strain *A,* to which *C* is resistant. Such a scenario reflects an additional benefit to being resistant: the potential to benefit from the inhibitors of neighbors without having to directly produce them.

In jointly considering resource and interference competition, we hypothesized facilitative interactions where *B* and *C* are engaged in exploitative competition for a shared resource at which *C* is inferior to *B*, and a nearby strain *A* produces a diffusible inhibitor to which *B* is more susceptible than *C.* In contrast to the case above, the suppression of our focal strain *C* by *B* is in this case not inhibitor-mediated but instead results from the ability of *B* to hold off invasion by *C* in the absence of *A*. We limited this analysis to the asymmetric pairwise interactions in the network that left the loser strain with ‘0’ invasion points vs. the winner, and that such a winner scored at least 0.5 when invading the loser. In other words, we only considered facilitation of loser strains whose fate otherwise would be (local) competitive exclusion.

**RESULTS**

**Phylogenetic patterns in trait variation**

Maximum growth rate (*rm*), lag time (*L*), and maximum yield (*K*) derived from growth curves (Fig. S1) varied substantially between *P. fluorescens* and *P. syringae* clades (Fig. 1a–b): *P. fluorescens* strains on average had growth rates 1.6 times higher than *P. syringae*, and although average *L* and *K* values were similar between clades, *P. syringae* exhibited far more variation in both *L* and *K* than *P. fluorescens* (Fig. 1b). The composite pattern is that strains from each clade differ systematically in both the distribution of, and correlations between, the life history characters measured here.

Genetic distance strongly predicted trait divergences across this suite of *Pseudomonas* spp. strains (mantel statistics [*m*] were above the 95% or 99% quantile of the permuted null distributions for each trait; Table S1). The correspondence between genetic distance and trait similarity was weaker when comparing strains within the same clade but in all cases showed *m* values corresponding to *p*≤0.028 (Table S1).

*Exploitative competition traits.* Pairwise soft-agar invasion assays (Fig. S2) revealed that the competitive abilities of *P. fluorescens* isolates was superior to *P. syringae* isolates across all three metrics (Fig. 1a–b). Within *P. syringae*, variation between sub-clades was evident (Fig. 1a), reflected in a moderate correlation between *DG* and overall competitive fitness (*Cw*) (95% < *m* < 99% null quantiles; Table S1). Variation in competitive indexes in *P. fluorescens* was slightly bimodal (Fig. 1b), with distances between strains exhibiting a strong correlation with genetic distance (*m* > 99% null quantile; Table S1).

*Interference competition traits.*Of the 40 strains assayed, 13 (all *P. fluorescens*) produced halos surrounding some subset of the resident strains they invaded, indicating the production of diffusible inhibitors (Fig. S2). Although we scored inhibition as a binary trait (see *Methods*), the strength and extent of inhibition varied among strain pairings, examples of which are shown in Fig. S3a–c (black arrows). Mean inhibition index (*Ct*) was 0.20, although 2 strains inhibited only one other, and 03A failed to inhibit any strains (Fig. S2). Inhibition interactions were predominately restricted to *P. fluorescens*–*P. syringae* pairings, although four *P. fluorescens* strains were susceptible to inhibition by two of the toxic strains (43A, 34A; Fig. S2). Resistance to toxin producers in *P. syringae* was variable, although the mean value was high at 0.72 (Fig. 1b). Genetic distance between strains was correlated neither with similarity in resistance values (*Cr*) in *P. syringae* to toxin-producers, nor with *Ct* in *P. fluorescens* (*m* < 90% quantiles; data not shown). Overall, only *P. fluorescens* exhibited toxin production, but resistance to such toxins was common among strains from both clades.

**Correlations between life history components**

*Growth traits.* Owing to the clear differences between clades, we analyzed the correlations between the traits for each clade separately. The canonical and expected negative correlation between *rm* and *K* was not observed for either clade, although the trend was weakly negative for *P. fluorescens*; for *P. syringae* it was positive (Pearson’s *r* = 0.47 [0.10–0.73 95% CI], *p* = 0.016; Fig. 2a). Instead of an *rm* trade-off with *K*, *P. fluorescens* strains exhibited a positive correlation between growth rate and with lag time: the faster growing strains had slower acceleration. Lag showed no correlation with *rm* for *P. syringae*, but was weakly positively correlated with maximum yield, *K* (Fig. 2a). Detailed scatterplots are presented in Fig. S4a for all growth trait comparisons.

*Resource environment.* Maximum growth rate, lag phase duration, and maximum yield were only loosely correlated between minimal media (MM) and a rich media (KB) (Fig. S5). When they were correlated, strains from *P. fluorescens* and *P. syringae* exhibited qualitatively distinct patterns. Maximum growth rate was positively correlated across environments for *P. syringae* but negative for *P. fluorescens*; the latter strains exhibited a trade-off such that the slowest growers in minimal media were nearly twice as fast as the rest when in rich media, and vice versa (Fig S5a). *P. fluorescens* lag time also exhibited a trade-off between environments, to which maximum growth rate was similarly positively correlated, as in MM. The between-trait correlations were conserved across environments for *P. syringae*, reflected in a positive correlation between growth rate and yield (Fig. S5b). However, we observed wide variance and no correlations between environments for both lag phase and yield on their own (Fig. S5a). Resource environment thus plays a potentially large role in coordinating the expression of traits related to these fundamental life history characters.

*Life history correlates of competitiveness.* Offense (*Co*) and defense (*Cd*) were strongly positively correlated overall with linear slopes near 1 (Fig. 2a; Fig. S4c); however, there is a dense and narrow band of strains with *Co* between 0.2 and 0.25 that showed a wide range of *Cd*values between 0.03 and 0.6 (Fig. S4c). Within this range, offense and defense appear uncorrelated. Lack of correlation around this range may indicate a saturating function may fit the data better; however, a second-order polynomial regression did not improve fit (log likelihood ratio test; *X2* = 0.8, *p* > 0.2, data not shown). Both offense and defense compared against the composite measure *Cw*, yielded similar near-1 slopes but with slightly offset elevations between the clades (Fig. S4c).

Competition and growth traits revealed opposing correlational patterns between the clades: *Cw* was positively correlated with *rm* for *P. syringae* (model *F3,21* = 11.5, adjusted *r2* = 0.57; Table 1), but negatively with both *rm* and *L* for *P. fluorescens*. When analyzed in a multiple regression, the correlation with *rm* disappeared, revealing a strong negative relationship with *L* as the sole driver of variation in *Cw* for *P. fluorescens* (model *F3,21* = 15.3, adjusted *r2* = 0.77; Table 1). *Cw* in *P. syringae*, on the other hand, was solely explained by *rm*; Table 1).

Principle component analysis of *Cw*, *Cr*, and *Ct* with the three growth traits revealed largely non-overlapping 95% confidence ellipses for the two clades (Fig. 2b). The first two principle component axes together explained 72.5% of the variation in the data. The loading vectors of competitiveness and lag phase were in opposing directions, indicating a negative correlation, while those for competitiveness and inhibitory capacity are largely collinear, indicating a positive correlation (Fig. 2b). The loading for resistance, *Cr*, falls close to co-linear with lag phase duration, a relationship that was not apparent in the pair-wise correlation analysis depicted in Fig. 2a. Interestingly, strain 08B—tentatively categorized as *P. syringae* in this analysis but phylogenetically sister to the clade—falls beyond the 95% confidence ellipses for both named clades (Fig. 2b).

**Genetic distance, trait matching, and competition outcomes**

Asymmetric competitiveness was the most frequent interaction outcome both for the total strain set and the within- and between-clade comparisons (Fig. S6a). 98% of between-clade pairings resulted in the asymmetric dominance of one strain (the *P. fluorescens* strain always won; Fig. S2). Within clades, however, *P. syringae* strains were more likely to exhibit reciprocal invasibility than *P. fluorescens*, while the proportion of asymmetric interactions was similar in both clades (20%; Fig. S6a). RI and RNI interactions became less frequent with increasing genetic distance (Fig. S6b; Table 2). Including pairings between very same strain (i.e. the most “intra-specific” form of competition assayed here) into this GLM increased the magnitude of the probability estimate for reciprocal competitive exclusion at *DG* ~ 0 (Table 2).

In binomial GLMs, the overall probability of an asymmetric outcome increased with genetic distance (Fig. S6b; Table S1). This finding is driven predominantly by the competitive superiority of *P. fluorescens* over *P. syringae.* When trait distances were included with genetic distance in interaction outcome GLMs, the effect of *DG* generally dropped out (Table S2). Within *P. fluorescens*, pairings between strains with larger differences in lag time more often resulted in asymmetric outcomes, while similarity in lag time predicted reciprocal invasibility (Table S2). *P. syringae* exhibited the same pattern of findings except for *rm* instead of *L*, although residual variation was still explained by *DG*, perhaps correlated with un-measured factors involved in competitiveness (Table S2).

Strains isolated from the same leaves were no more or less likely to display a particular competitive outcome than pairs of strains isolated from different leaves (Fisher’s exact tests, *p* > 0.3, data not shown), nor were winners more likely to have been at a higher abundance in leaves when they were sampled (Humphrey et al. 2014) (binomial sign test, *p* = 0.45, data not shown).

**Competitive interaction network and intransitivity**

*Toxin-mediated facilitation.* Our analysis of the patterns of pairwise interaction outcomes combined with the traits that predict them was expanded to include sets of three strains in order to evaluate the extent of intransitivity (non-hierarchical competitive asymmetries) within the bacterial interaction network. 8,203 trios were evaluated for facilitation based on the 641 pairs of strains that met the competitive asymmetry criteria (see *Methods*). Of these trios, 632 (7.7%) met the criteria of the inferior competitor being facilitated by the killing of the superior competitor by a third party to which the facilitated strain was resistant. Of all 40 strains, 39 were implicated at least once as a participant in a facilitative trio as one or more of the following roles: the facilitator, the knocked-out competitor, or the facilitated strain (‘A’, ‘B’, and ‘C’, respectively; Fig. 3a). Overall, 26 strains were facilitated, and 21 of these also served as the knocked-out competitor in a subset of the trios (Fig 4b, inset). Twelve of the 13 inhibitor-producing strains (all *P. fluorescens*) were implicated as facilitators (A strains) (Fig 4b, inset). In all but 6 of the 632 facilitative trios, the A strain was also exploitatively dominant to the C strain, even though such strains were resistant to their inhibitors (Fig 4b, arrows).

The number of trios in which a given strain played each role varied substantially among strains, with one strain in particular (43A) playing a disproportionate role as facilitator compared to other inhibitor-producing *P. fluorescens* (Fig. 3b). Three inhibitor-producing strains also played the role of the B strain in trios including an A strain to whose toxin it was susceptible (36A, 46A, 06B), and one *P. fluorescens* strain played all three roles (06B). For strains that exclusively played the B and C roles, the probability of playing a B role was weakly negatively correlated with the probability of playing a C role (Pearson’s *r*  = –0.30 [–0.56–0.12, 95% CI], *p*=0.059). The propensity towards B vs. C roles was explained by underlying differences in competitive fitness: the most facilitated strains (high C fraction) were among the least competitive (low *Cw*) in the populations, indicated by a negative correlation (*r*  = –0.76 [|0.86–0.58| 95% CI], *p*<10–5; Fig. 3c, Table 3). B strains were intermediate relative to the entire range of *Cw* values. Facilitator A strains had consistently higher *Cw*, owing to the generally higher competitiveness of *P. fluorescens* strains (Fig. 1a–b). Resistance (*Cr*) was strongly positively correlated with the probability of being facilitated (Pearson’s *r* = 0.57 [0.32–0.75 95% CI], *p*<10–4; Fig. 1d, Table 3), and *Cw* and *Cr* jointly considered in binomial GLMs independently (*Cw* and *Cr* are uncorrelated for *P. syringae*; Fig. 2a) contributed to variation in B and C roles (Fig. 3c–d, Table 3). A network of interactions between A and C strains is displayed in Fig S6a.

We also uncovered five trios whose mutual invasion asymmetries met the criteria for a rock–paper–scissor game out of the 9,604 possible trios of interactions evaluated (Fig. 3a). Nine unique strains were implicated in these trios. Each trio was comprised of distantly related *P. syringae* strains (mean *DG* between strains in R–P–S trios = 0.118 [0.115–0.122 95% CI]; Fig S6b). Strains 21A and 24C were implicated in three R–P–S trios that included several unique third-party strains (Fig S6b).

**DISCUSSION**

*P. fluorescens* and *P. syringae* strains differ substantially in life history across nearly all axes measured in the estimated mean values per clade, their variances, as well as the correlations among them. Exploitative competitiveness was substantially higher in *P. fluorescens* strains, and the underlying traits that correlated with variation in exploitative competitiveness were unique for each clade. Competitive dominance in within-clade competitions was largely explained by how dissimilar the life history traits were between the interacting pairs, and genetically more similar strains tended to display more similar life histories.

Such variation in competitiveness resulted in a large number of non-hierarchical interactions when we examined the asymmetries between all trios of strains. Despite being competitively superior, *P. fluorescens* may indirectly facilitate otherwise poor competitors that are nonetheless resistant to inhibitors to which their superior competitors are not. The intermediate frequencies of inhibitor production and resistance among these *Pseudomonas* spp. strains thus may indirectly elevate the phenotypic diversity maintained across the community, with the relatively poor competitors reaping the majority of the benefit from facilitative interactions. This result echoes a similar result from a theoretical study wherein the weakest competitor in an exploitative R–P–S trio experienced the largest increase in population size, arising from a tragedy of the commons that nonetheless maintains diversity (Frean and Abraham 2001).

**The life history correlates of exploitative competition are variable**

Selection for increased exploitative competitive ability is expected to increase maximum growth rate, perhaps at the expense of growth efficiency (i.e. yield), which can result in a tragedy of the commons whereby rapid but wasteful use of resources yields higher competitive ability (Pfeiffer et al. 2001; MacLean 2007). Consistent with this, overall exploitative competitive fitness (*Cw*) for *P. syringae* was positively correlated with maximum growth rate (*rm*) (Table 1). Notably, however, neither *P. syringae* nor *P. fluorescens* strains exhibited a canonical *r–K* trade-off: growth rate was positively correlated with yield in both minimal and rich media for *P. syringae*, and was uncorrelated with yield for *P. fluorescens* (Fig. 2, S5). In stark contrast to *P. syringae*, exploitative competitiveness for *P. fluorescens* was most correlated with having a shorter lag phase duration (1/*L*)*.* Interestingly, shorter lag phase was negatively correlated with growth rate for *P. fluorescens*, which was a pattern not found for *P. syringae*. These findings indicate that exploitative ability for *P. syringae* and *P. fluorescens* likely are constrained by separate underlying life history characters, and that the unique correlational structure among these life history characters may differentially constrain the evolution of exploitative ability for each clade.

We were surprised to uncover that maximum growth rate was correlated with a longer lag phase in *P. fluorescens*, as this pattern contradicts the traditional dichotomy between generally “fast” vs. “slow” life histories. *Escherichia coli* lines adapting to a glucose-limited environment exhibited coordinated increases in growth rate *and* shorter lag time after 10,000 generations (Vasi et al. 1994; Lenski et al. 1998). *E. coli* selected to persist in lag phase during periods of antibiotic stress incurred no pleiotropic cost of reduced maximum growth rate despite up to a 10-fold increase in lag time (Fridman et al. 2015). One explanation for our lag–growth correlation is that competitive fitness for *P. fluorescens* in the spatial microcosms we used may have more to do with space than resource use; thus, strains that preempt as much space as possible early on may reap the rewards of their territorial monopoly even when this comes at the expense of a decreased maximal rate of growth. One potential mechanism of this is the production of exudates that prevent physical expansion of competitor cells. This explanation rests on an intuitive physiological trade-off between exudate production and cell replication, but explains both the premium on short lag as well as its later costs.Thus, a straightforward hypothesis is that lag phase is causally affected by the amount of exudate production and exudate production trades off with maximum growth rate. Lag phase has received renewed attention as a distinct component of microbial life cycles (Rolfe et al. 2012), and characterizing the physiology of cells during this phase may reveal the nature of its linkage with maximum growth rate.

Our results suggest that *P. syringae* strains are generally less able to exert priority effects in the spatial context of our assays. However, if both strains were to compete in an unstructured environment where preemption of space was irrelevant, *P. syringae* strains with high growth rates might be expected to outcompete a variety of *P. fluorescens* strains with relatively lower growth rates (Fig 1). Thus, the lag phase–growth rate negative correlation in *P. fluorescens* resembles a colonization–competition trade-off. Spatial priority effects arising from territoriality have been shown theoretically to provide a mechanism for maintenance of colonization–competition trade-offs that would otherwise lead to competitive exclusion (Edwards and Schreiber 2010). More recently, a colonization–competition trade-off has been shown to underlie territoriality in *Vibrio* spp. based on the differential ability of clones to contest territory vs. disperse to new ephemeral habitats (Yawata et al. 2014). Either one of these mechanisms may contribute to the maintenance of diverse exploitation strategies in *P. fluorescens* across patchy and ephemeral leaf environments.

In light of these findings, we hypothesize that *P. fluorescens* may be a territorial species whose potential effect (among others) in the phyllosphere may be to exclude colonization by *P. syringae* strains. This prediction is consistent with the identity of *P. fluorescens* as a plant mutualist, although the evidence of this comes exclusively, to our knowledge, from studies of its indirect effects via plant defensive signaling or direct toxicity to pathogenic fungi following its colonizing of plant roots (Mendes et al. 2011; Hol et al. 2013). In addition to such indirect effects, the superior competitiveness of *P. fluorescens* vs. *P. syringae* noted in this study suggests that direct interactions may affect phyllosphere bacterial community assembly as well as plant disease risk from phytopathogenic isolates of *P. syringae*.

Growth of the laboratory strain Psm4326 in MM appeared attenuated compared to its four closest relatives in this strain collection (Fig. S1). This strain was also among the weakest competitors, both offensively and defensively. It is possible that this sensitivity arose as a consequence of domestication to the laboratory environment. This implies that the preservation of genotypic states from wild isolates is crucial in order to accurately infer relationships among life history traits if the goal is ultimately to understand how they are shaped by the selective milieu in the wild.

**Lack of trade-offs between exploitative and interference competition**

The manifestations of correlations between exploitative and interference competition may strongly depend on the underlying mechanisms of interference competition. Here, such an interference mechanism could range from direct injection of bacterial effectors via type VI secretion systems (Decoin et al. 2014), the production of subversive growth-regulating diffusible *N*-acylhomoserine lactones (AHLs) or enzymes that quench these signals typically involved in quorum sensing (QS) (Dulla and Lindow 2009), or the production of diffusible toxins (e.g. bacteriocins or phage-derived proteins). Trade-offs between toxin production and toxin resistance with intrinsic growth rate are often presumed in R–P–S models (e.g. Neumann and Jetschke 2010) and are necessary to permit co-existence of types. Interestingly, inhibition ability (*Ct*) or resistance (*Cr*) did not trade-off with any of the life history traits measured in this study (Fig. 2a), which is consistent with recent findings that toxin induction did not affect *in vitro* life history measures in *P. fluorescens* (Garbeva et al. 2011). Instead, we detected a positive correlation between inhibitory ability (*Ct*) and overall exploitative competitiveness for *P. fluorescens*. Although unexpected, such a positive correlation is nevertheless intuitive: megacolonies invading a resident strain presumably must reach a critical size in order for any toxicity to be detectable if induction is either density dependent or if the toxic effects are concentration-dependent. Cells may only reach such a critical density if their relative exploitative competitiveness enables them to do so, without which interference competitive ability is irrelevant.

**The interaction between exploitative and interference competition underlies intransitivity**

Irrespective of the underlying mechanisms of toxicity and resistance, the frequency of these traits in a community may have large indirect effects that generate intransitive asymmetries among diverse genotypes. Several theoretical studies on intransitivity call for increased empirical research in order to test the predictions that non-hierarchical competitiveness stabilizes diversity (Laird and Schamp 2006; Laird and Schamp 2008). Our results address this call by exploring some of the intransitive properties of an empirical interaction network measuring the joint outcomes of exploitative and interference competition. Instead of combining assortments of laboratory strains to explore community properties

(e.g. Eisenhauer et al. 2012a; Eisenhauer et al. 2012b; Eisenhauer et al. 2013), we used a collection of natural isolates derived from a local population of native bittercress. Intransitivity in bacterial communities has been principally considered empirically with respect to bacteriocin production and resistance (Czaran et al. 2002; Kerr et al. 2002; Majeed et al. 2011). In our interaction network, we found evidence that the interference competition may equalize fitness differences between competitors that otherwise have asymmetric exploitative abilities (Fig. 3b). Many strains that were facilitated by an inhibitor producer to which they were resistant were also the strain whose inhibition facilitated another (Fig. 3c–d). Intransitive facilitation of the sort explored here is only possible with an intermediate frequency of toxin resistance expressed by *P. syringae* (Fig. 3d). The fact that resistance is not more common among *P. syringae* suggest the existence of a cost of resistance that did not manifest itself in the assays conducted in our study.

We show that the gains from facilitation predominantly go towards weaker resource competitors (Fig. 3c). Only in a small subset of the facilitation trios could the facilitated strain invade the producer. When the facilitated strain does *not* pose a competitive threat to the facilitator—as is the case most of the time here—the gains from facilitation may be short-lived. However, the overall effect of this degree of intransitive facilitation may be to prolong periods between exclusion/extinction events, elevating the diversity that is observable at any given point within the system (Laird and Schamp 2006; Laird and Schamp 2008; Laird 2014). The additional form of intransitivity detected in our study is a pair of extended trios that have R–P–S invasion asymmetries, which are predicted to lead to frequency-dependent or cyclical invasion dynamics (Laird 2014). This prediction is awaiting an empirical test, and this system presents an excellent opportunity for doing so.

These conceptual implications of intransitivity speak to the large degree of diversity (both genetic and phenotypic) apparently maintained within this *Pseudomonas* spp. community despite clear pairwise competitive asymmetries between a large fraction of the strains. Due to the inherently spatial and ephemeral nature of the phyllosphere environment in a sub-alpine plant species, extensive variation along an absolute fitness gradient might be expected, as spatial structure protects low fitness genotypes from global exclusion (Amarasekare 2003; Laird and Schamp 2008; Kryazhimskiy et al. 2012). Still, multiple distinct genotypes occurring within the same leaf is common (Fig. 1a), and the potential for local competitive interactions within or on leaf surfaces is large (Lindow and Brandl 2003). The factors promoting co-existence, even if transient, may include competitive intransitivity mediated by some combination of exploitative and interference competition, as documented here. A predicted consequence of this for evolution as well as community assembly is that larger fitness differences are required between genotypes or species in order for deterministic processes like competitive exclusion of genotypes/species to take place at the landscape scale (Cvijovic et al., *in review*). This system is ripe for the modeling of how particular combinations of (1) life history traits and (2) inhibitor production and resistance traits can stabilize the prevalence of relatively poor competitors embedded in non-hierarchical interaction networks. The joint implications of the maintenance of otherwise poor competitors for ecosystem-level traits such as productivity or trophic flow through food webs and the rate of adaptive evolution within species, remains a compelling topic for further study.

**Conclusions**

We find that the competitive abilities of strains within a natural community of phyllosphere *Pseudomonas* spp. varied between the two major clades present, *P. fluorescens* and *P. syringae*. Variation in competitiveness was best explained by distinct life history traits in each clade: shorter lag time in *P. fluorescens*, and increased maximum growth rate in *P. syringae*. The lack of expected life history trade-offs between growth rate and yield, but the presence of different trait correlations that were distinct between clades, illustrates the evolutionary lability of the relationships among these fundamental dimensions of fitness. Nonetheless, conserved trait correlations within clades suggest that disparate life history strategies may allow for persistence of both clades in the environment. The *P. fluorescens* clade may contain early colonizing strains that contest territory to a greater extent, which may serve to directly buffer against leaf colonization from potentially phytopathogenic *P. syringae.* In contrast, a high degree of inhibitor resistance among *P. syringae* may prevent local exclusion when spatial structure releases them from direct exploitative competition with *P. fluorescens*. Finally, the combination of exploitative and interference competition in this system potentially stabilizes the co-existence of strains that might otherwise competitively exclude one another in isolation due to the fitness-equalizing force of inhibitor-mediated facilitation.

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**FIGURES**



**Fig. 1**. Phylogenetic distribution of life history trait variation within a *Pseudomonas* spp. community. **A**. Life history components are maximum growth rate (*rm*), lag phase (*L*), maximum yield (*K*), derived from individual microcosm growth experiments; and components of offensive (*Co*), defensive (*Cd*), overall (*Cw*) competitiveness, resistance to toxicity (*Cr*), and toxicity (*Ct*), measured on derived from a pairwise competitive interaction network (see *Methods*). Column- z-score of each trait value indicated by color. **B**. Smoothed frequency distributions of trait values for each measured trait by clade (*P. fluorescens* and *P. syringae*). Mean (µ) estimates per clade with ± 2x standard errors depicted to the right of the curves. Note the *x*-axis value scale modifiers to the right of the axis labels.

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**Fig. 2.** Pairwise correlations (**a)** and principle component analysis (PCA) (**b**) of six traits reflect dissimilarities between clades, as well reveal the correlational structure among traits across *Pseudomonas* spp.. **A**. Colors reflect magnitude of each *Pearson’s r* estimate where the FDR corrected *p* < 0.05; comparisons with FDR-corrected p<0.10 retained color but are italicized. **B**. PCA 95% envelopes per clade depicted as solid or dashed ellipses. Dots are labeled with strain Ids. Individual trait vector loadings are in blue for resource use traits and orange for interference traits).

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**Fig. 3.** Prevalence of intransitive interactions in a *Pseudomonas* spp. interaction network. **A**. Types of interaction trios resulting in facilitation (left) or rock-paper-scissors (R–P–S) competitive asymmetries. *N* = number of trios meeting the given criteria out of the total trios analyzed (see *Methods*). **B.** Frequency distributions of how often each strain played the facilitator (‘A’), the knocked-out intermediate (‘B’), or the facilitated (‘C’). Several strains played multiple roles; strains in facilitative trios with as well as without toxic intermediates are indicated with black triangles to the left of the strain Ids. Panel (**B**) inset displays the distribution of the number of unique strains that played each combination of roles. 06B played all three. The probability of playing A, B, or C roles in facilitative trios varied with (**c**) overall competitiveness, *Cw*, as well as (**d**) resistance, *Cr*. See Table 5 for GLM results.

**TABLES**

**Table 1.** Multiple regression model results for life history trait correlates of competitiveness.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Clade | variable | estimate | se | *t* | *p* |  | *F* | *r2* | *p* |
| *P. fluorescens* | Int. | 1.1 | 0.315 | 3.529 | 0.006 |  | 15.3 | 0.77 | 0.0004 |
|  | *rm* | –52 | 256 | –0.206 | 0.84 |  |  |  |  |
|  | *L* | -0.0008 | -0.0001 | –4.898 | 0.0006 |  |  |  |  |
|  | *K* | 0.214 | 0.495 | 0.433 | 0.67 |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |
| *P. syringae* | Int. | –0.693 | 0.129 | –5.356 | 0.0001 |  | 11.5 | 0.57 | 0.0001 |
|  | *rm* | 1016 | 217 | 4.681 | 0.0013 |  |  |  |  |
|  | *L* | -5.88E-05 | -7.08E-05 | –0.832 | 0.41 |  |  |  |  |
|  | *K* | 0.202 | 0.296 | 0.681 | 0.51 |  |  |  |  |

*rm* = maximum growth rate

*L* = lag phase duration

*K* = maximum growth yield

se = standard error of the coefficient estimate

**Table 2.** GLM results for pairwise interaction outcomes vs. genetic distance with and without self-interactions included.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Including self-interactions | | | |  | Excluding self-interactions | | | |
| Outcome | LOR (*DG*) | se | *z* | *p* |  | LOR  (*DG*) | se | *z* | *p* |
| RI | –0.17 | 0.02 | 7.25 | < 0.0001 |  | –0.11 | 0.03 | 3.62 | 0.0003 |
| RNI | –0.17 | 0.02 | 8.13 | < 0.0001 |  | –0.13 | 0.03 | 4.88 | < 0.0001 |
| AS. | 0.21 | 0.02 | -11.17 | < 0.0001 |  | 0.13 | 0.02 | -6.19 | < 0.0001 |

*DG* = genetic distance

RI = reciprocal invasibility

RNI = reciprocal non-invasibility

AS = asymmetric outcome

LOR = log odds ratio

se = standard error of the LOR estimate

**Table 3.** GLM results for probability of playing facilitator (A), knocked-out competitor (B), or the facilitated strain (C) in interaction trios.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Y | X | LOR | se | *z* | *p* |
| Pr( A ) | Int. | -1.87 | 0.49 | -3.80 | < 0.0001 |
|  | *Cw* | 2.19 | 0.53 | 4.10 | < 0.0001 |
|  | *Cr* | 0.45 | 0.29 | 1.54 | 0.12 |
| Pr( B ) | Int. | 0.02 | 0.12 | 0.14 | 0.89 |
|  | *Cw* | -0.85 | 0.11 | -7.74 | < 0.0001 |
|  | *Cr* | -1.90 | 0.20 | -9.70 | < 0.0001 |
| Pr( C ) | Int. | -3.71 | 0.24 | -15.76 | < 0.0001 |
|  | *Cw* | -2.66 | 0.18 | -15.16 | < 0.0001 |
|  | *Cr* | 2.48 | 0.29 | 8.45 | < 0.0001 |

A = facilitator role

B = knocked-out competitor role

C = facilitated role

Int. = intercept

*Cr* = resistance

*Ct* = toxicity

Y = dependent variable

X = predictor variatble

LOR = log odds ratio

se = standard error of the LOR estimate