Appendix S1 Extended Methods

Experimental regime

The Non-Mixed Ecology treatment has been previously published in a study that compared its effect relative to a life cycle without reproductive specialisation (Hammerschmidt *et al*. 2014). Here the effect of meta-population structure on the evolutionary transition to multicellularity is addressed. Groups of cells ('microcosms') in both the Non-Mixed and Mixed Propagule treatments of the present study experience identical two-phase life cycles driven by frequency-dependent selection. More specifically, each of the Non-Mixed and Mixed Propagule meta-population ecologies comprised 15 replicates of eight competing groups that were founded with *P. fluorescens* strain SBW25 (Silby *et al.* 2009), and propagated through ten generations of evolution (one generation equated to one WS-SM-WS life cycle (Hammerschmidt *et al*. 2014).

Maturation Phase (Fig. 1): Each group was founded by a single WS colony. Microcosms were incubated under static conditions for six days, after which they were checked for the presence of an intact mat at the air-liquid interface. If the mat was not intact, that line was deemed extinct.

Dispersal Phase (Fig. 1), Non-Mixed Ecology: All microcosms with viable mats were homogenised by vortexing and then individually diluted and plated on solid media. Agar plates were subsequently screened for SM colonies. Lines without SM colonies were deemed extinct. To ensure that only SM cells, and no WS cells, were transferred to the Dispersal Phase, all SM colonies were individually transferred to 200 µl liquid medium and incubated for 24 h under static conditions. Thereafter they were pooled and used to inoculate Dispersal Phase microcosms. Each mixture of SM cells arising from within each individually plated microcosm was used to inoculate one or more daughter microcosms in the Dispersal Phase. When a microcosm was deemed extinct at the end of the Maturation Phase, it was replaced by a pool of SM (dispersing) cells from another microcosm randomly chosen from the same population of eight (Fig. 1b).

Dispersal Phase (Fig. 1), Mixed Propagule Ecology: All microcosms with viable mats were homogenised by vortexing and then pooled prior to diluting and plating on solid media. Agar plates were subsequently screened for SM colonies. To ensure that only SM cells, and no WS cells, were transferred to the Dispersal Phase, all SM colonies were individually transferred to 200 µl liquid medium and incubated for 24 h under static conditions. Thereafter they were pooled and used to inoculate Dispersal Phase microcosms. Because all microcosms with viable mats were pooled prior to plating, only one SM mixture was generated, and this mixture was used to inoculate all eight microcosms entering the Dispersal Phase (Fig. 1c).

After three days of incubation under static conditions (during which new WS mats emerged), all microcosms in both treatments were individually plated on solid agar. The most dominant WS morphotype on each agar plate was selected to inoculate the next generation of the life cycle. If there were no WS colonies on the plate, the microcosm was deemed extinct. Figs 1b and 1c contrast the death-birth process of group competition in the Non-Mixed Ecology, with the physical mixing mode of competition in the Mixed Propagule Ecology.

Fitness assay

Cell-level and group-level fitness were assayed after ten life cycle generations: 15 representative clones (one per replicate population) were generated from each of the evolved treatments, in addition to 15 ancestral WS lines (each independently isolated from the earliest mats to emerge from the ancestral SM strain SBW25) (described in detail in Hammerschmidt *et al.* (2014). For each genotype, three replicate competition assays were performed in populations of eight microcosms over the timescale of one full life cycle (Fig. 1a) against a neutrally marked ancestral competitor (Zhang & Rainey 2007). In order to include the effects of both cell fitness and group fitness on the outcome of competition, all fitness assays were performed in the Mixed Propagule Ecology (Fig. 1c). To simulate a meta-population structure with eight competing groups,

four started with the marked reference strain and four started with the focal clone, the "SM mixture" used to inoculate the Dispersal Phase contained an equal volume of the marked reference strain for all focal strains. This ensured that the reference strain performed equally for each competition during the Maturation Phase. The single-celled bottleneck ensured that non-chimeric mat offspring could be counted at the end of the life cycle. Our proxy for group-level fitness is the proportion of 'offspring' mats produced at the end of one life cycle by the focal genotype relative to the marked reference strain, and cell-level fitness the total number of cells in the mat at the end of the Maturation Phase.

Model of selection regimes

The model simulates the evolutionary dynamics of metapopulations composed of $M = 8$ groups. Each group contained one or more lineages, which are the primary agents of selection. Each lineage in the metapopulation is characterized by three parameters: cell growth rate ω, transition probability p, and the number of cells in lineage $n(t)$. At the beginning of each simulation, each group in a metapopulation was seeded with a unique lineage. The growth rate and transition probability of each lineage were sampled from a bivariate normal distribution with means $\langle\omega\rangle = 1$ and $\langle p\rangle = 10^{-6}$, variances $\sigma_\omega = 0.5$ and $\sigma_p = 5 \cdot 10^{-7}$, and correlation coefficient (between growth rate and transition probability) $\rho = -0.5$. The initial population of each lineage was set to a single cell. The dynamics of growth during Maturation and Dispersal Phases were simulated identically. Lineages grew exponentially according to their growth rates ω_i until their combined size $\sum_i n_i$ reached the carrying capacity of the group $N = 10^6$ cells. Since each cell division in lineage i can result in a switch between phenotypes with probability p_i, the number of phenotype switches during growth was sampled from Poisson distribution with rate parameter $p_i n_i$. The size of a lineage at which a phenotype transition event occurred n_i^* was sampled from a uniform distribution between one and n_i. The moment

at which this event occurred was calculated as $t_i^* = log(n_i^*/n_i(0))/\omega_i$. Each phenotype switch event resulted in the emergence of a new lineage of another phenotype, with growth rate and transition probabilities equal to those in the maternal lineage. The newly emerged lineages also grew exponentially and were sampled only at the end of the growth phase. At the end of each Dispersal Phase of the life cycle, a single novel lineage phenotype was sampled with probability proportional to its representation within its group. At the end of each Maturation Phase, all novel phenotype lineages were sampled in numbers proportional to their sizes. Each sample seeded one group at the beginning of the next growth phase. However, groups in which no phenotype switch events occurred did not contribute any samples at the end of the growth phase. These groups were deemed extinct and were reseeded by another random sample from the metapopulation. Seeding after the Maturation Phase differed in the Mixed Propagule Ecology: all samples were pooled together and the resulting mixture of lineages seeded all groups for the next Dispersal Phase. For both ecologies, simulations lasted for 20 full cycles and 600 independent realizations were performed. The average growth rate and transition probabilities across all groups were recorded for each simulation run.

References

Hammerschmidt K, Rose CJ, Kerr B, Rainey PB. (2014). Life cycles, fitness decoupling and the evolution of multicellularity. *Nature* 515, 75–79.

Silby MW, Cerdeño-Tárraga AM, Vernikos GS, Giddens SR, Jackson RW, Preston GM. et al. (2009). Genomic and genetic analyses of diversity and plant interactions of *Pseudomonas fluorescens*. *Genome Biol.* 10, R51.

Zhang X-X, Rainey PB. (2007). Construction and validation of a neutrally-marked strain of *Pseudomonas fluorescens* SBW25. *J Microbiol Meth.* 71, 78–81.

Figure S1

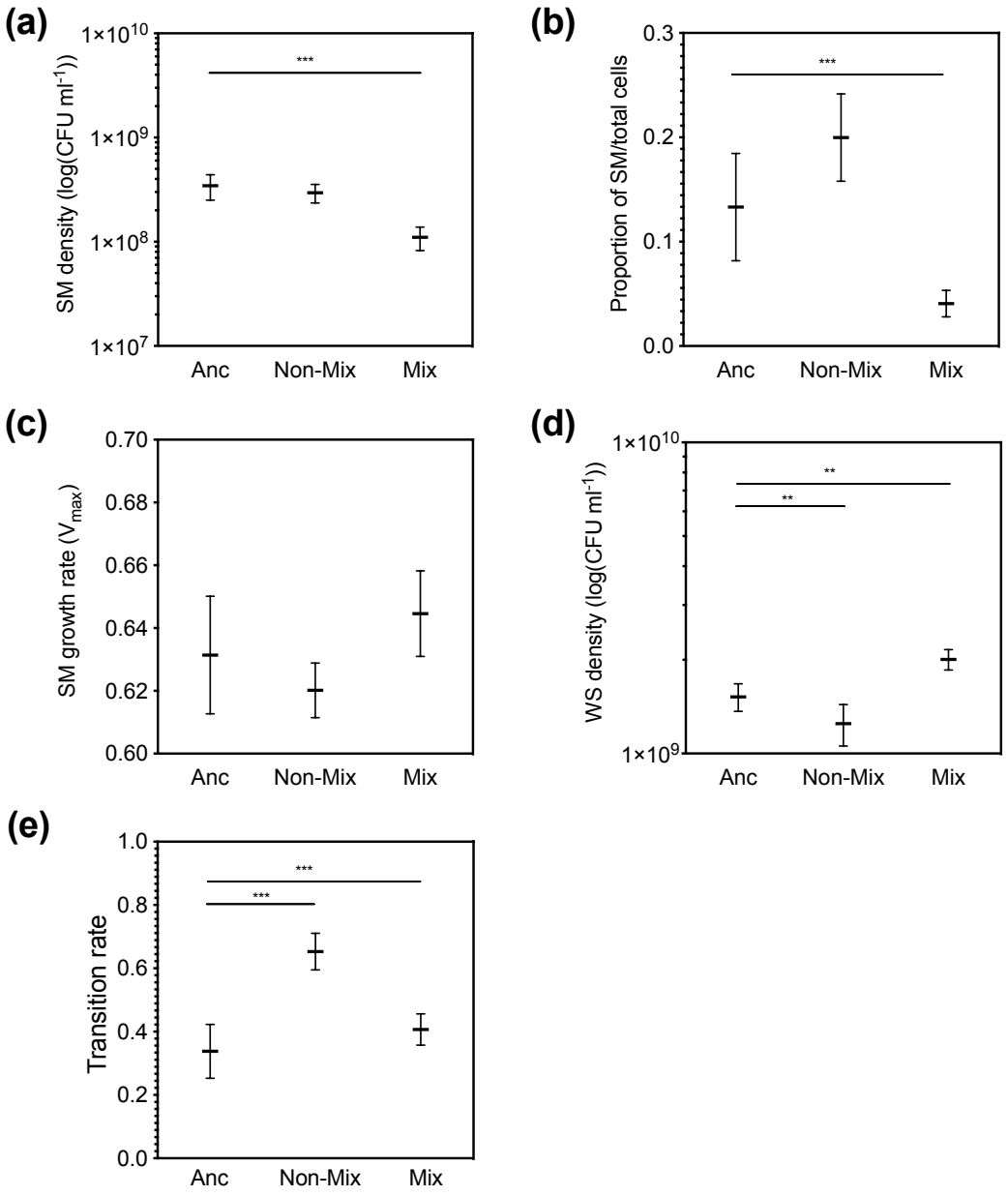
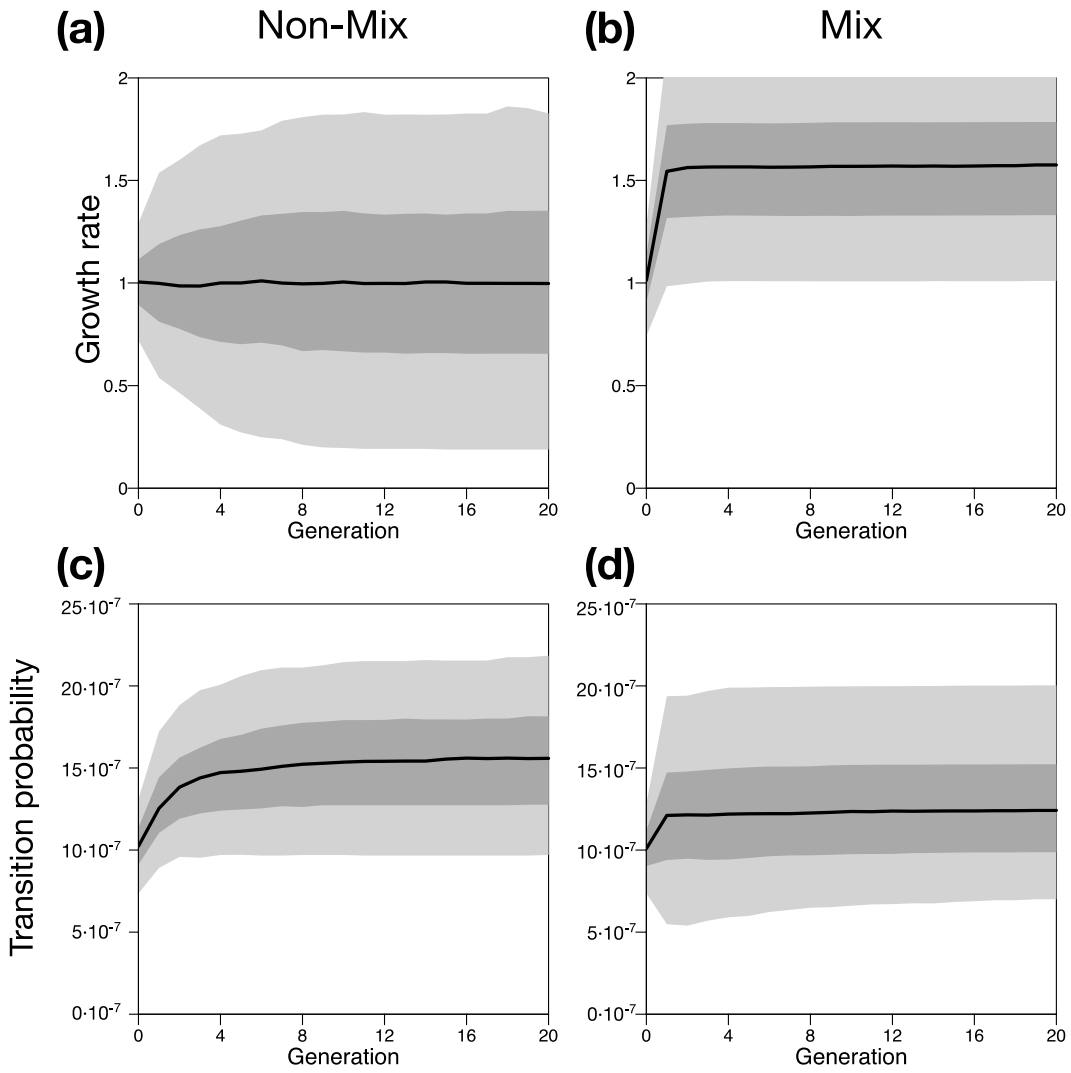


Figure S1

Changes in life cycle traits in the Non-Mixed (Non-Mix) and Mixed Propagule (Mix) Ecologies compared to the ancestral populations (Anc): (a) SM density, (b) Proportion of SM, (c) SM growth rate, (d) WS density, (e) Transition rate. Error bars are s.e.m., based on n = 14 (Non-Mix) and n = 15 (Anc, Mix). ** denotes significance at the level of P = 0.001 - 0.01, and *** at the level of P <0.001.



Supplementary Figure 2. Model dynamics with a random distribution of initial parameters (no trade-off) (a,b) average cell growth rate, and (c,d) average transition probability in the Non-Mixed Ecology (a,c) and the Mixed Propagule Ecology (b,d). Black lines represent median values across 600 independent realizations of the respective selection regime. Dark grey areas indicate a 50% confidence interval, while light grey areas indicate a 95% confidence interval.

Table S1

Effects of the meta-population structure on the level of selection. The red box highlights selection during different phases of the Mixed Propagule Ecology for two incompatible traits (parameters that are negatively correlated), leading to a conflict between levels of selection.

Ecology	Life cycle phase	Distribution of Variation	Level of Selection	Life-history requirement(s)	Trait selected
Non-Mixed	**MATURATION PHASE**	Between groups	Between groups	Produce SM cells	WS-SM transition rate
	DISPERSAL PHASE	Between groups	Between groups	Produce WS cells	SM-WS transition rate
Mixed Propagule	**MATURATION PHASE**	Between groups (low)	Between groups (weak)	Produce SM cells	WS-SM transition rate
	DISPERSAL PHASE	Within groups	Between cells	Produce WS cells AND Outcompete WS produced by other groups	WS density