

Evolution alters ecological mechanisms of coexistence in experimental microcosms

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Summary

1. Species coexistence can be promoted by both equalizing mechanisms that increase fitness equivalence among species and stabilizing mechanisms that decrease niche overlap among species. Strength of the coexistence mechanisms can be altered by evolution. In particular, populations evolved in sympatry may show niche divergence due to competition-mediated selection, whereas populations evolved in allopatry have a chance to become ecologically equivalent competitors which, on secondary contacts, may form ‘neutral communities’.

2. We addressed how evolution may change ecological mechanisms of coexistence with experimental microcosms of *Escherichia coli* strains that reproduce asexually and can be considered as analogues of species. We obtained five pairs of bacterial strains, within each of which the two strains could coexist stably. We then allowed the bacterial strains to evolve in a chemically defined nutrient medium for ~1100 generations, under sympatric (in two-strain mixtures) or allopatric scenarios (in monocultures). The strength of coexistence mechanisms was assessed based on reciprocal invasion assays and measurements of environmental carrying capacity.

3. Our data confirmed that coexistence could be weakened by either fitness difference or niche overlap among competitors. In allopatrically evolved strain pairs, fitness difference among competitors became larger or unchanged; and the magnitude of niche overlap often showed an increase rather than a decline. Sympatrically evolved competitors showed no consistent changing trend in the strength of the coexistence mechanisms. Overall, sympatrically evolved competitor pairs did not differ from allopatrically evolved ones in the magnitude of fitness difference, but had lower levels of niche overlap.

4. The results are consistent with the ‘character displacement’ view that allopatric populations of competing species occupy more similar niche space compared with sympatric populations. However, the pattern observed here resulted from allopatrically evolved competitors showing niche convergence, but not sympatrically evolved competitors consistently showing further niche divergence. The results also suggest that convergent evolution in allopatry followed by secondary contacts is an unlikely mechanism for the origin of ‘neutral communities’.

Key-words: allopatry, competition, experimental evolution, fitness difference, niche overlap, reciprocal invasion, species coexistence, sympatry

Introduction

A major advance in ecology in the past several decades is the recognition that two categories of processes may jointly operate to promote species coexistence, stabilizing mechanisms that increase negative intraspecific interactions relative to interspecific interactions and equalizing mechanisms that minimize inherent differences in species growth performance (Chesson 2000; Adler, HilleRisLambers &

Levine 2007). The outcome of competition (coexistence versus exclusion) is determined by the balance of the two types of forces. When competitors have similar growth performance (or say ‘ecological fitness’, reflecting the degree of adaptation to the environment, independent of other species), coexistence is more likely as competitive exclusion between such species can be very slow. However, even perfectly equivalent species cannot coexist stably in the long run. Stabilizing forces arising from niche differentiation can lead to stable coexistence, but only when fitness difference among species is not very large (Chesson 2000;

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Bell 2001; Hubbell 2001; Adler, HilleRisLambers & Levine 2007; Vellend 2010; Lankau 2011). Much attention has been paid to assessing the importance of the two categories of coexistence forces in recent years (Fukami *et al.* 2007; Levine & HilleRisLambers 2009; Zhang, Buckling & Godfray 2009; Siepielski *et al.* 2010; Carroll, Cardinale & Nisbet 2011; Narwani *et al.* 2013).

Now there is a resurgent interest in the effect of evolution on the maintenance of biodiversity and biodiversity–ecosystem functioning relationships, as the prevalence and ecological importance of rapid evolution have been increasingly appreciated and the distinction between ecological and evolutionary time-scales becomes blurred (Hairston *et al.* 2005; Fox & Vasseur 2008; Shores, Hegreness & Kishony 2008; Gravel *et al.* 2011; Lankau 2011; Lawrence *et al.* 2012; Kremer & Klausmeier 2013; Genung, Schweitzer & Bailey 2014; Zuppingier-Dingley *et al.* 2014; Fiegna *et al.* 2015). Both the stabilizing and equalizing mechanisms of species coexistence may change along with evolutionary dynamics. Studies of character displacement suggest that competition-mediated evolutionary change may lead to reduced niche overlap and thus more stable coexistence among competitors (Brown & Wilson 1956; MacArthur & Levins 1967; Dayan & Simberloff 2005; MacLean, Dickson & Bell 2005; Grant & Grant 2006; Silvertown *et al.* 2006). However, this has often been inferred only by the pattern that sympatric populations of competing species are less similar in traits associated with resource competition than allopatric populations. In many cases, conclusions about the stability of species coexistence have been drawn without rigorous tests, for example by the ‘mutual invasibility’ criterion (Siepielski & McPeck 2010).

Hypotheses have also been proposed for how evolution may affect the equalizing mechanisms of coexistence in sympatric populations of competitors. For instance, fitness difference among species may be exacerbated by evolution when adaptation to the environment in rare species is more limited due to lower supply of genetic variation or lower effectiveness of selection; alternatively, fitness difference can be reduced if high-abundance species need evolve more costly resistance to natural enemies (Lankau 2011). Evolutionary changes in allopatric populations of potential competitors also deserve attentions as these populations may get in contact with each other in future. As a matter of fact, the character displacement hypothesis implies that allopatric populations of potential competitors may show convergence in niche requirements. If their parallel adaptation also lead to convergence in growth performance (approaching the same fitness peak on an adaptive landscape; Fong, Joyce & Palsen 2005; Arendt & Reznick 2008), they may become ecologically equivalent and, on secondary contacts, form ‘neutral communities’.

Here, we report an experiment that addresses how evolution of species in sympatry or allopatry affects both stabilizing and equalizing forces that determine coexistence in

a microbial system. We obtained five pairs of *Escherichia coli* strains that reproduce asexually and thus can be considered as analogous to species. Strains within every pair can stably coexist in batch culture conditions with a chemically defined medium. We then established five groups of evolution lines with those strain pairs. Bacteria were allowed to evolve for ~1100 generations both in monocultures (in allopatry) and two-strain mixtures (in sympatry), with niche overlap and fitness difference between the evolved competitors examined.

Materials and methods

A schematic presentation of the experimental set-up, including strain screening, selection experiment and assessment of coexistence mechanisms, was provided (Appendix S1, Fig. S1, Supporting information). In this study, we used the terms ‘strains’ and ‘species’ interchangeably, considering asexual bacterial strains as analogous to species.

STRAINS AND CULTURE CONDITIONS

Bacterial strains were obtained from a previous experimental evolution study (Ni 2011), in which populations of *Escherichia coli* K-12 GM4792, and its variant K-12 GM4792 Lac⁺, evolved for ~1300 generations in several modified MOPS media (Neidhardt, Bloch & Smith 1974). Those evolution lines were propagated in batch cultures. They were grown at 37 °C in microcosms of 160 µL of nutrient media in 96-well microplates, on an orbital shaker at 330 rpm; and 1% of each culture was inoculated into a fresh microcosm every 24 h (i.e. ~6.64 generations of propagation per transfer).

In this study, we obtained five pairs of strains. Within each of those pairs, the two strains could coexist stably in a particular nutrient medium (Appendix S1, Table S1). The five pairs consisted of a total of nine strains (see details in Appendix S1, Table S2). These coexisting strain pairs were chosen by screening numerous bacterial isolates using reciprocal invasion tests. Briefly, bacterial isolates from several populations of two selection environments (nitrogen- or phosphorus-limiting medium, see notes in Table S1 in Appendix S1) in Ni (2011) were paired. The two isolates within each pair had opposite Lac makers and were thus distinguishable by colony colours when grown on LB agar plates with X-gal (40 µg/mL; Lac[−] colonies being white and Lac⁺ colonies blue). The two isolates were acclimated in monocultures for 48 h, then mixed at two volumetric ratios, 1 : 100 and 100 : 1; about 1.6 µL of each mixture was transferred to 160 µL of fresh medium and grown for 24 h. Initial and final densities of each competitor were estimated by plating diluted cultures on LB agar plates with X-gal. A Malthusian parameter was calculated for each competitor within each mixed community, $m = \ln(N_f/N_0)$, with N_0 and N_f the relevant initial and final densities, respectively (Lenski *et al.* 1991). Relative growth rate of a strain against its competitor was assessed as a selection coefficient: for a pair of competitors i and j , $S_i = m_i - m_j$. Relative growth rate when rare ($S_{\text{rare},i}$) for each competitor was assessed in appropriate mixed communities; for instance, $S_{\text{rare},i}$ is the relative growth rate of strain i in a mixture where strain i was initially rare and strain j was initially abundant (see more details in Appendix S1). $S_{\text{rare},i} > 0$ indicates that strain i can invade when rare. If both strains within a pair had positive S_{rare} values, this pair was considered as a stable-coexistence strain pair. Each reciprocal invasion assay was replicated three times. The five stable-coexistence strain pairs were used to perform the following selection experiment.

SELECTION EXPERIMENT

Bacteria within each stable-coexistence strain pair evolved in sympatry (in mixtures with competitors) or in allopatry (in monocultures) for ~1100 generations (160 transfers), with six replicates for each treatment. Each monoculture was initiated with 1.6 µL of acclimated culture of a single strain, each mixture with equivalent volumes of two competitors (0.8 µL of each). Each inoculum contained about 10^6 bacterial cells. In each microplate, every evolution line was neighboured by sterile wells, to minimize the chance of contamination. For every 50 generations, 100 µL of culture from each evolution line was mixed with 70% glycerol (1 : 1) and stored at -40°C . In the two-strain mixtures, competitors showed fluctuations in relative abundances, but no extinction event was observed (Appendix S1, Fig. S2). At the end of the selection experiment, cultures were plated on LB agar plates with X-gal. Six colonies were randomly chosen from each population in each evolution line (that is, six colonies from each monoculture, and twelve from each mixture). A total of 720 isolated colonies were separately grown in liquid medium and then stored at -40°C .

ASSESSMENT OF COEXISTENCE

Reciprocal invasion assays (see above for the protocol) were conducted to test for the strength of coexistence between competitors (i.e. mutual invasibility). The six colonies isolated from each population were grown in liquid medium and then pooled together with equal volumes; this pooled culture was used to represent an evolved population. For each strain pair, there were 24 evolved populations: 2 strains \times 2 evolution scenarios (sympatry or allopatry) \times 6 replicates. These evolved populations were then paired before invasion tests. Sympatrically evolved populations were grouped according to their original evolution line (that is, competitors from the same evolution line were grouped as one pair). Allopatrically evolved populations were randomly paired. For a pair of competing populations i and j , two values of relative growth rate when rare, $S_{\text{rare-}i}$ and $S_{\text{rare-}j}$, were calculated. The strength of coexistence was measured as the smaller value among the two, $\min(S_{\text{rare-}i}, S_{\text{rare-}j})$, as species coexistence requires every competitor to be capable of invading when rare, and thus, the smaller S_{rare} value is the key to assess whether or not two competitors can stably coexist (Chesson 2000; Siepielski & McPeck 2010). Each reciprocal invasion assay was replicated three times.

ESTIMATES OF FITNESS DIFFERENCE AND NICHE OVERLAP

A species' fitness, in an ecological sense, is a function of its degree of adaptation to the environment, independent of other species (Chesson 2000; Adler, HilleRisLambers & Levine 2007; Lankau 2011). Therefore, a measure of species fitness should be an absolute fitness component (such as environmental carrying capacity) that is relevant to competitive ability of the species of interest (Adler, HilleRisLambers & Levine 2007; Levine & HilleRisLambers 2009). Niche difference among species is a force to increase negative intraspecific interactions relative to interspecific interactions; it should therefore be measured with competition experiments. Several estimates of fitness difference and niche difference have been proposed (Levine & HilleRisLambers 2009; Carroll, Cardinale & Nisbet 2011; Chesson 2013; Chu & Adler 2015). In particular, Chesson (2013) derived analytical estimates for fitness difference and niche difference based on Lotka–Volterra competition model. Fitness difference between species i and j can be expressed as $K_i/K_j = [(\alpha_{ii} \times \alpha_{ij})/(\alpha_{ji} \times \alpha_{jj})]^2$ and niche difference, $\rho = [(\alpha_{ij} \times \alpha_{ji})/(\alpha_{ii} \times \alpha_{jj})]^2$, where K is the environmental carrying capacity, α_{ii} and α_{jj} are the intraspecific competition coefficients, and α_{ij} and α_{ji} are interspecific competition coefficients. In certain

experimental systems (e.g. grass species), the competition coefficients can be measured by replacement series competition experiments (Chu & Adler 2015).

In our microbial experimental system, organisms grow very rapidly to approach equilibrium densities; population-level, but not individual-level, growth performance is easily measurable. Therefore, we may assess the environmental carrying capacity for species (strains), but cannot directly measure the intraspecific and interspecific competition coefficients. In this study, fitness difference, between competitors i and j , was measured as the difference in the equilibrium population densities when grown in monocultures, $\log_{10}(K_i/K_j)$. A value of zero indicates equal fitness, while larger values suggest greater fitness differences. Niche overlap was assessed based on the reciprocal invasion tests. The slope in the relationship between a species' relative growth rate and its initial relative frequency in a community indicates how strongly a species' growth is affected by interspecific versus intraspecific competition. A more negative slope suggests a stronger effect of intraspecific competition relative to interspecific competition, and thus larger magnitude of niche differentiation (MacLean, Dickson & Bell 2005; Adler, HilleRisLambers & Levine 2007; Levine & HilleRisLambers 2009). For a pair of species i and j , the two competitors yield a same slope value (MacLean, Dickson & Bell 2005). We used the slope values to measure niche overlap, with larger (less negative) values indicative of greater extent of niche overlap (see more details in Appendix S1).

STATISTICAL ANALYSIS

General linear model was used for assessing the effect of fitness difference and niche overlap on the strength of coexistence across all evolved competitor pairs. Differences between sympatrically and allopatrically evolved competitor pairs in fitness difference, niche overlap and the strength of coexistence were analysed using mixed-effects linear models, where strain pair ID was included as a random factor. One-sample t -tests were performed for the difference of the evolved competitor pairs from the ancestral competitor pairs in the measures of fitness difference, niche overlap and strength of coexistence. The analyses were carried out in the R environment (R Core Team 2014).

Results

Across all the evolved competitor pairs, the strength of coexistence decreased with increasing values of fitness difference, or increasing values of niche overlap ($R^2 = 0.598$; fitness difference, $F_{1,56} = 9.512$, $P = 0.003$; niche overlap, $F_{1,56} = 71.11$, $P < 0.001$; interaction, $F_{1,56} = 2.61$, $P = 0.112$; Fig. 1).

Overall, sympatrically evolved competitor pairs did not differ in fitness difference from allopatrically evolved competitor pairs ($F_{1,54} = 1.152$, $P = 0.288$; Fig. 2a), but showed lower levels of niche overlap ($F_{1,54} = 30.90$, $P < 0.001$; Fig. 2b), and higher coexistence strength ($F_{1,54} = 9.84$, $P = 0.003$; Fig. 2c). Compared with the ancestral competitor pairs, allopatrically evolved competitor pairs showed unchanged (pair B, C, and D) or increased (pair A and E) fitness difference; and the magnitude of niche overlap more likely increased rather than decreased (increased in pair A, C and D, unchanged in pair B, and slightly decreased in pair E; Fig. 2). The sympatrically evolved competitor pairs showed no consistent trends (fitness difference increased in pair B and C, and

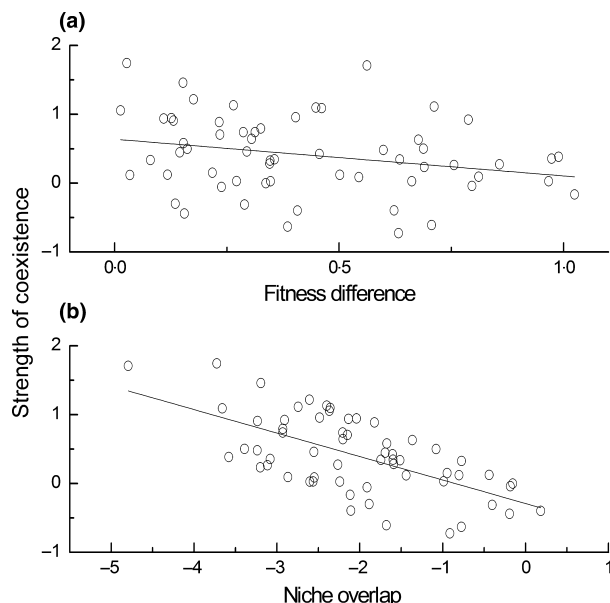


Fig. 1. The relationship between the strength of coexistence and the measure of fitness difference (a) or the measure of niche overlap (b).

unchanged in the remaining ones; niche overlap increased in pair A and C, decreased in pair B and unchanged in pair D and E; Fig. 2).

Discussion

In our study, allopatrically evolved competitor pairs showed higher levels of niche overlap compared with the sympatrically evolved ones. Together with earlier work (Zupping-Dingley *et al.* 2014; Fiegna *et al.* 2015), our finding provides unambiguous experimental evolution evidence for the conventional ‘character displacement’ view that allopatric populations of competing species may occupy more similar niche space compared with sympatric populations (Brown & Wilson 1956; Dayan & Simberloff 2005; Grant & Grant 2006; Silvertown *et al.* 2006).

The character displacement pattern observed in our study, however, resulted from allopatrically evolved competitors showing niche convergence, but not sympatrically evolved competitors consistently showing further niche divergence. As a matter of fact, there was no consistent evolutionary trend in the extent of niche difference among the five strain pairs; certain strain pairs that evolved in sympatry even showed higher levels of niche overlap relative to their ancestors (pair A and C, Fig. 2b), although coexistence was maintained in all our sympatric evolution lines throughout the selection experiment (Appendix S1, Fig. S2). Thus, the direction of niche evolution (divergence versus convergence) in sympatric populations of competing species might be historically contingent, and evolution of coexisting species under competition may not universally lead to further niche differentiation. Note that the results should not be over interpreted to question the generality of competi-

tion-mediated niche evolution (Zupping-Dingley *et al.* 2014; Fiegna *et al.* 2015), as our experiment was performed using strain pairs that had showed strong coexistence in the beginning. We intentionally chose stable-coexistence strain pairs to perform our experiment for the potential to study both niche divergence and convergence.

Batch cultures of bacteria in a chemically defined nutrient media are characterized by periodic changes in resource availability. Maintenance of genetic diversity has often been documented in such seasonally fluctuating environments (Turner, Souza & Lenski 1996; Rainey *et al.* 2000; Barrett, MacLean & Bell 2005; Maharjan *et al.* 2006; Tan, Kelly & Jiang 2013); meanwhile, theoretical considerations suggested that selection in temporally heterogeneous environments may favour generalists of highest geometric mean fitness, with evolution of niche convergence and low levels of diversity expected (Reboud & Bell 1997; Kassen & Bell 1998; Kassen 2002). In the present study, coexistence in all sympatric evolution line was maintained throughout the experiment, further suggesting ample opportunities for niche differentiation in temporally heterogeneous environments (Le Gac *et al.* 2012; Abrams, Tucker & Gilbert 2013). A variety of mechanisms were proposed to explain the maintenance of diversity in such seemingly simple environments, including differences in resource use (Tilman 1982), growth rate-yield trade-offs (Levin 1972; Novak *et al.* 2006; Gudelj *et al.* 2007), or niche construction resulting from the activities of the bacteria themselves such as cross-feeding where secretion of metabolic by-products by one strain may promote the persistence of its competitors (Turner, Souza & Lenski 1996; Rozen & Lenski 2000; Le Gac *et al.* 2012).

Unfortunately, we were not able to identify the specific mechanisms for niche partitioning among our bacterial strains. Intriguingly, our ancestral strains, which had previously evolved in either nitrogen- or phosphorus-limiting medium, showed no predictable patterns in nitrogen or phosphorus requirement (Ni 2011), ruling out difference in nitrogen/phosphorus resource use ratio as a probable explanation for coexistence. We also estimated exponential growth rate and growth yield of the evolved populations in monocultures; there was not a relationship between the estimated magnitude of niche difference and the strength of the growth rate-yield trade-off (Appendix S1). One possible reason for the coexistence of our bacterial strains is trade-offs between uptake of the major carbon source (glucose) and use of metabolic by-products. We did not do rigorous tests for this cross-feeding mechanism in the present study as we observed only small differences among our strains in growth profiles such as colony size or population density during stationary phase and we imagine that the cross-feeding mechanism is not easy to verify or falsify unless the coexisting competitors show obvious differences in growth profiles (Turner, Souza & Lenski 1996; Rozen & Lenski 2000).

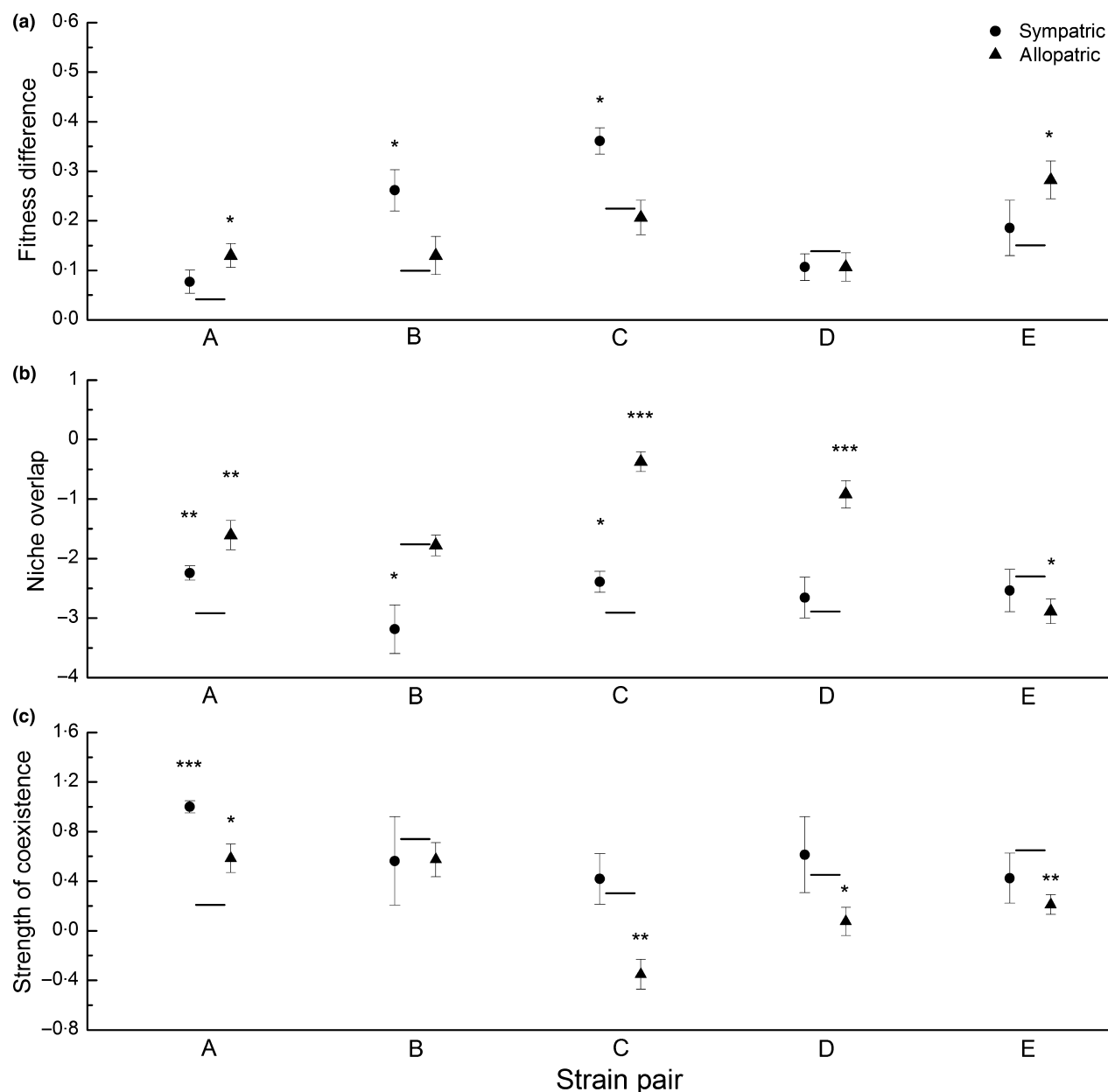


Fig. 2. Fitness difference (a), niche overlap (b) and strength of coexistence (c) measured for sympatrically and allopatrically evolved competitor pairs. Reference lines indicate the ancestral strain pairs. Asterisks indicate significant difference from ancestral values (one-sample *t*-test, *: $0.01 < P \leq 0.05$, **: $0.001 < P \leq 0.01$, *** $P < 0.001$). Data show mean \pm SE ($n = 6$).

Fluctuations in the relative abundances of competitors were observed in some, although not all, of the sympatric evolution lines (Appendix S1, Fig. S2). This implies a possibility of arms race-like co-evolution between competitors, where advantages obtained by one strain through adaptive evolution may be overcome by its competitor later with corresponding adaption (MacLean, Dickson & Bell 2005). A recent study reported asymmetrical co-evolution between two *E. coli* strains that coexisted through a cross-feeding mechanism, where one strain encroached the other's niche over time (Le Gac *et al.* 2012). Such asymmetrical co-evolution may also have occurred in certain evolution lines in our study, particularly in microcosms of

strain pair E where one strain maintained its dominance over time in most of the replicate populations (Appendix S1, Fig. S2). It is noteworthy that divergence was also observed among replicate microcosms of certain strain pairs in terms of the identity of the strain that achieved dominance (Appendix S1, Fig. S2), suggesting the existence of chance effect in niche evolution.

Our allopatrically evolved competitors did not show a decline in fitness difference (Fig. 2a), although they often showed niche convergence. Thus, convergent evolution in niche occupation was not coincided with a convergence in ecological fitness. For a particular strain pair (pair C), the allopatrically evolved competitors could not coexist any

more when getting secondary contacts (Fig. 2c). This finding suggests that convergent evolution in allopatry followed by secondary contacts is unlikely to be a major mechanism for the origin of 'neutral communities'.

Acknowledgements

We thank Chuan Ni for technical assistance. This study was funded by the National Natural Science Foundation of China (31222010, 31421063, and 31030014), and the 111 project (B13008). The authors declare no conflict of interest.

Data accessibility

All data used in this manuscript are present in the manuscript and its supporting information.

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Received 26 May 2015; accepted 5 November 2015
Handling Editor: Scott Carroll

Supporting Information

Additional Supporting information may be found in the online version of this article:

Appendix S1. Supplementary methods and results.

Appendix S2. Data used in this study.

Data S1. Methods.

Figure S1. Schematic presentation of the experimental setup.

Figure S2. Community composition of the sympatric evolution lines over time, shown as the proportional abundance of one particular strain within each evolution line.

Table S1. The formula of the modified MOPS medium used in the present study.

Table S2. Information about strain pairs used for the experimental evolution study.