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Supporting Information for

Manipulation of natural transformation by AbaR-type islands promotes fixation of antibiotic resistance in *Acinetobacter baumannii* populations

Rémi Tuffet[a,b,‡], Gabriel Carvalho[b‡], Anne-Sophie Godeux[a,c,‡], Fanny Mazzamurro[d,e], Eduardo P. C. Rocha[d], Maria-Halima Laaberki[a,c,#@], Samuel Venner[b,#@], Xavier Charpentier[a,#,@]

[a] CIRI, Centre International de Recherche en Infectiologie, Inserm, U1111, Université Claude Bernard Lyon 1, CNRS, UMR5308, École Normale Supérieure de Lyon, Univ Lyon, 69100, Villeurbanne, France
[b] UMR CNRS 5558 – LBBE "Laboratoire de Biométrie et Biologie Évolutive", Université Claude Bernard Lyon 1, Villeurbanne, France.
[c] Université de Lyon, VetAgro Sup, 69280 Marcy l'Etoile, France.
[d] Institut Pasteur, Université Paris Cité, CNRS UMR3525, Microbial Evolutionary Genomics, Paris, 75015, France.
[d] Collège Doctoral – Sorbonne Université, F-75005 Paris, France – France
‡, contributed equally. #, co-senior authors.
@, Correspondence to:
Maria-Halima Laaberki, maria-halima.laaberki@vetagro-sup.fr (experimental work)
Samuel Venner, samuel.venner@univ-lyon1.fr (computational work)
Xavier Charpentier, xavier.charpentier@univ-lyon1.fr

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Supporting text

Computational model

We have developed a stochastic computational model, inspired by an earlier model (1). The model is composed of two compartments: bacterial cells (intracellular compartment) and extracellular DNA (eDNA within the extracellular compartment). Bacteria possess several insertion sites on their chromosome which can be occupied by two types of DNA each. Bacteria can uptake eDNA from the extracellular compartment and replace their own using transformation. Bacteria release DNA into the extracellular compartment upon cell lysis which can be enhanced by a stochastic bactericidal stress.

In the first stage, the environmental fluctuations only concern exposure to stress 1 (which occurs randomly, see Fluctuating stress below), for which resistance is conferred by AbaR (Figure 3 and S3). Insertion sites of AbaRs include three genes, *comM*, *pilT* and *pho.* These sites can be occupied by their respective wild-type allele (*comM*, *pilT* or *pho*) or by their respective AbaR genomic island (*comM::*AbaR, *pilT*::AbaR or *pho*::AbaR). In accordance with experimental results, the frequency of natural transformation can be affected by the insertion of an AbaR that inactivates the gene (see Figure 2 and SI appendix Table S2) This frequency is unchanged when the *pho* gene is inactivated, is reduced when the *comM* gene is inactivated and is dropped to 0 when the *pilT* gene is inactivated. The $n=2^3=8$ different bacterial genotypes, along with their resistance phenotypes, natural transformation phenotypes and replication costs are listed in Table S3.

In the second stage, we model more complex environmental fluctuations. In addition to stress 1, we have introduced either a new stress that reduces the survival of non-resistant bacteria (see Flucutating stress below), or a change in resource for bacterial growth (bacteria that do not have the allele adapted to this new resource have a lower growth capacity, see Fluctuating resource below). In the latter two cases, adaptation to the new environment is conferred by a new single nucleotide polymorphism (SNP) that can be acquired by transformation and that integrates into a new site. This additional site can be occupied by a wild type allele or by the SNP. The inactivation of the *comM* gene, by insertion of an AbaR, only slightly inhibits the acquisition of SNPs by transformation, whereas insertion of an AbaR in the *pilT* gene totally inhibits it (see figure 2C). The $n=2^4=16$ different bacterial genotypes, along with their resistance phenotypes, natural transformation phenotypes and replication costs are listed in Table S4.

Model processes

Bacterial population growth follows a logistic model and the number of replicating cells per genotype i and per time step dt, $G_{i,t+dt}$ is determined using a binomial distribution:

$$G_{i,t+dt} \sim Bin\left(\mu_{i,t}.\,dt, N_{i,t}\right) \quad (1)$$

where $$\mu_{i,t} = \left(\mu_{max} - \frac{\mu_{max} - k_b}{K} N_{tot,t}\right) * \left(1 - c_i\right). \quad (2)$$

$N_{i,t}$ is the number of cells with the genotype i at time t. $\mu_{i,t}$ is the replication rate of genotype i at time t. μ_{max} is the maximal growth rate per capita. k_b is the constant basal lysis rate per capita, which allows the extracellular compartment to be supplied, independently of the presence of stress. $N_{tot,t}$ is the total number of cells in the population (considering all genotypes) at time t. c_i is the cost, in terms of cell replication, induced by all DNA types possessed by the genotype i. K is the carrying capacity. The number of lysed cells per time step $L_{i,t+dt}$ is calculated using a binomial distribution:

$$L_{i,t+dt} \sim Bin\left(k_{i,t}.\,dt, N_{i,t}\right) \quad (3)$$

where $k_{i,t}$ is the lysis rate of genotype i at time t which depends on both the basal lysis rate and exposure to environmental stress (see Modeling environmental fluctuations). To optimize calculation time, the natural transformation mechanism is modeled as two successive processes. First the number of competent cells engaging in a transformation event during a time step $C_{i,t+dt}$ is determined using the following binomial distribution:

$$C_{i,t+dt} \sim Bin\left(T_{i,t}.\,dt, N_{i,t}\right) \quad (4)$$

$$T_{i,t} = T_{max,i} * \left(\frac{A_{tot,t}}{1 + A_{tot,t}}\right) \quad (5)$$

where $T_{i,t}$ is the transformation rate at time t, $T_{max,i}$ is the maximal transformation rate per capita (i.e. the expected rate of transformation in the absence of any limiting constraint: (i) when the eDNA is not limiting, (ii) transformation is not inhibited and (iii) the cell acquires a short sequence - either a WT allele or a SNP - of the genotype i), $A_{tot,t}$ is the total number of eDNA at time t .

Second, the number of competent cells undergoing a transformation event $C_{i,t+dt}$ is distributed between the different bacterial genotypes using a multinomial distribution as follows:

$$\left(C_{t+dt}^{i \to j}\right)_{j=0,\ldots,n-1} = Multinomial\left(C_{i,t+dt}, \gamma_{i,.}\right)$$

where $\gamma_{i,.}$ is a probability vector that takes into account the relative frequency of the different eDNA molecules, corrected by their acquisition success rate (see Table S5). This success rate depends on both the bacterial genotype (transformation inhibition or not) and the molecule to be integrated (AbaR on the one hand, or SNP and WT on the other) (see Table S6).

Cells undergoing a transformation event change their genotype according to the DNA type integrated (see Table S6). The overall variation of a genotype i during a time step is summarized by:

$$N_{i,t+dt} = N_{i,t} + G_{i,t+dt} - L_{i,t+dt} - C_{t+dt}^{i \to !i} + C_{t+dt}^{!i \to i} \qquad (6)$$

where $C_{t+dt}^{i \to !i}$ is the number of competent cells going from the genotype i to a different genotype and $C_{t+dt}^{!i \to i}$ is the number of cells becoming of genotype i from a different genotype.

In the extracellular compartment, eDNA is degraded at a constant rate per capita Dg_j. The number of degraded eDNA molecules j per time step, $D_{j,t+dt}$ is determined using a binomial distribution

$$D_{j,t+dt} \sim Bin\left(Dg_j.dt, A_{j,t}\right) \qquad (7)$$

where $A_{j,t}$ corresponds to the number of eDNA of type j. The extracellular compartment is supplied by eDNA from lysed cells, each lysed cell releasing DNA molecules corresponding to their DNA composition. In addition, eDNA is added at a marginal rate simulating residual arrival from neighboring populations (open system), which prevents the total loss of the various eDNAs in the system. The number of eDNA molecules j added per time step M_j is defined by:

$$M_j = M_{input,j} * dt \qquad (8)$$

where $M_{input,j}$ is the number of molecules of eDNA of type j,is residual and set to be orders of magnitude lower than the DNA released by cell lysis. The overall variation of eDNA of type j in the extracellular compartment during a time step is determined as follows by:

$$A_{j,t+dt} = A_{j,t} - D_{j,t+dt} + M_j + \sum_{i=1}^{n} \left[L^j_{i,t+dt} - C^j_{i,t+dt} \right] \quad (9)$$

where $L^j_{i,t+dt}$ is the number of cells of genotype i (containing DNA of type j) which are lysed and $C^j_{i,t+dt}$ is the number of DNA molecules of type j which are acquired by competent cells.

Modeling environmental fluctuations

Fluctuating stress

The bacterial population is exposed either to a single stress (stress 1, Figure 3, Figure 4D) or to two stresses (stress 1 and 2, figure 4A, 4B and 4C). In all these simulations AbaRs confer resistance to stress 1 while the SNP confer resistance to stress 2 (Figure 4, rows 1-3) .

When there is only stress 1 (Figure 3 and 4D), the lysis rate for genotype i at time t is therefore calculated as follows:

$$k_{i,t} = k_b + I_{1,t} * \left(1 - r_{1,i}\right) \quad (10)$$

where $I_{1,t}$ is the intensity of stress 1 at time t, $r_{1,i}$ is the stress resistance provided by the genotype i.

Environmental fluctuations are modeled by a stochastic stress whose duration, frequency and intensity are variable. Stress 1 is modeled as a sequence of stress-free periods (whose durations are drawn from $\exp\left(f_1\right)$) and stressful periods (whose durations are drawn from $\exp\left(\frac{1}{d_1}\right)$) and stress intensities are drawn from $Unif\left(0,0.75\right)$. We set the maximum stress intensity at 0.75 to avoid population extinctions.

When there are 2 stresses (AbaR and SNP confer antibiotic resistance, see Figure 4A, 4B, 4C), the lysis rate for genotype i at time t is calculated as follows:

$$k_{i,t} = k_b + I_{1,t} * \left(1 - r_{1,i}\right) + I_{2,t} * \left(1 - r_{2,i}\right) \quad (11)$$

$I_{1,t}, I_{2,t}$ are the intensities of stresses 1 and 2 at time t (the term $I_{1,t} * \left(1 - r_{1,i}\right) + I_{2,t} * \left(1 - r_{2,i}\right)$ is truncated to 1 if necessary), $r_{1,i}, r_{2,i}$ are the stress resistances of the genotype i. Stress 1 increases the lysis rate of cells not carrying AbaRs whereas the lysis rate of cells carrying any AbaR remains at the basal rate k_b. The lysis rate of cells carrying the SNP remains at the basal rate in presence of stress 2 and the lysis rate of

cells carrying both AbaR and SNP always remains at the basal rate. Stress 2 is modelled as stress 1 for its duration, frequency and intensity (see above).

Fluctuating resource

In the last situation modelled, the SNP confers a selective advantage when the bacterial population has access to a new resource over a given period (Figure 4D). Under this scenario, we consider two SNP: SNP_0 and SNP_1. SNP_0 is advantageous in environment 0 (it does not incur replication costs) but does not allow optimal exploitation of the resource in environment 1 (increases the replication cost) and inversely for SNP_1. Unlike the situation with exposure to stress 2, here, the SNPs only influence the replication parameter c_i (see equation (2)). The environmental switches are modeled as a change from environment 0 to 1, or from environment 1 to 0.

Statistical analysis and figures. All the statistical analyses and figures were produced on R4.3.1 (additional packages: ggplot2 (2), forcats (3), ggpubr (4), scales (5)).

Table S1: Gene content and acquisition time of the abaR disrupting *comM* in strains with abaR-disrupted *comM*

gembase: genome identifier; Nb.genes: number of genes in abaR; tniC: present if yes otherwise absent; tniA: present if yes otherwise absent; orf4: present if yes otherwise absent; CSL: presence of the left-end conserved sequence if yes otherwise absent, CSR: presence of the right-end conserved sequence if yes otherwise absent, ST : sequence type; Acquisition.node: node at which all genes for the abaR were gained, MGE/Defense system: mobile genetic elements and defense systems detected in the region of interruption, AMR: antimicrobial resistance gene detected in the region of interruption

gembase	Nb genes	tniC	tniA	orf4	CSL	CSR	ST	Acquisition.node	MGE/Defense system	AMR
ACBA.0922.00001	20	yes	yes	yes	yes	yes	1	Node105	ISL3	
ACBA.0922.00002	11	no	yes	yes	no	yes	437	Node95	IS701	
ACBA.0922.00005	16	yes	yes	yes	yes	yes	2	Node417	IS91	tetracycline, aminoglycoside, streptomycin
ACBA.0922.00013	8	no	no	yes	no	yes	2	Node417		
ACBA.0922.00017	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00023	16	yes	yes	yes	yes	yes	2	Node417	IS91	tetracycline, aminoglycoside, streptomycin
ACBA.0922.00025	23	yes	yes	yes	yes	yes	1	Node105		
ACBA.0922.00034	20	yes	yes	yes	yes	yes	1	Node105		
ACBA.0922.00051	11	yes	yes	yes	yes	yes	39	ACBA.0922.00051		
ACBA.0922.00063	9	no	no	yes	no	yes	437	Node95		

										sulfonamide, aminoglycoside, streptomycin, gentamicin, cephalosporin, phenicol, tetracycline,
ACBA.0922.00066	60	yes	yes	yes	yes	yes	1	Node105	ISL3, Integron_complete, IS6, ISL3	chloramphenicol
ACBA.0922.00090	33	yes	yes	yes	yes	yes	367	ACBA.0922.00090	ISL3	sulfonamide
ACBA.0922.00115	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00133	9	no	no	yes	no	yes	494	ACBA.0922.00133		
							122			
ACBA.0922.00185	9	yes	yes	yes	yes	yes	0	ACBA.0922.00185		
ACBA.0922.00200	13	yes	yes	no	yes	no	923	ACBA.0922.00200	IS5	
ACBA.0922.00212	15	yes	yes	yes	yes	yes	32	Node11		
ACBA.0922.00241	5	no	no	yes	no	yes	2	Node417	IS91	aminoglycoside, streptomycin
ACBA.0922.00251	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00263	15	yes	yes	yes	yes	yes	32	Node11		
ACBA.0922.00273	8	yes	yes	yes	yes	yes	2	Node417	IS91, NA	aminoglycoside, streptomycin
ACBA.0922.00289	20	yes	yes	yes	yes	yes	1	Node105	ISL3, ISL3	
ACBA.0922.00290	16	yes	yes	yes	yes	yes	2	Node417	IS91	aminoglycoside, tetracycline, streptomycin
ACBA.0922.00295	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00318	20	yes	yes	yes	yes	yes	1	Node105	ISL3, ISL3	
ACBA.0922.00334	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00336	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00338	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00353	17	no	no	yes	no	yes	1	Node105	Integron_complete	sulfonamide, aminoglycoside, streptomycin, gentamicin
ACBA.0922.00356	16	yes	yes	yes	yes	yes	2	Node417	IS91	tetracycline, aminoglycoside, streptomycin

ACBA.0922.00361	20	yes	yes	yes	yes	yes	20	Node105	ISL3, ISL3	
ACBA.0922.00362	10	yes	yes	yes	yes	yes	318	Node62		
ACBA.0922.00365	10	yes	yes	yes	yes	yes	632	ACBA.0922.00365		
ACBA.0922.00368	16	yes	yes	yes	yes	yes	2	Node417	IS91, IS91	tetracycline, aminoglycoside, streptomycin
ACBA.0922.00372	9	no	no	yes	no	yes	2	Node417	IS91	aminoglycoside, tetracycline, streptomycin
ACBA.0922.00381	20	yes	yes	yes	yes	yes	1	Node105		
ACBA.0922.00386	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00387	5	no	no	yes	no	yes	15	Node62		
ACBA.0922.00388	7	no	no	yes	no	yes	745	Node417	IS91	tetracycline, aminoglycoside, streptomycin
							101			
ACBA.0922.00400	15	yes	yes	no	yes	no	7	ACBA.0922.00400	new, new	
ACBA.0922.00406	10	yes	yes	yes	yes	yes	84	Node62		
ACBA.0922.00410	31	yes	yes	yes	yes	yes	-	ACBA.0922.00410	IS91, IS3	sulfonamide
ACBA.0922.00420	10	yes	yes	yes	yes	yes	103	ACBA.0922.00420		
ACBA.0922.00421	16	yes	yes	yes	yes	yes	1	Node105		
ACBA.0922.00428	16	yes	yes	yes	yes	yes	2	Node417	IS91	tetracycline, aminoglycoside, streptomycin
ACBA.0922.00436	10	yes	yes	yes	yes	yes	318	Node62		
ACBA.0922.00440	18	yes	yes	yes	yes	yes	2	Node417	IS91	tetracycline, aminoglycoside, streptomycin
ACBA.0922.00446	10	yes	yes	yes	yes	yes	15	Node62		
ACBA.0922.00447	10	yes	yes	yes	yes	yes	15	Node62		

ACBA.0922.00450	16	yes	yes	yes	yes	yes	1	Node105	
ACBA.0922.00453	10	yes	yes	yes	yes	yes	15	Node62	
ACBA.0922.00465	10	yes	yes	yes	yes	yes	318	Node62	
ACBA.0922.00467	9	yes	yes	no	yes	no	2	Node417	
ACBA.0922.00470	10	yes	yes	yes	yes	yes	15	Node62	
ACBA.0922.00471	15	yes	yes	yes	yes	yes	-	Node417	aminoglycoside, tetracycline, streptomycin
ACBA.0922.00475	16	yes	yes	yes	yes	yes	1	Node105	
ACBA.0922.00476	33	yes	yes	yes	yes	yes	271	ACBA.0922.00476	sulfonamide
ACBA.0922.00479	16	yes	yes	yes	yes	yes	1	Node105	
ACBA.0922.00480	7	yes	yes	no	yes	no	2	Node417	
ACBA.0922.00482	10	yes	yes	yes	yes	yes	15	Node62	

Table S2: Parameters used in the model.

While the values of time step dt and carrying capacity K are arbitrary, the value of the eDNA input $M_{input,j}$ is set at $1E2$ to add on average one eDNA molecule of each type j at each time step dt. The values of μ_{max} and k_b are chosen so that $\mu_{max} - k_b = \frac{2^{dt}-1}{dt}$ to ensure that one time unit corresponds to one generation of AbaR-free cells and in the absence of density dependence and stress exposure. Finally the rate of eDNA degradation Dg_j is set at 0.16 so that the amount of extracellular eDNA is divided by 1E5 in 24h as documented in (6) (i.e. 72 generations using the approximation that the generation time is 20 minutes).

Parameter	Description	Value(s)	Unit
dt	Time step	0.01	Unitless
μ_{max}	Maximal growth rate	0.795555	GT^{-1}
k_b	Constant basal lysis rate	0.1	GT^{-1}
c	Cost of SNP	0.01 or 0.1	Unitless
K	Carrying capacity	1E7	cells
$T_{max,i}$	Maximal transformation rate of genotype i	1E-3 or 0	GT^{-1}
Dg_j	eDNA degradation rate	$0.16 \forall j$	GT^{-1}
$M_{input,j}$	eDNA molecules input	$1E2 \forall j$	molecules.GT^{-1}

Table S3: Genotypes of the 3 sites model (*comM*, *pilT*, *pho*)

List of the 8 bacterial genotypes of the 3 sites model, with their simplified name, numbering (used in the transition matrix S5), complete genotype, maximal transformation rate (effective transformation rate depend on bacterial genotype and eDNA types and are listed in Table S5), as well as resistance and replication phenotypes. List of the 6 types of extracellular DNA molecules with their numbering.

Name	Number	Genotype			Phenotype		Cost c_i
		comM	pilT	pho	Maximal transformation rate $T_{max,i}$	Resistance	
AbaR-free	0	WT	WT	WT	1E-3	No	0
pho::AbaR	1			AbaR	1E-3	Yes	0.01
pilT::AbaR	2		AbaR		0	Yes	0.01
comM::AbaR	4	AbaR			1E-3	Yes	0.01
AbaR-multi	3		AbaR	AbaR	0	Yes	2*0.01
	5	AbaR		AbaR	1E-3	Yes	2*0.01
	6	AbaR	AbaR		0	Yes	2*0.01
	7	AbaR	AbaR	AbaR	0	Yes	3*0.01
Number		0	1	2	3	4	5
eDNA molecule		comM	comM::AbaR	pilT	pilT::AbaR	pho	pho::AbaR

Table S4: Genotypes of the 4 sites model (*comM, pilT, pho,* SNP)

List of the 16 bacterial genotypes of the 4 sites model, with their simplified name, numbering (used in the transition matrix S5), complete genotype, maximal transformation rate, as well as resistance and replication phenotypes.

List of the 8 types of extracellular DNA molecules with their numbering.

Name	#	Genotype				Maximal transformation rate $T_{max,i}$	Resistances		Cost c_i
		comM	pilT	pho	SNP		Stress 1	Stress 2	
AbaR-free	0	WT	WT	WT	0	1E-3	No	No	0
AbaR-free+SNP	1	WT	WT	WT	1	1E-3	No	Yes	c
pho::AbaR	2			AbaR	0	1E-3	Yes	No	0.01
pho::AbaR+SNP	3			AbaR	1	1E-3	Yes	Yes	0.01+c
pilT::AbaR	4		AbaR		0	0	Yes	No	0.01
pilT::AbaR+SNP	5		AbaR		1	0	Yes	Yes	0.01+c
AbaR-multi	6		AbaR	AbaR	0	0	Yes	No	2*0.01
AbaR-multi+SNP	7		AbaR	AbaR	1	0	Yes	Yes	2*0.01+c
comM::AbaR	8	AbaR			0	1E-3	Yes	No	0.01
comM::AbaR+SNP	9	AbaR			1	1E-3	Yes	Yes	0.01+c
AbaR-multi	10	AbaR		AbaR	0	1E-3	Yes	No	2*0.01
AbaR-multi+SNP	11	AbaR		AbaR	1	1E-3	Yes	Yes	2*0.01+c
AbaR-multi	12	AbaR	AbaR		0	0	Yes	No	2*0.01
AbaR-multi+SNP	13	AbaR	AbaR		1	0	Yes	Yes	2*0.01+c
AbaR-multi	14	AbaR	AbaR	AbaR	0	0	Yes	No	3*0.01
AbaR-multi+SNP	15	AbaR	AbaR	AbaR	1	0	Yes	Yes	3*0.01+c
eDNA molecules nomenclature									
Number		0	1	2	3	4	5	6	7
eDNA molecule		comM	comM::AbaR	pilT	pilT::AbaR	pho	pho::AbaR	SNP0	SNP1

Table S5: Transformation event probabilities used in the model.

The probability of success of the transformation undertaken is equal to 1 when (i) the transformation is not inhibited (i.e. when the cell involved in the transformation is of WT or Δ*pho* genotype) and (ii) the cell acquires a short allele (WT or SNP). In other situations, the probability of a successful transformation is lower, either because of the high length of the acquired allele (AbaR acquisition), or because the *comM* or *pilT* gene is no longer functional (Δ*comM* or Δ*pho*). The probability of a successful transformation is then calculated as the ratio of the probabilities drawn at random from the normal distributions (mean and SE) obtained from the log-transformed frequencies of the experiments (Figure 2). The 'reference' probability (an estimate of the probability of transformation without inhibition and corresponding to the acquisition of a short sequence) P1 and P5, differs according to experimental design 1 or 2, respectively. The distributions of the relative probabilities (mean and SD for 1000 draws) are given for information.

Experimental design	Event	Random draw of log-transformed probabilities	Transformation exp	Relative probability	Relative probability distributions (mean±sd)
Experimental design 1 (model of gene transfer, Figure 2B)	Deletion of AbaR by WT cells	$P_1 \sim N(-8.415965, 0.26905$	$Q_1 = \frac{\exp(P_1)}{1+\exp(P_1)}$	$R_1 = \frac{Q_1}{Q_1} = 1$	
	Acquisition of AbaR by WT cells	$P_2 \sim N(-9.840681, 0.15379$	$Q_2 = \frac{\exp(P_2)}{1+\exp(P_2)}$	$R_2 = \frac{Q_2}{Q_1}$	2.57E-1 ±8.28E-2
	Deletion of AbaR by ΔcomM cells	$P_3 \sim N(-14.19787, 0.29826$	$Q_3 = \frac{\exp(P_3)}{1+\exp(P_3)}$	$R_3 = \frac{Q_3}{Q_1}$	3.34E-3 ±1.41E-3
	Acquisition of AbaR by ΔcomM cells	$P_4 \sim N(-16.39911, 0.23570$	$Q_4 = \frac{\exp(P_4)}{1+\exp(P_4)}$	$R_4 = \frac{Q_4}{Q_1}$	3.72E-4 ±1.37E-4
Experimental design 2 (model of gene and allelic transfer, Figure 2C)	Acquisition of SNP by WT cells	$P_5 \sim N(-11.75745, 0.34040$	$Q_5 = \frac{\exp(P_5)}{1+\exp(P_5)}$	$R_5 = \frac{Q_5}{Q_5} = 1$	
	Acquisition of SNP by ΔcomM cells	$P_6 \sim N(-13.70786, 0.21012$	$Q_6 = \frac{\exp(P_6)}{1+\exp(P_6)}$	$R_5 = \frac{Q_6}{Q_5}$	2.04E-1 ±6.04E-2

Table S6: Matrix of transformation probabilities γ (3 sites model)

Matrix of transition probabilities from an initial genotype to a new genotype by natural transformation for the 3 insertion site model. The probabilities shown in the table are the probabilities of transformation once a cell and an eDNA molecule have met. The sum of probabilities is therefore equal to 1 for each line because in the event of non-transformation, the genotype of the cell remains unchanged. For the model with 4 insertion sites (3 AbaR and 1 SNP), the γ matrix is constructed in a similar way, but is 16x16 in size.

Initial genotype \ Final genotype		AbaR-free	pho::AbaR	pilT::AbaR	AbaR-multi	comM::AbaR	AbaR-multi	AbaR-multi	AbaR-multi
		0	1	2	3	4	5	6	7
AbaR-free	0	$1-\sum_{j\neq i}\gamma_{0,j}$	$R_2\frac{A_{5,t}}{A_{tot,t}}$	$R_2\frac{A_{3,t}}{A_{tot,t}}$	0	$R_2\frac{A_{1,t}}{A_{tot,t}}$	0	0	0
pho::AbaR	1	$R_1\frac{A_{4,t}}{A_{tot,t}}$	$1-\sum_{j\neq i}\gamma_{1,j}$	0	$R_2\frac{A_{3,t}}{A_{tot,t}}$	0	$R_2\frac{A_{1,t}}{A_{tot,t}}$	0	0
pilT::AbaR	2	0	0	1	0	0	0	0	0
AbaR-multi	3	0	0	0	1	0	0	0	0
comM::AbaR	4	$R_3\frac{A_{0,t}}{A_{tot,t}}$	0	0	0	$1-\sum_{j\neq i}\gamma_{4,j}$	$R_4\frac{A_{5,t}}{A_{tot,t}}$	$R_4\frac{A_{3,t}}{A_{tot,t}}$	0
AbaR-multi	5	0	$R_3\frac{A_{0,t}}{A_{tot,t}}$	0	0	$R_3\frac{A_{4,t}}{A_{tot,t}}$	$1-\sum_{j\neq i}\gamma_{5,j}$	0	$R_4\frac{A_{3,t}}{A_{tot,t}}$
AbaR-multi	6	0	0	0	0	0	0	1	0
AbaR-multi	7	0	0	0	0	0	0	0	1

Table S7: Bacterial strains and plasmids used in this study

Name/Genotype	Source	Comments
strains used for the measure of acquisition of a single nucleotide polymorphism		
M2 WT	(7)	*A. nosocomialis* M2 Wild-type strain
M2 *comM::[sacB aacC4]*	This study	Sucrose sensitive and apramycin resistant
M2 *ΔcomM*	This study	Deletion into the *comM* gene
M2 *pho::[sacB aacC4]*	This study	Sucrose sensitive and apramycin resistant
M2 *Δpho*	This study	Deletion into the *pho* gene
M2 *attn7::[lacZ aphA]*	This study	Natural transformation with assembly PCR
M2 *ΔcomM attn7::[lacZ aphA]*	This study	Kanamycin resistant
M2 *Δpho attn7::[lacZ aphA]*	This study	Kanamycin resistant
M2 *pilT::km*	(8)	Not naturally transformable ; Kanamycin resistant
M2 *rpoB*(Rif^R)	This study	Spontaneous mutation in *rpoB* gene ; Rifampicin resistant
M2 *rpoB*(Rif^R) *comEC::aacC4*	This study	Rifampicin resistant, apramycin resistant
Strains used for bacterial competitions		
M2 *hu-sfgfp aacC4*	(9)	Fluorescent ; apramycin resistant
M2 *comM::[*AbaR4*]*	This study	Natural transformation with genomic DNA from *A. baumannii* 40288 strain
M2 *comM::[*AbaR1*]*	This study	Natural transformation with genomic DNA from *A. baumannii* AYE strain
AYE WT	(10)	*A. baumannii* AYE Wild-type strain
M2 *comM::[*AbaR4*] hu-sfgfp aacC4*	This study	Fluorescent ; apramycin resistant
M2 *comM::[*AbaR1*] hu-sfgfp aacC4*	This study	Fluorescent ; apramycin resistant
M2 *ΔcomM hu-sfgfp aacC4*	This study	Fluorescent ; apramycin resistant
Strains used for the measure of acquisition and deletion of the *L* fragment		
M2 *pho::[*AbaR4*]*	This study	Natural transformation with genomic DNA from *A. baumannii* AB0057 strain
M2 *comM::[sacB aacC4] pho::[*AbaR4*]*	This study	Sucrose sensitive and apramycin resistant
M2 *ΔcomM pho::[AbaR4]*	This study	Deletion into the *comM* gene
M2 *ΔcomM pho::[AbaR4 sacB aacC4]*	This study	Intermediate genotype
M2 *ΔcomM pho::[*AbaR4 *Δ'aacC4]*	This study	Intermediate genotype
M2 *ΔcomM pho::[*AbaR4 *tniA::(sacB aacC4) Δ'aacC4]*	This study	Intermediate genotype
M2 *ΔcomM pho::[*AbaR4 *tniA1(Oc) Δ'aacC4]*	This study	Intermediate genotype
M2 *ΔcomM pho::[AbaR4 tniA1(Oc) aacC4::(aphA Lj)]*	This study	Intermediate genotype
M2 *rpoB*(Rif^R) *ΔcomM pho::[AbaR4 tniA1(Oc) aacC4::(aphA Lj)]*	This study	Recipient strain for deletion ; deletion into the *comM* gene ; Rifampicin resistant
M2 *comEC::tetA ΔcomM pho::[*AbaR4 *tniA1(Oc) aacC4::(aphA Lj)]*	This study	Donor strain for acquisition ; not naturally transformable
M2 *ΔcomM pho::[*AbaR4 *tniA1(Oc) aacC4 ΔLj]*	This study	Intermediate genotype
M2 *rpoB*(Rif^R) *ΔcomM pho::[*AbaR4 *tniA1(Oc) aacC4 ΔLj]*	This study	Recipient strain for acquisition ; deletion into the *comM* gene ; Rifampicin resistant
M2 *comEC::tetA ΔcomM pho::[*AbaR4 *tniA1(Oc) aacC4 ΔLj]*	This study	Donor strain for deletion ; not naturally transformable
M2 *pho::[AbaR tniA1(Oc) aacC4::(aphA L)]*	This study	Intermediate genotype
M2 *rpoB*(Rif^R) *comM$^+$ pho::[*AbaR4 *tniA1(Oc) aacC4::(aphA Lj)]*	This study	Recipient strain for deletion ; rifampicin resistant
M2 *comM$^+$ pho::[*AbaR4 *tniA1(Oc) aacC4 ΔLj]*	This study	Intermediate genotype
M2 *rpoB*(Rif^R) *comM$^+$ pho::[*AbaR4 *tniA1(Oc) aacC4 ΔLj]*	This study	Recipient strain for acquisition ; Rifampicin resistant
Plasmids		
pASG-4	(9)	Not replicative in *Acinetobacter*
pMHL-7		Contains the [*sacB aacC4*] cassette
pMHL-2	(9)	Contains the *aacC4* gene
pXDC116	Derivative of pXDC61 (11)	Contains the Kan^R cassette

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Primer name	Sequence 5' to 3'	Template ; annealing site	Use (genetic construct)
mlo-104	AAGATATCGGTCTCCAAGC	M2 genomic DNA ; *rpoB* gene	*rpoB*(Rif^R)
mlo-105	AGTACGGCCTTCGTCAT	M2 genomic DNA ; *rpoB* gene	*rpoB*(Rif^R)
mlo-97	ATACCGCCGTAGAATGCC	M2 genomic DNA ; 2-kbp Upstream *comM* gene	*ΔcomM*
comM-Rev	TTAAGAGTGATTACCTCGATAAGA	M2 genomic DNA ; 3' of the *comM* gene	*ΔcomM*
asg-100	AATTTCCAGTGCACGACG	M2 genomic DNA ; *comM* gene	*ΔcomM*
asg-76	CGGCGTGCACTAGAAATTCCACGGGTGAAATTAC	M2 genomic DNA ; *comM* gene	*ΔcomM*
Apra-For	ATCAAGGCCCGATCCTTGGAGCCCTTG	pMHL-2; Begining of *aacC4* gene	*[aacC4 ΔL] ; aacC4::aphA*
mlo-29	TCATGAGCTCAGCCAATCGACTGG	pMHL-2; End of Apra^R cassette	*[aacC4 ΔL] ; [sacB aacC4] ; [*AbaR4 *aacC4::(aphA L)]* (L=2, 4-kbp)
asg-58	ATCACCCATCACATATACCTGCCG	pMHL-2 ; Upstream *sacB* gene	*[sacB aacC4]*
asg-85	ACCCTGTATTTCACGTAG	M2 genomic DNA ; 2-kbp Upstream *pho* gene	*pho::[sacB aacC4] ; pho::[*AbaR4 *sacB aacC4] ; Δpho*
asg-86	CGGCAGGTATATGTGATGGGTGATTACGACGGCTCACATATTGGTC	M2 genomic DNA ; *pho* gene	*pho::[sacB aacC4] ; pho::[*AbaR4 *sacB aacC4] ; Δpho*
asg-87	CCAGTCGATTGGCTGAGCTCATGACTAAAGCAAGCGGTG	M2 genomic DNA ; *pho* gene	*pho::[sacB aacC4]*
asg-88	AACCAGCAATAACACC	M2 genomic DNA ; 2-kbp Downstream *pho* gene	*pho::[sacB aacC4] ; pho::[*AbaR4 *sacB aacC4] ; Δpho ; pho::[*AbaR4 *Δ'aacC4]*
asg-97	ATCACCCATCACATATACCTGCCGTTGGGACGCAATTGG	M2 genomic DNA ; *pho* gene	*Δpho*
asg-108	TTCAGTTGTCTCAAAGGACGC	AbaR4 island	*pho::[*AbaR4 *sacB aacC4] ; pho::[*AbaR4 *Δ'aacC4] ; [*AbaR4 *aacC4::(aphA L)]* (L=2, 10-kbp)
asg-137	CGGCAGGTATATGTGATGGGTGATGTCAGTTCCACAAATAAATCAGAGT	M2 genomic DNA ; Begining of 3'half *pho* gene	*pho::[*AbaR4 *sacB aacC4]*
asg-77b	GTCAGTTCCACAAATAAATCAGAGT	M2 genomic DNA ; Begining of 3'half *pho* gene	*pho::[*AbaR4 *Δ'aacC4]*
asg-138	ACTCTGATTTATTTGTGGTGACTCATGCCCTCGTGGTCAG	pMHL-2 ; *aacC4* gene	*pho::[*AbaR4 *Δ'aacC4]*
mlo-74	TGACAGAGCTGACGCC	M2 genomic DNA ; Begining of *pho* gene	*tniA::[sacB aacC4] ; tniA1(Oc)*
asg-159	GACCACATTGGGTTATAGAATGATACGACTA	AbaR4 island ; *tniA* gene	*tniA1(Oc)*
asg-160	TAGTCGTATCATTCTATCCAATGTGGTTGTC	AbaR4 island ; *tniA* gene	*tniA1(Oc)*
asg-161	CGGCAGGTATATGTGATGGGTGATTATTGACAGCATTAGATTC	AbaR4 island ; *tniA* gene	*tniA::[sacB aacC4]*
asg-162	CCAGTCGATTGGCTGAGCTCATGATAGGGCGAATGAATATGATGTG	AbaR4 island ; *tniA* gene	*tniA::[sacB aacC4]*
R18	TGAGAGACGCTACTC	AbaR4 island	*tniA::[sacB aacC4] ; tniA1(Oc)*
comEC-For	TGGTGTCGCTGTACTAATTACGGT	M2 genomic DNA ; *comEC* gene	*comEC::aacC4*
asg-67	GGGTTGAGATATACTCGCG	M2 genomic DNA ; 2-kb upstream *comEC* gene	*comEC::tetA*
comEC-Rev	CTTCAGTTCACGCATCAAGCTTGT	M2 genomic DNA ; *comEC* gene	*comEC::aacC4 ; comEC::tetA*
asg-168b	GGAAGTATCAGAATTGGTTAATCAATGATATGTTGCTCAG	M2 genomic DNA ; *comEC* gene	*comEC::tetA*
asg-169	CATATATAATAATTAAGTTTATTTTTCAAAAAGGCTCAATATACTG	M2 genomic DNA ; *comEC* gene	*comEC::tetA*
asg-148	GACTTGAGGCGCAGCGTCGTCCAGACCTGACC	pMHL-2 ; *aacC4* gene	*aacC4::aphA*
kan-F	CTGCGCCTCAAGTGTTC	pXDC116 ; Kan^R cassette	*aacC4::aphA*
kan-R	TGCCTCGTGAAGAAGG	pXDC116 ; Kan^R cassette	*aacC4::aphA*
R3	CAAGGGCTCCAAGGATCGGGCCTTGATTTACCATATGTGC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=2-kbp)
F4.2	CCTTCTTCACGAGGCATTAAGGCAAGATTAGC	AbaR4 island	[AbaR aacC4::(aphA L)] (L=2-kbp)
oxa23-Rev	ATTTCTGACCGCATTTCCAT	*bla*$_{OXA-23}$ gene	*[*AbaR4 *aacC4::(aphA L)]* (L=4-kbp)
R5	CAAGGGCTCCAAGGATCGGGCCTTGATTATTCGAGAATGG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=4-kbp)
F6.2	CCTTCTTCACGAGGCTAGACAGTGATGAG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=4-kbp)
F7	TTTGAGACTGAATAAGG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=6-kbp)
R7	CAAGGGCTCCAAGGATCGGGCCTTGATAGATCATCGCAGTAGAC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=6-kbp)
F8.2	CCTTCTTCACGAGGCAAGTTCATTGTCTACTGCG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=6-kbp)

asg-107	TCTTTCGTATCTGAATTTCCACG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=6-kbp)
asg-84	CATTCCAATAAGTTCGACTTCTG	AbaR4 island	*[AbaR4 aacC4::(aphA L)]* (L=8-kbp)
R9	CAAGGGCTCCAAGGATCGGGCCTTGATTGTTACTTGGGTG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=8-kbp)
F10.2	CCTTCTTCACGAGGCAAGTACCAGAAGC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=8-kbp)
oxa23-D	CAGTGCTTTTAGTTGTTGTGA	*bla*$_{OXA-23}$ gene	*[*AbaR4 *aacC4::(aphA L)]* (L=8-kbp)
F11	AAAGCGTATCTTGCTG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=10-kbp)
R11	CAAGGGCTCCAAGGATCGGGCCTTGATTCGCCATGGCAGTG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=10-kbp)
F12.2	CCTTCTTCACGAGGCAAACTGCCATGGCG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=10-kbp)
F13	TTTCTCAGATACAGCC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=12-kbp)
R13	CAAGGGCTCCAAGGATCGGGCCTTGATAAGCACCGTAATTCTC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=12-kbp)
F14.2	CCTTCTTCACGAGGCAATGAGAATTACGGTGC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=12-kbp)
asg-79	AGAAGTCGTTATTGG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=12-kbp)
mlo-111	CACTAGAAGCGCCAAGTACGA	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=14-kbp)
R15	CAAGGGCTCCAAGGATCGGGCCTTGATATCTGAGAGACGC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=14-kbp)
F16.2	CCTTCTTCACGAGGCATTTCTCAGATACAGCC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=14-kbp)
R16	ATGGAAGCACCGTAATTC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=14-kbp)
F17	TGTCATTTACAGCAATAGAATAGAG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=16-kbp)
R17	CAAGGGCTCCAAGGATCGGGCCTTGATACAAGCTTTACTGATGTG	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=16-kbp)
F18.2	CCTTCTTCACGAGGCAATCAGTAAAGCTTGTC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=16-kbp)
R18	TGAGAGACGCTACTC	AbaR4 island	*[*AbaR4 *aacC4::(aphA L)]* (L=16-kbp)

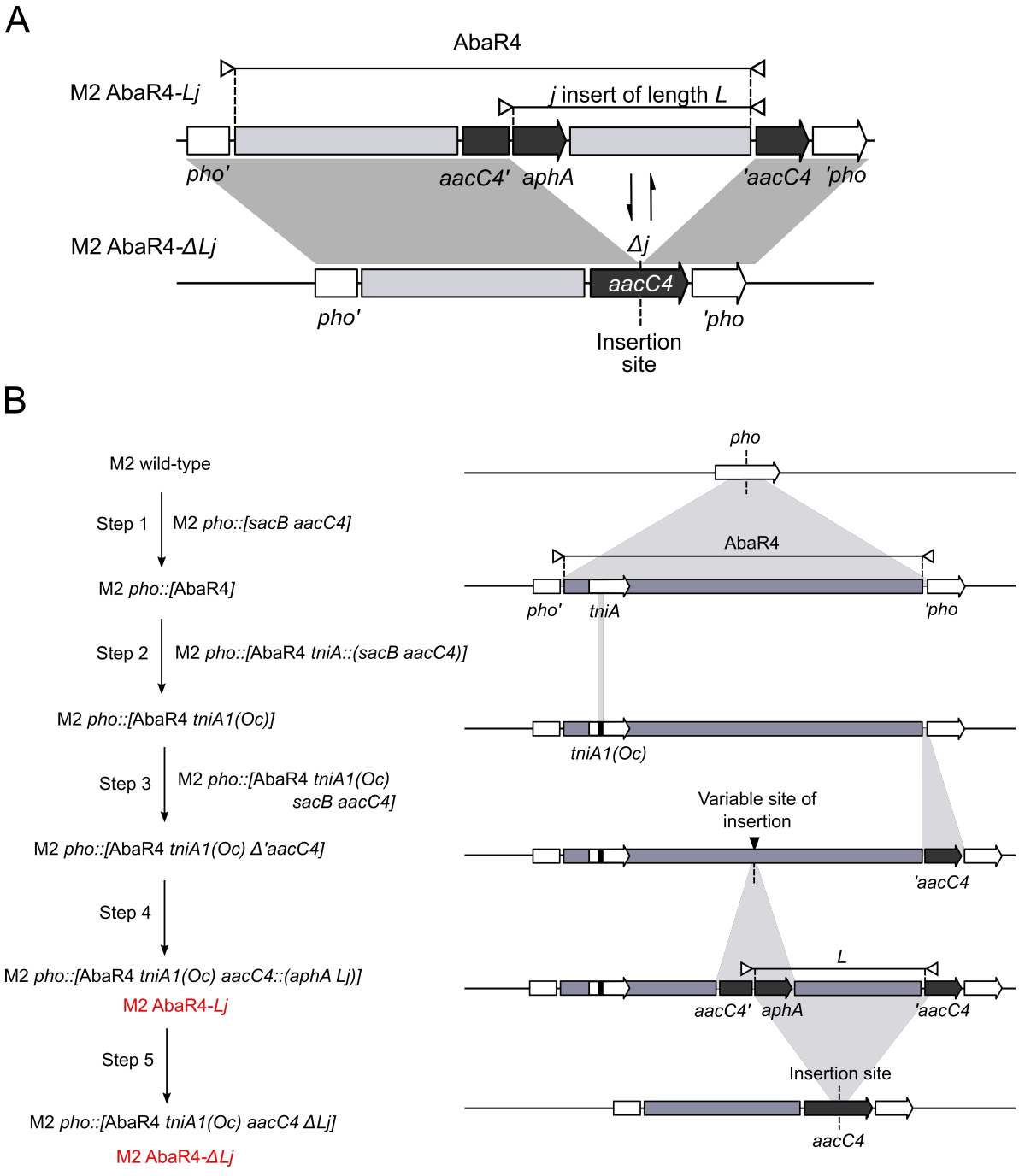


Figure S1. Strategy and mutant construction to quantify events of acquisition or deletion of a heterologous DNA fragment occuring within mixed populations.

A. Genotypes of the M2 AbaR4-*Lj*/*ΔLj* couples used to quantify events of acquisition or deletion. In the M2 AbaR4-*Lj* genotype, the *j* insert of length *L* is part of an AbaR4 inserted in the *pho* gene, is delimited by the two halves of an *aacC4* apramycin resistance gene (*aacC4'* and *'aacC4*) and contains an *aphA* aminoglycoside resistance gene inserted directly downstream the 3'half of *aacC4*. In the M2 AbaR4-Δ*Lj* genotype, the *j* fragment has been removed to reconstruct the *aacC4* gene. The acquisition of *j* induces the expression of the *aphA* gene and the interruption of the *aacC4* gene leading to a kanamycin resistant/apramycin sensitive phenotype, whereas its deletion induces the reconstruction of an intact *aacC4* gene and the elimination of the *aphA* gene

leading to a kanamycin sensitive/apramycin resistant phenotype.

B. Chromosomal modifications used to generate the genotypes carrying the *j* fragment with a variable length. The *A. nosocomialis* M2 wild-type strain was modified to insert a large AbaR4 island into the *pho* gene (**step 1**). This was achieved by transforming the M2 *pho::*[*sacB aacC4*] intermediate with purified genomic DNA extracted from the *A. baumannii* AB0057 strain which naturally carries the AbaR4 island inserted into its *pho* gene. To avoid any transposition of the AbaR4 island, a point mutation leading to the generation of a stop codon and a change into the open reading frame was inserted into the coding sequence of the *tniA* gene of the AbaR4, generating the M2 *pho::*[AbaR4 *tniA1*(Oc)] strain (**step 2**). The M2 *pho::*[AbaR4 *tniA1*(Oc) Δ*'aacC4*] strain was then obtained by transforming the M2 *pho::*[AbaR4 *tniA1*(Oc) *sacB aacC4*] intermediate with the 3'half of the *aacC4* gene resulting to its insertion between the end of the AbaR4 and the 3'half of the *pho* gene (**step 3**). This parental strain was then transformed with different genetic constructs composed of the 5'half of the *aacC4* gene and a kanamycin resistance gene (*aphA*) flanked by 2-kbp regions that are homologous to different insertion sites of the AbaR4. It generated 8 different M2 *pho::*[AbaR4 *tniA1*(Oc) *aacC4::*(*aphA Lj*)] genotypes in which an heterologous DNA fragment labelled *j,* had a variable *L* length of 2, 4, 6, 8, 10, 12, 14 and 16-kbp (**step 4**). Finally, each of these genotypes, renamed M2 AbaR-*Lj*, were transformed with a PCR product of the *aacC4* gene to remove the *j* fragment and reconstruct the intact *aacC4* gene (**step 5**). The resulting M2 *pho::*[AbaR4 *tniA1*(Oc) *aacC4* Δ*Lj*)] genotypes were renamed M2 AbaR4-Δ*Lj.* The *ΔcomM* derivatives were obtained by transforming the M2 *ΔcomM* strain with genomic DNA extracted from the different M2 AbaR4-*Lj* and M2 AbaR4-Δ*Lj* strains.

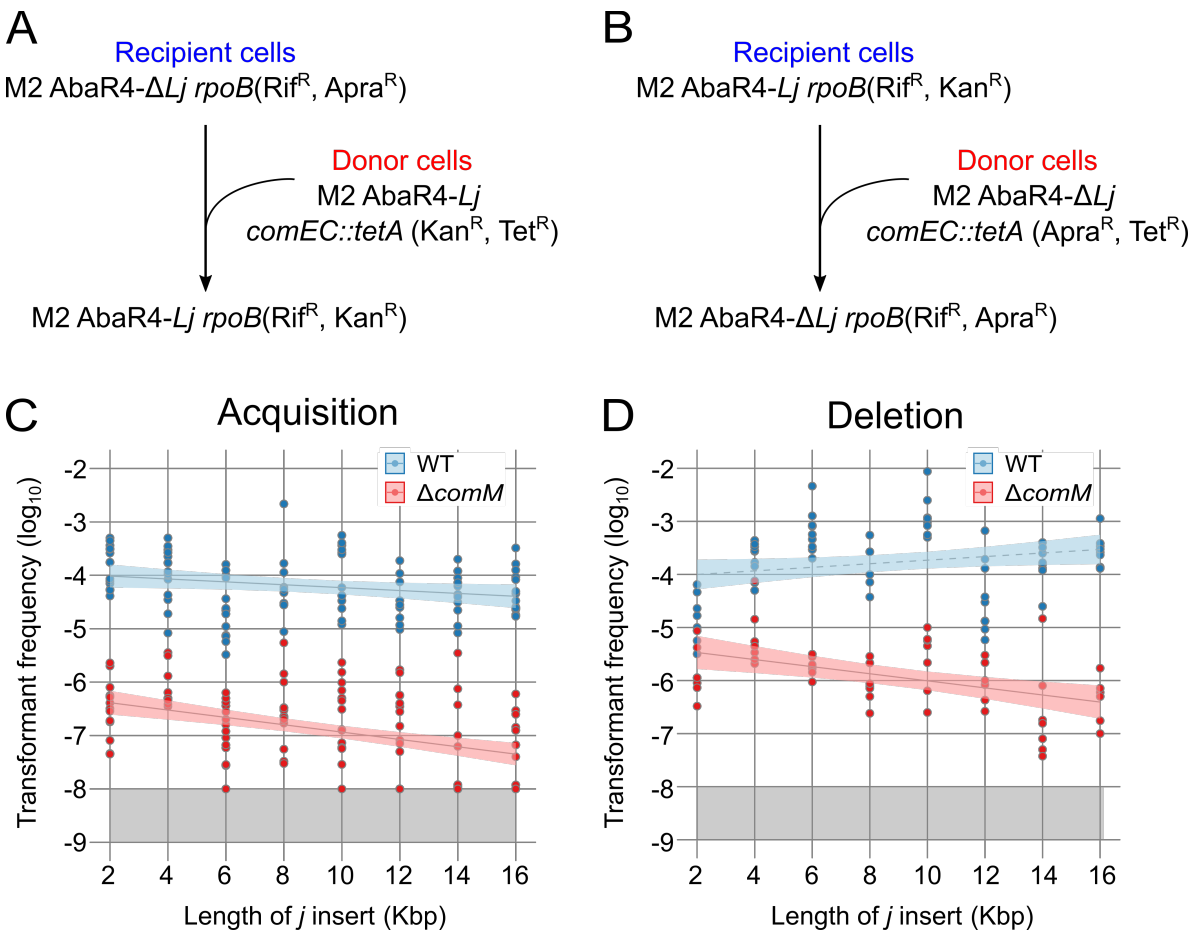


Figure S2. Quantification of rates gene transfer (acquisition and deletion), as a function of sequence length, occurring within populations.

A. and B. Genotypes of derivatives used as recipient or donor to generate the acquisition and the deletion of the *j* fragment. Acquisition events were obtained by naturally transforming M2 AbaR4-Δ*Lj rpoB2*(Rif^R) recipient cells with non-transformable M2 AbaR4-*Lj comEC::tetA* and deletion events were obtained by naturally transforming M2 AbaR4-*Lj rpoB2*(Rif^R) recipient cells with non-transformable M2 AbaR4-*ΔLj comEC::tetA*. Natural transformation frequencies of acquisition (C) or deletion (C) of a heterologous *j* fragment with variable length in mixed culture. Transformant frequencies are represented using a log transformation of the ratio of the number of transformants to the number of total recipient cells. The genetic backgrounds of the *comM* gene are represented for acquisition and for deletion of the *j* fragment (*comM*$^+$ in black and Δ*comM* in blue). Detection limit (*F*=-8) is indicated by the light grey area. Experimental events, considered as under this limit, were given the detection limit value. A regression linear model is represented for each condition, as a full line when the slope is significantly different from 0 or as a pointed line if not. Transformant frequencies represent the ratio between the number of transformants and the total number of recipient cells. The limit of detection for transformants frequencies is indicated by the grey area ($F < 10^{-8}$).

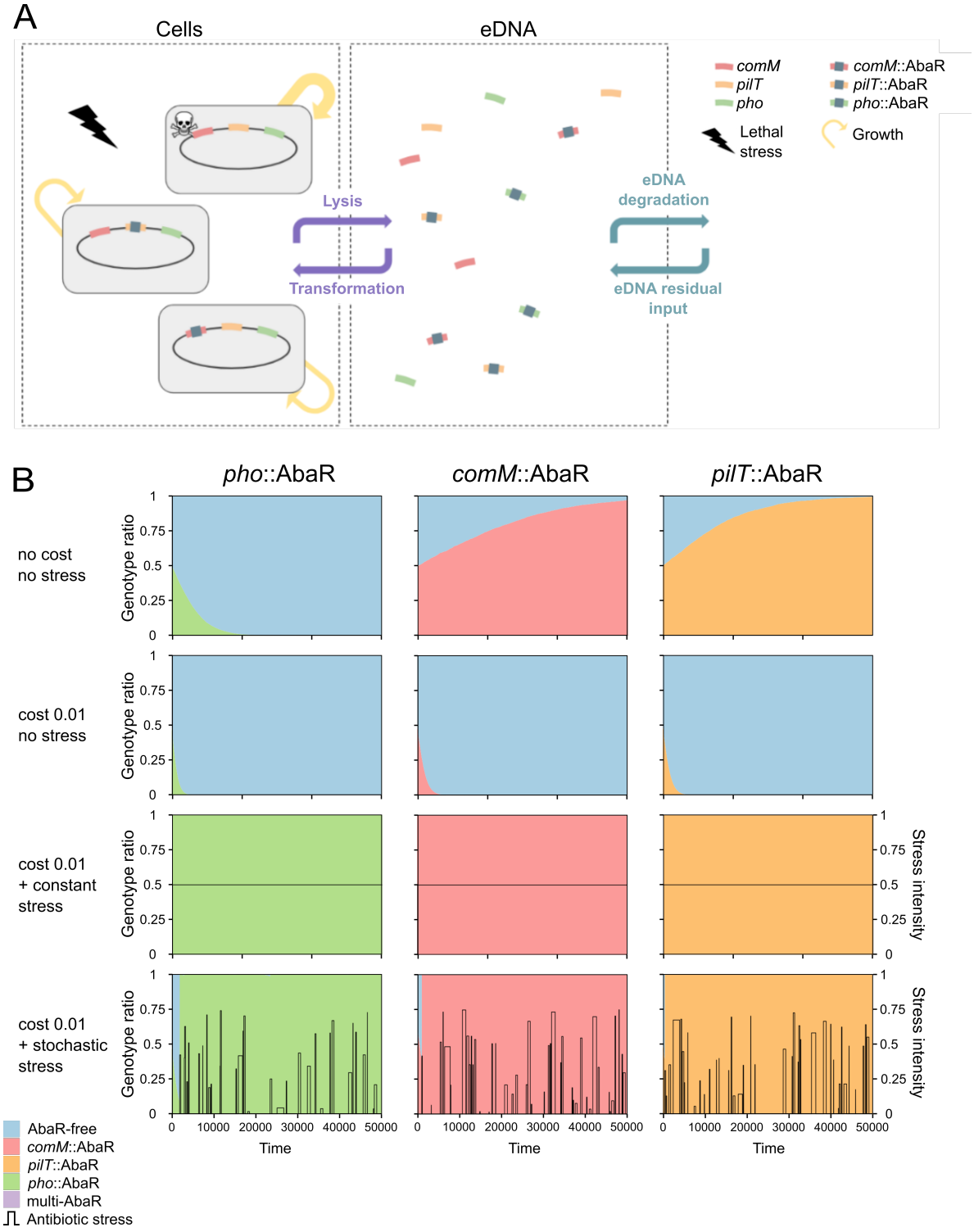
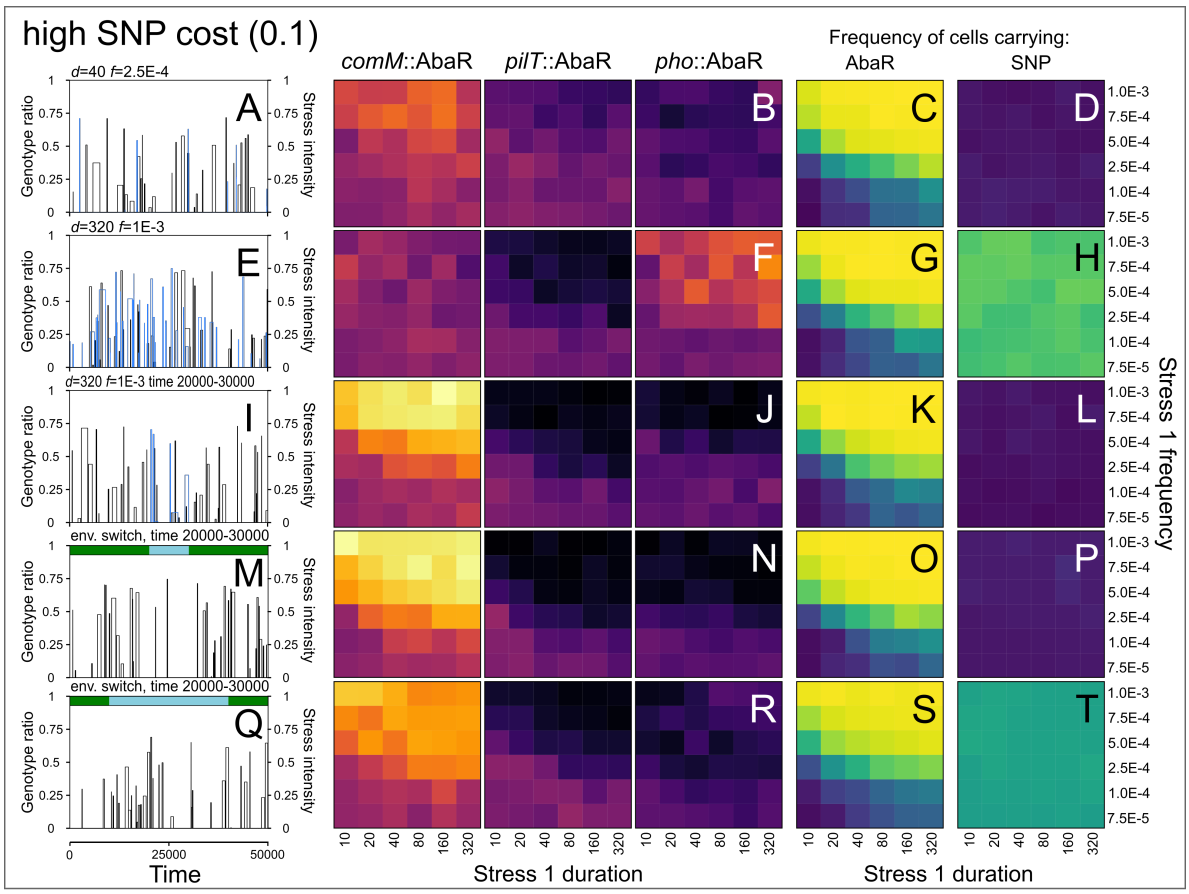
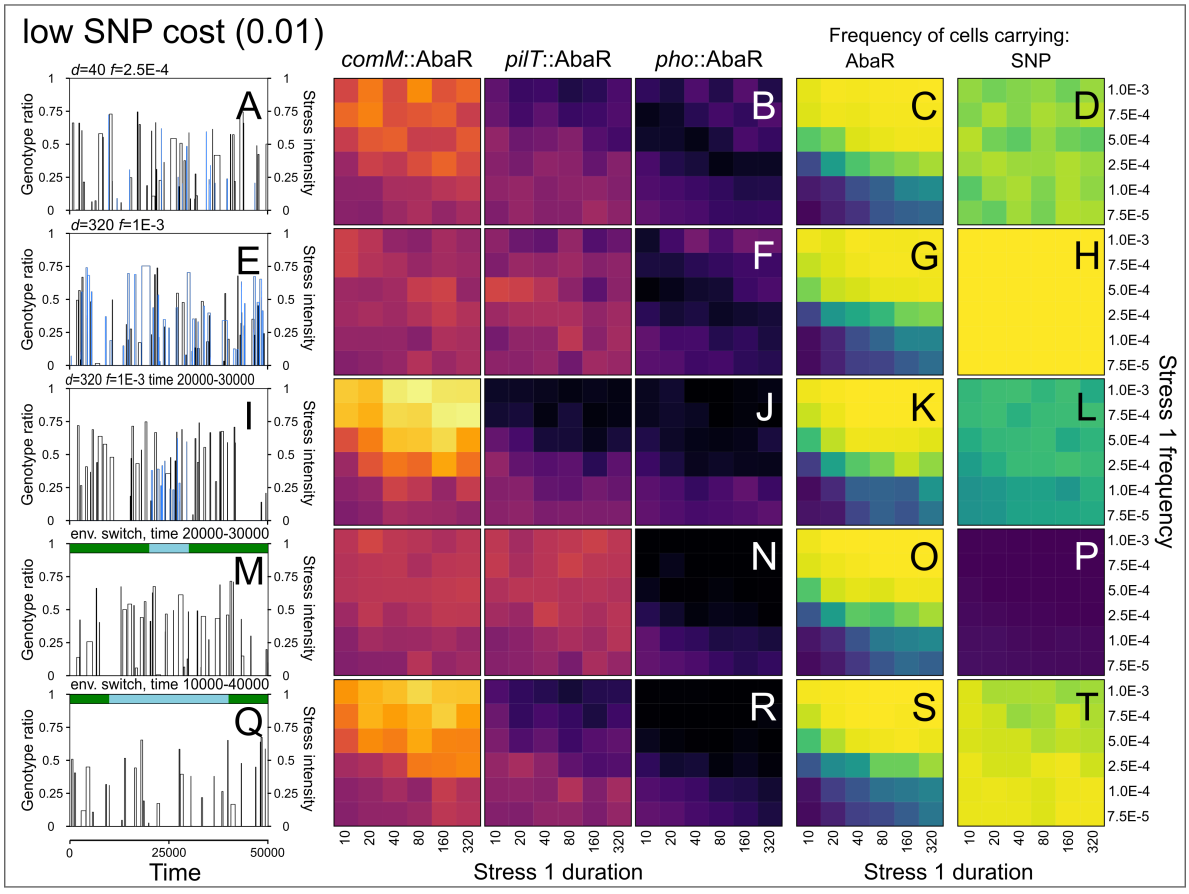


Figure S3. A mathematical model of *A. baumannii* population involving HGT by natural transformation. A. Schematic representation of the stochastic computational model for modeling AbaR dynamics within a bacterial population. The model comprises two compartments, one composed of bacterial cells, the other of extracellular DNA (eDNA). The bacterial population grows according to a logistic model. The bacterial cells have three AbaR insertion site in their chromosome (*comM*, *pilT*, *pho*) at which two types of alleles from the eDNA compartment can be integrated by

transformation and replace their current DNA: wild-type allele and AbaR carrying resistance (*comM*::AbaR, *pilT*::AbaR, *pho*::AbaR). The integration of a WT allele is costless for cells, whereas the integration of AbaR causes a decrease of cell replication. Bacterial populations are faced with stochastic stresses of random duration, frequency and intensity. In the absence of stress, cells are lysed at a basal rate. Under stress exposure, the lysis rate of WT cells increases but remains unchanged for cells with an AbaR carrying resistance. Each lysed cell releases its DNA and fuels the extracellular compartment with eDNA. AbaR and WT alleles are constantly added to the extracellular environment at a marginal rate simulating residual arrival from neighboring populations. The WT alleles and AbaR are degraded at a constant rate in the extracellular compartment.

B. Examples of simuled dynamics of bacterial genotypes carrying AbaR (only one type per simulation) in competition with AbaR-free cells (wild-type) represented as Mueller plots. Simulations were initialized with an equal number of AbaR-free cells and AbaR-carrying cells (either *pho*::AbaR -first column-, or *comM*::AbaR -second column-, or *pilT*::AbaR -third column-) and with an equal number of each DNA types. As AbaRs are site-specific (for example, an AbaR specialising in insertion into the pho gene cannot insert into the *comM* or *pilT* gene), the results in each column relate to only one AbaR type. First row, when AbaRs carry no cost and confer no antibiotic resistance. Second row, when AbaRs carry a cost on fitness (0.01) and confer resistance to a stress, but no stress is applied. Third and fourth rows, same as in the second row, but a stress is applied either continuously or stochastically.


high SNP cost (0.1)
comM::AbaR
pilT::AbaR
pho::AbaR
Frequency of cells carrying:
AbaR
SNP
d=40 f=2.5E-4
d=320 f=1E-3
d=320 f=1E-3 time 20000-30000
env. switch, time 20000-30000
env. switch, time 20000-30000
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
Genotype ratio
Stress intensity
Time
Stress 1 duration
Stress 1 duration
Stress 1 frequency


low SNP cost (0.01)
comM::AbaR
pilT::AbaR
pho::AbaR
Frequency of cells carrying:
AbaR
SNP
d=40 f=2.5E-4
d=320 f=1E-3
d=320 f=1E-3 time 20000-30000
env. switch, time 20000-30000
env. switch, time 10000-40000
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
S
T
Genotype ratio
Stress intensity
Time
Stress 1 duration
Stress 1 duration
Stress 1 frequency

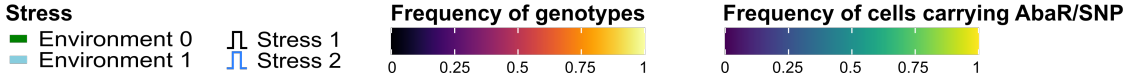

Stress
Environment 0
Environment 1
Stress 1
Stress 2
Frequency of genotypes
Frequency of cells carrying AbaR/SNP

Figure S4. Frequency of AbaR islands, island-carrying cells and SNP-carrying cells in fluctuating environments.

This figure is an extension of figure 4 and corresponds to the results of the competition between the three types of AbaR islands, which differ in their insertion strategy (initial number of cells: AbaR-free = K/10, initial number of each eDNA type = K), with K being the carrying capacity. Environmental fluctuations have two components: the bacterial population is exposed to stochastic stress 1 (to which resistance is conferred by AbaR) and another environmental change 2 (to which advantage is conferred by a SNP acquired by allelic transfer). The grey boxes show the dynamics of environmental change 2 (left column). In panels B, F, J, environmental change 2 corresponds to stochastic exposure to a new stress (stress 2) which from top to bottom are (B) rare and short (mean peak duration *d*=40, mean peak frequency *f*=2.5E-4), (F) frequent and long (mean peak duration *d*=320, mean peak frequency *f*=1E-3), and (J) frequent and long over a short period (20000-30000 time units). Lastly, environmental change 2 is not a stress but a change in resource availability occurring between 20000 and 30000 time units, or occuring between times 10000 and 40000, respectively (panel N and R). The ratio of bacterial genotypes carrying AbaR were assessed as a function of stress 1 frequency and duration. In addition to the information in Figure 4, this figure shows the frequency of AbaR-carrying cells (5th column) and the frequency of SNP-carrying cells (6th column). This figure also shows the results obtained when the cost of the SNP in terms of cell replication is low (0.01) (bottom panel), which are qualitatively similar to those presented in the main text (high cost equal to 0.1, top panel). However, when the period with the new resource is of short duration and the SNP costs are low (panel N at the bottom), there is no obvious advantage of *comM*::AbaR over *pilT*::AbaR. In the complete absence of SNP costs, we would have the same situation as in Figure 3 (without stress 2). In that sense, for a low SNP cost (0.01), and if the duration of exposure to the new environment is short (panel N) we obtain the same situation as in Figure 3. On the contrary, if exposure to the new environment is long enough, the advantage of *comM*::AbaR (which can acquire the advantageous SNP) has a significant effect and *comM*::AbaR can become fixed (panel R).

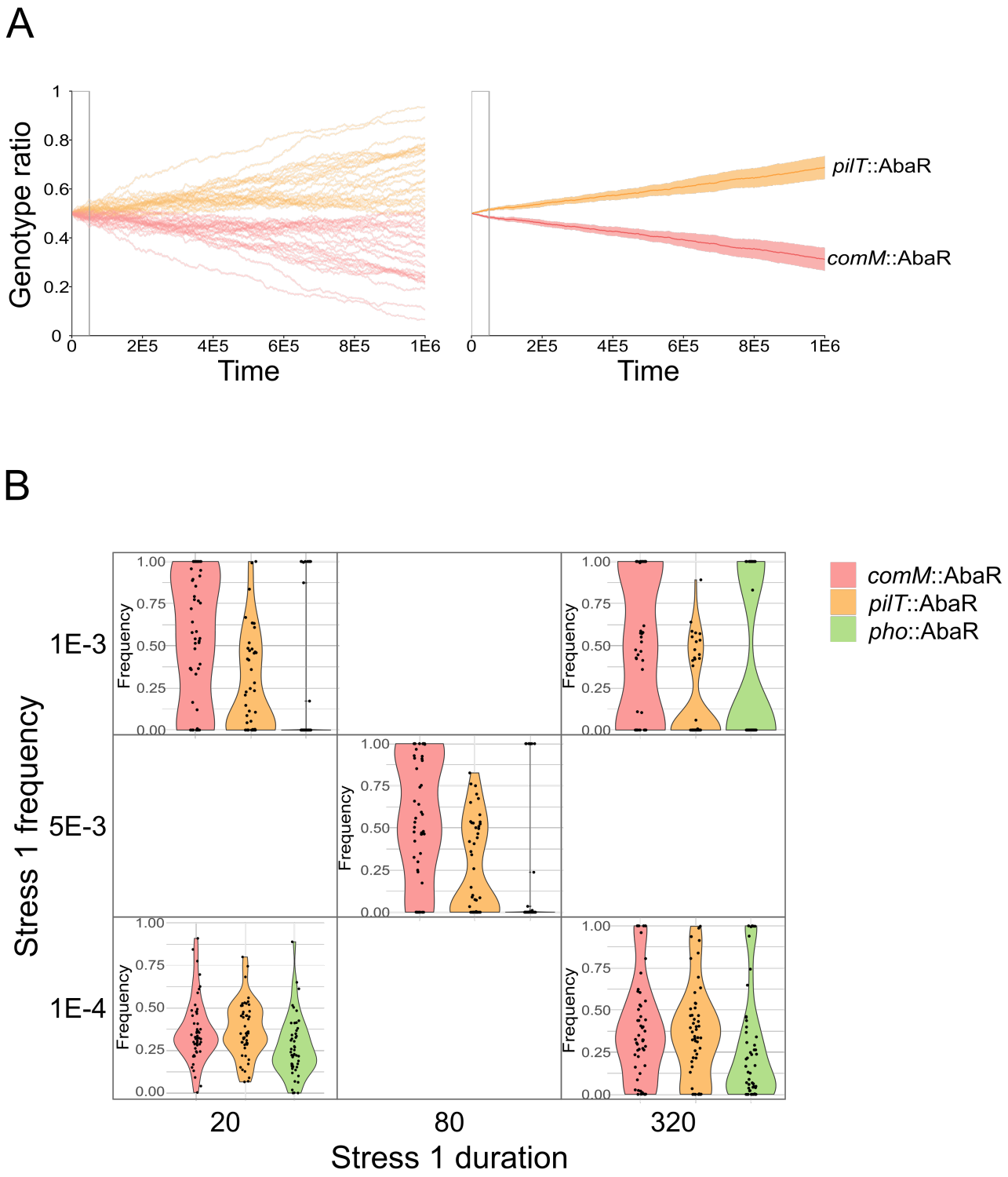


Figure S5. Illustration of the quasi-neutrality between *comM*::AbaR and *pilT*::AbaR (panel A) and the variability between simulations in a given regime of environmental fluctuations (panel B). A. The quasi-neutrality between *comM*::AbaR and *pilT*::AbaR is illustrated from their long-term dynamics under continuous stress (intensity 0.5). Under the initial conditions, the two genotypes are represented at the same frequency (K/2 each and K molecules of each eDNA types). Left panel: 25 independent dynamics. Right panel: Mean and confidence interval (95%) of the 25 simulations. We show that over the very long term, we can detect a small

selective advantage of AbaR inserting into the *pilT* gene (which completely inhibits transformation and therefore cannot be eliminated from the genome) over AbaR inserting into *comM* (which considerably - but not totally - reduces the probability of being eliminated). This selective advantage selective advantage is so small that the extinction of *comM*::AbaR never occurs in any trajectory (which lasts 1E6 time units equivalent to 1E6 generations without density dependence) and that the extinction of *comM*::AbaR in all trajectories would require an infinitely long time. B. The variability between simulations under a given regime of environmental fluctuations is illustrated from 50 simulations of the 4-sites model, with stress 2 being rare and short (Figure 4A). The different panels show the frequency distribution of the 3 AbaR genotypes (violin diagram and dots) with different stress 1 regimes. Note that when stress 1 is frequent (5E-4 or 1E-3), the *pho*::AbaR genotype becomes fixed or extinct, depending on the distribution of stress 2 along the trajectory. In that sense, the *pho*::AbaR genotype has an advantage when stress 2 becomes frequent (see figure 4B).

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