**Abstract**

Bacteria of the genera *Xenorhabdus* and *Photorhabdus* both colonize and influence the behavior

of nematodes, *Steinernema* and *Heterorhabditis* respectively. The nematodes infect, colonize and

ultimately kills off an insect host, with the help of the bacteria. Thus the bacteria are symbiotic of two

hosts, helping the nematode to reproduce optimally while killing the insect. Many studies focused on

the symbiotic association between the bacteria and their respective hosts, but they scarcely focused on

cooperation among bacteria. We will show that the life cycle of the bacteria is favorable to bacterial

cooperation, as the life cycle allows for high relatedness among bacteria. We here investigated a trait of

these bacteria that resemble a helping behavior: phenotypic variation. Phenotypic variation is observed

experimentally in both *Xenorhabdus* and *Photorhabdus*, bacteria are found in two phenotypic states,

controlled by a promoter switch. We provide equations connecting phenotypic variation frequency to

demographic and migration parameters. We argue that the tripartite system bacteria-nematode-insect

is an invaluable to study cooperation in experimental evolution.

**Keywords.** cooperation, phenotypic variation, relatedness, *Xenorhabdus*, *Photorhabdus* ,

**Introduction**

Studies of *Photorhabdus* and *Xenorhabdus* bacteria have

significant potential to reveal both common and distinct

aspects of pathogenesis and mutualism, as each of these

bacteria naturally enter into both types of relationships

during their life cycle: they are pathogens of insects and

are mutualists of nematodes from the genus *Heterorhabditis*

and *Steinernema* respectively [11, 1, 26]. Most

studies have been conducted on the species *Xenorhabdus*

*nematophila* and *Photorhabdus luminescens*, and

their respective host *Steinernema carpocapsae* and *Heterorhabditis*

*bacteriophora*. For simplicity, we refer to

these bacteria as *Xenorhabdus* and *Photorhabdus*.

The mutualistic relationship between the bacteria

and the nematode is not obligate, as both partners can

survive in the absence of the other. Thus, aposymbiotic

nematodes can be obtained and assessed for responses

to diverse bacterial and environmental stimuli. However,

symbiotic bacteria are required for nematodes to

reproduce efficiently [24].

Nematodes are either found in insect hosts or in the

soil (Figure 1). The soil-dwelling vector stage, called

the infective juvenile (IJ), is encased in a double cuticle,

and is non-feeding owing to its closed mouth and

anus. Prior to the IJ stage, ingested bacteria colonize

the nematode. *Xenorhabdus* colonize a discrete intestinal

location known as the vesicle [3], which is the lumen

between nematode epithelial cells at the anterior end of

the intestine. The *X. nematophila* population within

an IJ *S. carpocapsae* nematode is founded by one or

two individual bacterial cells that grow to fill the vesicle.

The mature population contains between 50 and

100 colony-forming unit [19]. The specificity is stringent

since other *Xenorhabdus* species do not colonize

*S. carpocapsae* IJs. Conversely, *Photorhabdus* bacteria

occupy a substantial fraction of the lumen of the nematode

gut[9]. The population is also founded by one or

two individuals [26]. Subsequently to colonization, the

IJ nematode then serves as a vector, carrying bacteria

into a susceptible insect, in which it is released from

its nematode vector and rapidly kills the insect. The

bacteria are capable of killing insects in the absence of

nematodes.

Haemolymph supports vigorous exponential growth

[26] of bacteria, immediately upon release from the nematode.

By contrast, the nematode vesicle or gut is

comparatively nutrient limiting. The insect carcass thus

provides nutrients for the propagation of both nematodes

and bacteria. In response to a signal, possibly

nutrient deprivation or space limitation, bacteria reassociates

with the nematode, and the pair leaves in

search of a new insect host to repeat the cycle. Nematodes

are easily propagated; several hundred thousand

IJs can be generated from the infection of a single insect

host.

Despite the similarity between the life cycles of

*Photorhabdus* and *Xenorhabdus* bacteria, the molecular

components of the regulatory networks controlling

pathogenicity and mutualism in *Photorhabdus* and

*Xenorhabdus* are different [9], suggesting a convergent

evolution.

Although *X. nematophila* and *Photorhabdus* models

are emerging as an invaluable tool for elucidating microorganism–

host interactions, many studies focused on

the symbiotic association between the bacteria and their

hosts, but they scarcely focused on interactions between

bacteria. We here seek to evaluate the potential for cooperation

among bacteria. We will show that the life cycle

of the bacteria is favorable to bacterial cooperation.

To that aim we will formulate a condition for cooperation

under simple scenarios consistent with knowledge of

nematode-bacterial mutualism. Our analysis uses wellestablished

formalism to break down the condition for

cooperation into the effect of different partners and their

genetic relatedness. We provide new results for relatedness,

derived as a function of demographic parameters,

experimentally accessible. The relatedness in computed

under a bacterial exponential stochastic growth process

in the insect. Although this framework can be adapted

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Figure 1: **The bacteria life cycle.** The infective juvenile nematode containing bacteria (nematode–bacteria

complex) enters a susceptible insect host through natural openings that include the mouth, anus and spiracles.

After entering the insect blood system, the nematode releases the bacteria. The bacteria undergo an exponential

growth until the population reaches approximately 109 bacteria, the growth is followed by a plateau. Together,

the nematode and bacteria overcome insect immunity and kill the insect. The insect cadaver is used as a nutrient

source and is protected from opportunistic infection and scavenging by metabolites produced by bacteria. Within

this environment, nematodes reproduce sexually and progeny develop through four juvenile stages. Some nematodes

develop into infective juveniles after being recolonized by their respective bacteria. The pair then exits the

depleted insect carcass in search of a new host.

to study manifold aspects of inclusive fitness of bacteria,

we have focused on a particular evolutionary strategy.

The bacteria can be found in two distinct phenotypic

variant, whereas each variant exhibits dramatically different

physiologies, required for initiating mutualistic or

pathogenic life-styles [1, 7]. A stochastic promoter inversion

causes the switch between the two variants [26]. We

will formulate conditions for a candidate evolutionarily

stable strategy [17], where the evolutionary strategy under

investigation is the frequency at which the switch

occurs. This study is inspired by the tripartite symbiosis

bacteria-nematode-insect, our aim is also to provide

results more widely applicable. The student derived the

mathematical equations presented in this report, he also

wrote C++ codes to validate the equations.

**Phenotypic variation and inclusive**

**fitness**

Both *Xenorhabdus* and *Photorhabdus* are found in the

insect in two phenotypic variants [1, 7], denoted type

I and II, controlled by a stochastic promoter inversion

switch [26]. Type I are known to be more virulent toward

the insect than type II [29, 8]. Type I are also

known to produce crystal proteins [4, 2] that supposedly

nourish the nematode and support nematode growth

and development [26]. The nematode also feeds off and

exploit the growing bacteria [30]. Furthermore, subsequently

to the post-exponential phase of growth [5], type

I also produces bactericides [13, 10] or fungicides that

eliminate unwanted guests, thus increasing the foraging

of the insect by the nematodes. On the other hand,

type II bacteria are known to better associate with the

nematode [23], by producing proteins involved in recognition

by the nematode [25, 12]. Moreover, studies have

shown that nematodes did not emerge from insect when

infected by type II solely [27, 26], suggesting the type I

are required for nematode growth.

We hereby intend to model this phenomenon, using

simplifying assumptions in order to produce analytic

prediction. We assume that type I does not colonize

any nematodes, but instead increase the number of nematodes

that make it to the next cycle, or equivalently

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Figure 2: **The bacterial fitness along the life cycle.** The life cycle is the same as in figure 1 but we introduce

phenotypic variation, namely type I and II. Type I are more virulent toward the insect, support nematode growth

and development, eliminate unwanted guests but cannot associate with the nematode. On the other hand, type

II only associate to the nematode. The phenotype under investigation, *z* is the probability that a lineage has not

switched to type II by the time of colonization of a nematode by a bacterium. *z*R

0 is the proportion of type I in

the insect, and the survival of nematodes migrating out of the insect is *f*(*z*R

0 ). The average success of nematodes

migrating out of all insects is *f*(¯*z*). The fitness of a focal lineage is (1 − *z*•)*f*(*z*R

0 )[(1 − *z*R

0 )*f*(¯*z*)]−1. We assume all

bacteria switch back from type II to I during the soil dwelling stage.

increase the survival of nematodes in the soil. On the

other hand type II are the ones that can colonize the

nematodes. This phenotype is only expressed after the

exponential growth of bacteria, which holds experimental

grounds [26]. All bacteria switch back from type II

to I during the soil dwelling stage of nematodes.

We seek to model the phenotypic variation as an evolutionary

strategy acting upon fitness. The phenotype

under investigation is not expressed by a single bacteria,

but instead should be seen as a phenotype expressed by

a lineage of bacteria. The resulting outcome on the bacteria

at the end of the lineage is its type (I or II). *z* is the

probability that a lineage has not switched to type II by

the time of colonization of a nematode by a bacterium.

The fitness of a focal lineage is a function of its own

phenotype (*z*•), of the average phenotype of all lineages

of bacteria in the same insect (*z*R

0 ), and the average phenotype

of the total population over all insect hosts (¯*z*).

By definition, *z*R

0 is thus the proportion of type I in the

insect by the time of colonization of nematodes by bacteria,

and 1 − *z*R

0 is the proportion of type II. Similarly,

¯*z* is the proportion of type I over all insects by the time

of colonization of nematodes by bacteria, and 1 − ¯*z* is

the proportion of type II.

Thus for a lineage, the probability that it ends colonizing

a nematode is 1−*z*•, and this lineage is competing

with lineages that colonize nematodes with probability

1 − *z*R

0 . Thus by solely taking into account colonization

of the nematode, the fitness of a focal lineage is

(1 − *z*•)*/*(1 − *z*R

0 )

However, the fitness of a focal lineage depends also

on the survival of the nematodes migrating out the same

insect. While in turn, the survival of the nematode depends

of the average phenotype of the lineages. We define

a function *f*, which is the survival of nematodes as

a function of the proportion of type I. Upon colonization,

the focal lineage is then carried over by nematodes

from the same insect, thus with survival *f*(*z*R

0 ), competing

with nematodes of average survival *f*(¯*z*). Thus by

solely taking into account survival of the nematodes, the

fitness of a focal lineage is *f*(*z*R

0 )*/f*(¯*z*)

Taking into account the effect of both colonization

and survival of the nematode, and under the model of

infinite number of insects, the fitness *w*(*z*•*, z*R

0 *,* ¯*z*) of a

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focal lineage is:

*w*(*z*•*, z*R

0 *,* ¯*z*) = 1 − *z*•

1 − *z*R

0

*f*(*z*R

0 )

*f*(¯*z*) (1)

We assume the success of nematodes *f* is an increasing

linear mapping of the proportion of type I: *f*(*x*) =

*\_*(*x* + ), *\_ >* 0, *\_* \_ 0 thus leading to:

*w*(*z*•*, z*R

0 *,* ¯*z*) = 1 − *z*•

1 − *z*R

0

*z*R

0 +

¯*z* +

(2)

A framework to study evolutionary strategies is to look

upon change in frequency of alleles over a life cycle

[18, 17]. The change in frequency \_*p* of a mutant allele

(frequency *p*) with phenotype *z* + *\_* in a wild-type

population with phenotype *z* is then given for a mutant

with small phenotypic deviation *\_* by Lehmann &

Rousset (2014) [17]:

\_*p* = *p*(1 − *p*)*\_*

\_

*@w*

*@z*•

\_\_\_\_

*z*\_

+ Cov(*z*•*, z*R

0 )

V(¯*z*)

*@w*

*@z*R

0

\_\_\_\_

*z*\_

\_

+ *o*(*\_*)*.*

(3)

The regression coefficient Cov(*z*•*, z*R

0 )

V(¯*z*) is also known as

the relatedness (*R*), introduced initially by Hamilton

[16] and refined subsequently [15]. Statically, the relatedness

is the covariance between the mutant allele

frequency in a focal individual and that in a recipient

relative to the variance in mutant allele frequency in the

population.

A condition for a candidate evolutionarily stable

strategy (*z*\_) is that no deviant mutant should invade

the resident population. At equilibrium, the change of

frequency of the mutant is null (\_*p* = 0) and the equation

(3) leads to:

*@w*

*@z*•

\_\_\_\_

*z*\_

+ *R*

*@w*

*@z*R

0

\_\_\_\_

*z*\_

= 0 (4)

We then need to compute the partial derivative of the

fitness with respect to *z*• and *z*R

0 and evaluate them at

*z*:

*@w*(*z*•*, z*R

0 *,* ¯*z*)

*@z*•

\_\_\_\_

*z*•=*z*R

0 =¯*z*=*z*

= 1

*z* − 1 (5)

*@w*(*z*•*, z*R

0 *,* ¯*z*)

*@z*R

0

\_\_\_\_

*z*•=*z*R

0 =¯*z*=*z*

= 1 +

(1 − *z*)(*z* + ) *.* (6)

Condition (4) then implies:

1

*z*\_ + *R*

1 +

(1 − *z*\_)(*z*\_ + ) = 0 (7)

)

*z*\_ = *R*(1 + )*,* (8)

In the special case = 0 the phenotype is equal to the

relatedness:

*z*\_ = *R.* (9)

It is worth noting that if *R <*

+ 1, then the candidate

evolutionarily stable strategy for a lineage is to switch

necessarily to type II by the time of infection (*z*\_ = 1).

The next section is dedicated to derive the relatedness

*R* under various models of migration and infection.

**Relatedness as a function of demographic**

**and migration parameters**

We seek to derive relatedness as a function of demographic

and migration parameters. The framework is

largely inspired from Rousset [21]. We developed two

models. In the first model, the insect is infected by nematodes

arriving simultaneously. Moreover, the migration

of nematodes is correlated, meaning the nematodes

disperse in an aggregated pattern [22]. In the second

model, the insect is infected by nematodes arriving consecutively,

but the migration is uncorrelated, meaning

dispersal resulted in a random or uniform distribution.

**Simultaneous infections, correlated migration**

The relatedness (*R*) is a regression coefficient, but *R*

can also be computed in terms of probability of identity

[21]. Let *Qi* be the probability of identity of genes from

different bacteria, within the same insect (*Q*w) and in

different insect (*Q*b). Here we consider the probability of

identity in the infinite allele model so that any mutation

would lead to a different allele. Let *μ* be the mutation

rate. The regression coefficient *R* can be computed as

[21]:

*R* = lim

*μ*!0

*Q*w − *Q*b

1 − *Q*b

(40)

The *Q*w and *Q*b are obtained by solving recursions describing

their change over successive life cycle, where

parameters of these equations are relevant demographic

and migration parameters (see Box 1). Thus the relatedness

can be expressed as:

*R* '

1 − *\_*(1 − ) + 1*/n*I

*.* (41)

*n*I denotes the total number of insects. *\_* is the probability

that two infecting nematodes come from the same

insect in the previous cycle. *\_* describes a correlated

migration, nematodes preferentially follow other nematodes

migrating out of the same insect. is the probability

that two randomly chosen bacteria in the same

insect originate from the same nematode. Under the

assumption of a large number of insects (*n*I \_ 1), the

relatedness *R* is:

*R* '

1 − *\_*(1 − ) (42)

5

**Box 1: Proof of the equation** (41)

Let *Qi* be the probability of identity (PI) of genes

from different bacteria. Within the same insect (*Q*w)

and in different insect (*Q*b). *\_* is the probability that

two nematode infecting the same insect come from the

same insect. *\_* is the correlation coefficient of migration.

During the soil dwelling stage of nematodes and

upon infection of the same insect, the PI within the

same nematode is *Q*1 and the PI in different nematodes

is *Q*2. is the probability that two randomly

chosen bacteria in the same insect originate from the

same nematode, whenever nematodes arrive consecutively

() or simultaneously (). We write the next

generation identities *Q*0

w and *Q*0

b as.

8>>>>><

>>>>>:

*Q*1 = 1 (10a)

*Q*2 = *\_Q*w + (1 − *\_*)*Q*b (10b)

*Q*0

w =  *Q*1 + (1 − )*Q*2 (10c)

*Q*0

b = *Q*w

*n*I

+ *n*I − 1

*n*I

*Q*b (10d)

• Upon infecting the same insect, bacteria in the

same nematode (10a) are all clonal, originating

from one bacterium that colonize the nematode.

• Upon infecting the same insect, bacteria in different

nematodes (10b) come from the same insect

with probability *\_* and thus have PI *Q*w, or

come from different insect with probability 1−*\_*

and thus have PI *Q*b.

• In the same insect (10c), bacteria come from the

same nematodes with probability and thus

have PI *Q*1, or come from different nematodes

with probability 1 − and thus have PI *Q*2.

• In different insect (10d), bacteria come from the

same insect with probability 1*/n*I and thus have

PI *Q*w, or come from different insects with probability

1 − 1*/n*I and thus have PI *Q*b.

Putting together all the parts, in the infinite allele

model with mutation rate *μ*, the recursion equations

for *Q*0

w and *Q*0

b are thus:

8<

:

*Q*0

w = (1 − *μ*)2[+ (1 − )(*\_Q*w + (1 − *\_*)*Q*b](11a)

*Q*0

b = (1 − *μ*)2

\_

*Q*w

*n*I

+ *n*I − 1

*n*I

*Q*b

\_

*.* (11b)

At equilibrium, *Q*0

w = *Q*w and *Q*0

b = *Q*b and the recursion

equations (11) can be solved explicitly and we

have:

*Q*w − *Q*b

1 − *Q*b

= (1 − *μ*)2

1 − (1 − *μ*)2*\_*(1 − ) + (1 − *μ*)2*/n*I

*.*

(12)

And thus by taking the limit for low mutation rate

(*u* ! 0), the relatedness is [21]:

*R* = lim

*μ*!0

*Q*w − *Q*b

1 − *Q*b

(13)

=

1 − *\_*(1 − ) + 1*/n*I

*.* (14)

Moreover can be derived explicitly (see Box 2) under

assumption of a simultaneous infection by the nematodes.

The insect is infected by *d* nematodes initially.

Each nematode *i* (1 \_ *i* \_ *d*) releases *ki* bacteria inside

the insect. Then all *d* lineages of bacteria enter a

stochastic exponential growth process (at rate *\_*) simultaneously.

For a large number of bacteria carried by the

nematode (*ki* \_ 1), we have proved (see Box 2) that

the stochastic part of the growth process is negligible.

Thus is nearly independent of the time at which the

growth is stopped in first approximation. Moreover, is

nearly independent of *\_*, and solely depends on the number

of nematodes infecting the insect and their carrying

capacities:

'

X*d*

*i*=1

*ki* P*d*

*i*=1 *ki*

!2

*.* (43)

If each nematode carries the same number of bacteria,

reduces to:

'

1

*d*

(44)

The relatedness finally simplifies:

*R* '

1

*d* − *\_*(*d* − 1) (45)

If either the number of nematodes infecting the insect

(*d*) is small or *\_* close to one, the relatedness is close to

one (see figure 3).

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**Box 2. Proof of the equation** (43) An insect is

infected by *d* nematodes. Each nematode *i* (1 \_ *i d*)

releases his bacteria inside the insect. The *d* colonies

of bacteria enter a stochastic growth process at time

*t* = 0. In this context, *Ni*(*t*) denotes the discrete random

variable describing the population size of bacteria

originating from the *i*th nematode, at time *t*.

*N*+(*t*) = P*d*

*i*=0 *Ni*(*t*) denotes the discrete random

variable describing the size of the whole population of

bacteria at time *t*. The random variable *Zi* describes

the frequency that two randomly chosen bacteria at

time *t* are originating from the *i*th nematode. By definition

we have:

*Zi* = *Ni*(*t*)(*Ni*(*t*) − 1)

*N*+(*t*)(*N*+(*t*) − 1) *.* (15)

The expectation of the sum of *Zi* over all nematodes

is thus (*t*), the probability that two randomly chosen

bacteria originate from the same nematode.

(*t*) = E

"

X*d*

*i*=1

*Zi*(*t*)

#

=

X*d*

*i*=1

E[*Zi*(*t*)] (16)

Thus we need to evaluate E[*Zi*(*t*)]. The growth process

is modeled as a discrete space, continuous time

Markov process. Each bacterium has an constant division

rate *\_* and they never die. Thus all bacteria is

independent of one another. This model depicts an

exponential growth. *ki* denotes the number of bacteria

initially contained in the *i*th nematode. The random

variable *Xi*(*t*) = *Ni*(*t*)−*ki* is thus the number of

births in the *i*th lineage. Under our model, *Xi*(*t*) is a

negative binomial[6, p. 158]:

*Xi*(*t*) \_ NB

􀀀

*ki, e*−*\_t*\_

() (17)

P(*Xi*(*t*) = *x*) =

\_

*x* + *ki* − 1

*x*

\_􀀀

*e*−*\_t*\_*ki* 􀀀

1 − *e*−*\_t*\_*x*

*.*

(18)

Consistently with previous notations, *k*+ = P*d*

*i*=0 *ki* is

the total number of bacteria released initially by the

*d* nematodes. Equivalently *X*+(*t*) = *N*+(*t*) − *k*+ is

the total number of bacteria born. Since all *Xi*(*t*) are

independent of one another, *X*+(*t*) is also a negative

binomial [14]:

*X*+(*t*) \_ NB

􀀀

*k*+*, e*−*\_t*\_

*.* (19)

Basic calculus leads to the distribution of *Xi*(*t*) conditional

on *X*+(*t*) = *x*+; this is a negative hypergeometric

and independent of *e*−*\_t*:

P(*Xi*(*t*) = *x*|*X*+(*t*) = *x*+)

=

\_

*x* + *ki* − 1

*x*

\_\_

*x*+ − *x* + *k*+ − *ki* − 1

*x*+ − *x*

\_

\_

*x*+ + *k*+ − 1

*x*+

\_ *.*

(20)

Leading to expectation for *Xi*(*t*)(*Xi*(*t*) − 1) conditional

on *X*+(*t*):

E[*Xi*(*t*)(*Xi*(*t*) − 1)|*X*+(*t*)]

= *ki*(*ki* + 1)

*k*+(*k*+ + 1)*X*+(*t*)(*X*+(*t*) − 1)*.* (21)

And the expectation of *Ni*(*t*)(*Ni*(*t*) − 1) conditional

on *N*+(*t*) is:

E[*Ni*(*t*)(*Ni*(*t*) − 1)|*N*+(*t*)]

= *ki*(*ki* + 1)

*k*+(*k*+ + 1)*N*+(*t*)(*N*+(*t*) − 1) −

2*ki*(*k*+ − *ki*)

*k*+(*k*+ + 1) *N*+(*t*)*.*

(22)

Leading in turn to the expectation of *Zi*(*t*) conditional

on *N*+(*t*).

E[*Zi*(*t*)|*N*+(*t*)]

= *ki*(*ki* + 1)

*k*+(*k*+ + 1) −

2*ki*(*k*+ − *ki*)

*k*+(*k*+ + 1)

1

*N*+(*t*) − 1*.*

(23)

By the law of conditional expectation:

E [*Zi*(*t*)] = E[E [*Zi*(*t*)|*N*+(*t*)]] (24)

= *ki*(*ki* + 1)

*k*+(*k*+ + 1) −

2*ki*(*k*+ − *ki*)

*k*+(*k*+ + 1) E

\_ 1

*N*+(*t*) − 1

\_

(25)

= *ki*(*ki* + 1)

*k*+(*k*+ + 1) − 2*e*−*\_t ki*(*k*+ − *ki*)

*k*+(*k*2+

− 1) *.* (26)

By summing E[*Zi*(*t*)] over all *i*, we evaluate the probability

of identity (*t*):

(*t*) = *k*+ +P*d*

*i*=1 *k*2

*i*

*k*+(*k*+ + 1) − 2*e*−*\_t k*2+

−

P*d*

*i*=1 *k*2

*i*

*k*+(*k*2+

− 1) *.* (27)

Under the assumption that the number of bacteria

carried by each nematode is large enough (*ki* \_ 1),

(*t*) becomes independent of *t* and does not depend

anymore on the bacteria growth rate:

'

X*d*

*i*=1

*ki* P*d*

*i*=1 *ki*

!2

*.* (28)

This approximation can be understood intuitively.

If the initial number of bacteria if large enough,

the probability that two randomly chosen bacteria

originate from the same nematode is independent of

time, and equals the sum over all nematodes of their

squared initial proportions.

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0 0*.*2 0*.*4 0*.*6 0*.*8 1

0*.*2

0*.*4

0*.*6

0*.*8

1

*d* = 20

*d* = 5

*d* = 3

*d* = 2

*d* = 1

Correlated migration coefficient (*\_*)

Relatedness (*R*)

0 1 2 3 4 5

0*.*2

0*.*4

0*.*6

0*.*8

1

*d* = 2

*d* = 3

*d* = 4

Arrival rate relative to growth rate (*!*)

Relatedness (*R*)

Average value for d=4

Average value for d=3

95% confidence bounds

1 − 2*!*B1*/*2(1 + *!,* 1 − *!*)

Figure 3: **Relatedness as a function of demographic parameters. Left panel:** Model of simultaneous

infection. Plot of the relatedness against *\_* (45) for several values of *d* ranging from 1 to 20. *\_* is the probability

that two infecting nematodes come from the same insect. *d* is the number of nematodes infecting the insect. If

either the number of nematode is smalls or *\_* close to one, the relatedness is close to one. **Right panel:** The

nematodes arrive consecutively, the time between two arrivals is Exp(*\_* ). The bacteria growth is exponential, with

divisions at rate *\_*. Estimated relatedness for 3 and 4 of nematodes (blue and purple solid lines) are plotted against

the parameter *!* = *\_\_*−1. For the case *d* = 2, and under the assumption of a deterministic growth of bacteria,

the relatedness is analytically derived (black solid line). Red solid lines are the confidence bounds estimated from

1000 replicate simulations of growth within an insect. If either the number of nematode is small or the arrival

rate of nematodes is small or the growth rate of bacteria is large, the relatedness is close to one.

**Consecutive infections, uncorrelated migration**

The assumption of consecutive infections mainly affects

the computation of . The analysis of this scenario is

otherwise similar to the previous one. R shall be given

by equation (41). However, differs from the previous

case. is the probability that two randomly chosen

bacteria in the same insect originate from the same nematode,

whenever nematodes arrive consecutively. In

the case of uncorrelated migration, *\_* = 1*/n*I. Thus we

have:

*R* '

1 +

*n*I

*.* (46)

Under the assumption of an important number of insects

(*n*I \_ 1), the relatedness is:

*R* ' (47)

We seek to derive under assumption of a consecutive

infections by the nematodes. The insect is infected

by nematodes arriving successively. We assume

that the time elapsed between two nematode arrivals

is exponentially distributed, with parameter *\_* .

*\_* is a rate measured per unit of time. We assume

a stochastic growth process for the bacteria, the division

rate is *\_*, *\_* is also measured per unit of time.

We could not derive an analytic formula of for this

general case, instead we estimated it by simulating

this process. The results of these estimates are depicted

in figure 3. the C++ code is publicly available

at https://github.com/ThibaultLatrille/ISEM\_M1\_

cpp\_simulation. An analytical approximation in terms

of the parameter *!* = *\_\_*−1 can be derived in the case

*d* = 2 (Box 3), is a decreasing function of *!*:

' 1 − 2*!*B1*/*2(1 + *!,* 1 − *!*)*,* (48)

where B*x*(*a, b*) is the incomplete beta function:

B*x*(*a, b*) =

Z *x*

0

*ta*−1(1 − *t*)*b*−1d*t.* (49)

This approximation, derived under the assumption that

bacterial growth is deterministic matches well the simulation

results (Figure 3).

The relatedness increases with decreasing number of

infecting nematodes, as in the previous model. The relatedness

also increases with decreasing arrival rate of

nematodes or equivalently with increasing growth rate

of bacteria. Increasing growth rate or decreasing arrival

rate leads to a population dominated by bacteria from

the first nematode arrived.

The previous models could be combined to take into

account both consecutive infections and correlated dispersal.

Nematodes move in aggregate, the aggregates

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**Box 3. Proof of the formula** (48)

We derive an analytic formula for for two nematodes

arriving successively. We assume the first nematode

arrive at time *T* before the second (and last) nematode,

where *T* \_ exp(*\_* ). Each nematode carries the

same number of bacteria, *k* \_ 1. The bacteria carried

by the nematodes grew in a deterministic fashion

at rate *\_*. Under all these assumptions, the number

of bacteria in the insect when the second nematode

arrives is the random variable *ke\_T* +*k*. Where *ke\_T*

bacteria are from the first one arrived and *k* are from

the second one.

Since *k* \_ 1, the random variable *ke\_T* is always far

greater than 1. Thus we can make use of the equation

(43) to derive :

= E

"\_

*ke\_T*

*k* + *ke\_T*

\_2

+

\_

*k*

*k* + *ke\_T*

\_2#

(29)

= E

"\_

*e\_T*

1 + *e\_T*

\_2

+

\_ 1

1 + *e\_T*

\_2#

(30)

=

Z 1

0

\_

*e\_t*

1 + *e\_t*

\_2

+

\_ 1

1 + *e\_t*

\_2!

*\_e*−*\_t*d*t.*

(31)

The evaluation of this integral is not straightforward.

Instead we derive the cumulative distribution function

*F*1(*p*) and *F*2(*p*) of *e\_T*

1 + *e\_T* and 1

1 + *e\_T* respectively.

*F*1(*p*) = P

\_

*e\_T*

1 + *e\_T*

\_ *p*

\_

= P

\_

*T* \_ *\_*−1ln

\_

*p*

1 − *p*

\_\_

= 1 − (1 − *p*)*!p*−*!* for 1*/*2 \_ *p* \_ 1*,* (32)

where *!* = *\_/\_*.

*F*2(*p*) = P

\_ 1

1 + *e\_T*

\_ *p*

\_

= P

\_

*T* \_ *\_*−1ln

\_1 − *p*

*p*

\_\_

= (1 − *p*)−*!p!* for 0 \_ *p* \_ 1*/*2*.* (33)

Hence the the probability density function *f*1(*p*) and

*f*2(*p*) are:

*f*1(*p*) = d*F*1(*p*)

d*p*

= *!*(1 − *p*)*!*−1*p*−*!*−1 for 1*/*2 \_ *p* \_ 1*,*

(34)

*f*2(*p*) = d*F*2(*p*)

d*p*

= *!*(1 − *p*)−*!*−1*p!*−1 for 0 \_ *p* \_ 1*/*2*.*

(35)

And

=

Z 1

1*/*2

*p*2*f*1(*p*)d*p* +

Z 1*/*2

0

*p*2*f*2(*p*)d*p* (36)

= 1 − 2*!*

Z 1*/*2

0

(1 − *x*)−*!x!*d*x* (37)

= 1 − 2*!*B1*/*2(1 + *!,* 1 − *!*)*,* (38)

where B*x*(*a, b*) is the incomplete beta function:

B*x*(*a, b*) =

Z *x*

0

*ta*−1(1 − *t*)*b*−1d*t.* (39)

Thus the probability of identity is solely dependent

on one parameter, *!*, the ratio between arrival rate of

nematodes and growth rate of bacteria.

arrive consecutively. The number of nematodes per aggregate

is *d*. In an aggregate, *phi* is the probability that

two nematodes come from the same insect. is the

probability that two randomly chosen bacteria in the

same insect originate from the same aggregate. Thus

shall be computed in terms of the number of aggregate,

the time elapsed between two aggregate arrivals and the

bacterial growth rate. Using a similar framework to derive

*R*, we have:

*R* =

*d*(1 − *\_* ) + *\_*

(50)

**Discussion**

The parasitic life cycle of *Xenorhabdus* and *Photorhabdus*

has an influence on their relatedness, and thus on

the potential for cooperation among bacteria. Under

simplifying assumptions, we derive the candidate evolutionarily

stable strategy for phenotypic variation probability

switch. This strategy is derived as a function of

relatedness. The relatedness is subsequently derived as

a function of a few demographic parameters, experimentally

accessible.

Our model of simultaneous infection can be compared

to outcomes of evolutionary experiments [28]. Indeed,

there is no experimental barrier to manipulate parameters

such as *d* or *\_* in the laboratory, thus the relatedness

can be controlled and manipulated [28]. However,

estimation of these parameters in the field seems

complicated under this model of migration. In the case

of consecutive infection, if the division rate (*\_*) of bacteria

can be hardly controlled but can be measured, the infection

rate by nematodes (*\_* ) can be controlled in laboratory

experiments. The number of nematodes infecting

the insects can also be controlled. Moreover, estimating

the arrival rate of nematodes is accessible in field experiments,

assuming one can lure nematodes to a trap containing

the insect and measure the arrival times. Thus

all demographic parameters can be estimated. More-

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over, the relatedness can be estimated independently by

various methods [20], and the results can be confronted

to the prediction of demographic model to assess the

validity of our model.

We focused on phenotypic variation since it holds

experimental grounds, but experiments show contradictory

results on many aspects [26]. It is still unknown

whether type I also colonize the nematode and to what

extend which type contributes to the survival of nematodes

[30]. We investigated a plausible simple scenario

in order to give insight on the relation between phenotypic

traits and relatedness.

Our model might not describe known and future

results, but the framework can be incremented and

adapted easily to take into account for these results. The

fitness function can be adapted to study other aspects of

helping behaviors, not solely phenotypic variation. By

taking into account cooperation at the bacterial level,

we hope this work will serve and drive new experiments

on the tripartite system bacteria-nematode-insect.

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