

Quantitative steps in symbiogenesis and the evolution of homeostasis

S. A. L. M. KOOIJMAN¹, P. AUGER², J. C. POGGIALE³ and B. W. KOOIJ¹

¹ *Department of Theoretical Biology, Institute of Ecological Science, Vrije Universiteit, de Boelelaan 1087, 1081 HV Amsterdam, The Netherlands*
(e-mail: bas@bio.vu.nl; kooij@bio.vu.nl)

² *Université Claude Bernard, Lyon 1, UMR CNRS 5558, 43 Bd du 11 Novembre 1989, 69622 Villeurbanne Cedex, France*
(e-mail: pauger@biomserv.univ-lyon1.fr)

³ *Centre D'Océanologie de Marseilles (O.S.U.) U.R.A. 41 Campus de Luminy Case 901, 13288 Marseille Cedex 9, France*
(e-mail: poggiale@com.univ-mrs.fr)

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ABSTRACT

The merging of two independent populations of heterotrophs and autotrophs into a single population of mixotrophs has occurred frequently in evolutionary history. It is an example of a wide class of related phenomena, known as symbiogenesis. The physiological basis is almost always (reciprocal) syntrophy, where each species uses the products of the other species. Symbiogenesis can repeat itself after specialization on particular assimilatory substrates. We discuss quantitative aspects and delineate eight steps from two free-living interacting populations to a single fully integrated endosymbiotic one. The whole process of gradual interlocking of the two populations could be mimicked by incremental changes of particular parameter values. The role of products gradually changes from an ecological to a physiological one. We found conditions where the free-living, epibiotic and endobiotic populations of symbionts can co-exist, as well as conditions where the endobiotic symbionts outcompete other symbionts. Our population dynamical analyses give new insights into the evolution of cellular homeostasis. We show how structural biomass with a constant chemical composition can evolve in a chemically varying environment if the parameters for the formation of products satisfy simple constraints. No additional regulation mechanisms are required for homeostasis within the context of the dynamic energy budget (DEB) theory for the uptake and use of substrates by organisms. The DEB model appears to be closed under endosymbiosis. This means that when each free-living partner follows DEB rules for substrate uptake and use, and they become engaged in an endosymbiotic relationship, a gradual transition to a single fully integrated system is possible that again follows DEB rules for substrate uptake and use.

Key words: symbiogenesis, endosymbionts, organelles, mitochondria, plastids, synthesizing units, substitutable and supplementary compounds, syntrophy, mixotrophy, dynamic energy budget, reserves, weak and strong homeostasis.

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I. INTRODUCTION

The literature on population dynamics has a strong focus on competition and predation, but much less so on mutualism. This is remarkable, because collaboration in the form of symbioses based on reciprocal syntrophy is basic to biodiversity, and probably to the existence of life (Reisser, Meier & Kurmeier, 1983; Ahmadjian & Paracer, 1986; Smith & Douglas, 1987; Douglas, 1994). The merging of two independent populations of heterotrophs and autotrophs into a single population of mixotrophs occurred frequently in evolutionary history (Reisser, 1992; Thingstad *et al.*, 1996). This process is known as symbiogenesis (Margulis, 1993). Recent molecular evidence has revealed the importance of symbiogenesis to evolution and the coexistence of organisms (Delwiche, 1999; Douglas, 1998). Originally free-living cyanobacteria became integrated into chlorophytes (including plants), rhodophytes and glaucocystophytes; rhodophytes became integrated into heterokontophytes, haptophytes and cryptophytes, while chlorophytes became integrated into euglenophytes and chlorarachniophytes; heterokontophytes and haptophytes became integrated into dinoflagellates, which themselves (especially *Gymnodinium adriaticum*) engaged into endosymbiotic relationships with animals (corals, other cnidarians and molluscs). Alveolates, to which dinoflagellates, ciliates and sporozoans belong, generally specialized in kleptoplastides (i.e. functional plastids that are acquired by feeding). Apart

from dinoflagellates, cryptophytes (especially *Chrysi-diella*) and diatoms engaged in endosymbiotic relationships with radiolarians and foraminiferans, and chlorophytes did so with animals (sponges, *Hydra*, rotifers and *Platyhelminthes*). Intracellular chloroplast populations seem to behave more dynamically in kleptomantic and endosymbiotic relationships (Stoecker & Silver, 1990), compared to fully integrated systems. The coupling of the dynamics of the subsystems can be tight as well as less tight.

Endosymbiotic relationships are not always stable on an evolutionary time scale. All of the algal groups have colourless representatives, which imply that they are heterotrophic. Many species with chloroplasts are known to have heterotrophic abilities as well, which classify them as mixotrophs. Strict photoautotrophy is probably rare, and mixotrophy the rule. Half of the species of dinoflagellates, for instance, do not have chloroplasts, probably due to evolutionary loss (Cavalier-Smith, 1998). The integration is a step-wise process, where plastids' genome size is reduced by gene loss, substitution and transfer to the hosts' genome, possibly to economize metabolism (Cavalier-Smith, 1987). Chloroplasts of chlorophytes have a typical genome size of 100 genes, but the genome sizes of chloroplasts of rhodophytes and glaucocystophytes are substantially larger. The chloroplasts of cryptophytes and chlorarachniophytes still contain a nucleomorph with some chromosomes (Maier, Douglas & Cavalier-Smith, 2000), believed to be derived from their earlier

rhodophyte and chlorophyte hosts. The endosymbionts of radiolarians, foraminiferans and animals maintained their full genome. The tightness of the integration is, therefore, reflected at the genome level.

Chloroplast evolution and transmission is just one example of symbiogenesis. Mitochondria which crawl around in eukaryotic cells (Bereiter-Hahn, 1990) became endosymbionts long before (Margulis, 1970; Taylor, 1974) or simultaneous with (Cavalier-Smith, 1987) the plastids; no eukaryotes with chloroplasts are known that lack mitochondria. Mitochondria evolved from once free-living relatives of the typhus bacterium *Rickettsia* (Anderson *et al.*, 1998), the α -group of purple bacteria. This evolutionary origin illustrates the narrow borderline between parasitic and symbiotic relationships. The shape of the cristae of mitochondria is nowadays an important criterion in tracing the evolutionary relationships among protists (Patterson, 1999), which points to a slow intracellular evolution. Hydrogenosomes are generally thought to have evolved from mitochondria in anaerobic or micro-aerobic environments. As testified by the presence of mitochondrion-derived genes, some parasitic or commensal groups (entamoebidae, microsporidia, diplomonads, parabasalids, some rumen ciliates, several groups of ciliates) lost their mitochondria (Roger, 1999). Such genes have not been found (yet) in the amitochondriate oxymonads, retortamonads, *Postgaardi* and *Psalteriomonas*. The absence of mitochondria is perhaps primitive in the pelobionts and the free-living *Trimastix* (Patterson, 1999). Other organelles, such as peroxisomes and glyoxisomes, probably also have endosymbiotic origins (Cavalier-Smith, 1987; Katz, 1999); they follow a growth and fission pattern that is only loosely coupled to the cell cycle. Organelles such as centrioles, undulipodia, and the nuclear membrane, possibly have an endosymbiotic origin (Margulis, 1993), but others oppose this point of view.

It is tempting to think of eukaryotes as chimaeras of prokaryotes as proposed in the Serial Endosymbiosis Theory (Margulis, 1970). The absence of phagocytosis in prokaryotes, however, has been used as argument in favour of an independent origin of prokaryotes and eukaryotes (Cavalier-Smith, 1987; Woese, 1981). The predatory bacterium *Daptobacter* can penetrate the cytoplasm of its bacterial prey *Chromatium*. This may be analogous to early events in the symbiotic acquisition of cell organelles (Guerrero, 1991). The existence of host membranes around organelles seems to be inconsistent with this interpretation. However, the outer membrane of the double membrane around mitochondria might be of negibacterial origin (Cavalier-Smith, 1987). If so, the problem arises if the third phagocytotic

host membrane was lost or never existed. Similarities in the DNA and RNA code and in the whole biochemical and metabolic organization of prokaryotes and eukaryotes suggest an evolutionary link. While the symbiotic origins of mitochondria and plastids in eukaryotes are nowadays generally accepted, the deep roots of eukaryotes remain elusive. We probably have to wait for further research on eukaryotes where the absence of mitochondria is primitive. Arguments exist for the existence of mitobionts in the remote past (Mikelsaar, 1987), from which mitochondria and prokaryotes developed; the mitobionts differentiated before they associated with various groups of eukaryotes.

Generally little is known about the population dynamics of intracellular organelles. Mitochondria constitute some 20 % of the volume of mammalian cells, but this varies per tissue and individuals' condition. Their number per cell varies between 1000 and 1600 in human liver cells, 500 and 750 in rat myoblasts, some 80 in rabbit peritoneal macrophages, and 1 in mammalian sperm cells (Bereiter-Hahn, 1990). In yeast and many unicellular algae, a single giant mitochondrion alternates cyclically with numerous small mitochondria. In the case of a single mitochondrion, the growth and division of the mitochondrion must be tightly linked to that of the cell. Mitochondria can fuse, resulting in a smaller number of larger mitochondria, and can even form a network, as observed in gametes of the green alga *Chlamydomonas*, for instance. Chloroplasts can transform reversibly into non-green plastids (proplastids, etioplastids and storage plastids) with other cellular functions. The evolution of organelles strongly suggests an increasingly strong coupling between species that were once more independent. This places eukaryotic cellular physiology firmly in an ecological perspective, and motivates the application of ecological methods to sub-cellular regulation problems. This mutually dependent dynamics is the focus of the present review, and includes that of intracellular parasites.

The physiological basis of endosymbiosis is probably always reciprocal syntrophy, where each species uses the products of the other species. The classic example is a heterotroph-alga/chloroplast symbiosis. The heterotroph feeds on nitrogen-containing organic sources (such as animal prey by corals) and produces ammonia as a waste product; the alga or chloroplast requires light and carbon dioxide and produces carbohydrates as a waste product (especially if its growth is nutrient limited) (Muscatine, 1977; Muscatine, Falkowski & Dubinsky, 1983; Muscatine *et al.*, 1984); ammonia-carbohydrate exchange forms the basis of the symbiosis. Mitochondria receive pyruvate, FAD, GDP, P,

and NAD from the cytoplasm, and return FADH, GTP, NADH and intermediary metabolites from the Krebs cycle. One-way syntrophy, where one species uses the product of the other, but not *vice versa*, is here treated as a special case of reciprocal syntrophy.

This focus on metabolic aspects of symbioses is not meant to imply that other aspects are unimportant. An intriguing example is the α proteobacterial symbiont *Wolbachia* of *Ecdysozoa* (including arthropods and nematodes), which affects sex determination (Werren, 2000; Charlat & Mercot, 2001; Jiggins *et al.*, 2001; Moret, Juchault & Rigaud, 2001). Other well-known examples are animal symbionts of plants, which promote pollination and seed dispersal. Symbioses on the basis of a mixture of syntrophic and non-metabolic relationships also exist: the Latin-American tree *Cecropia* stores glycogen in specialized plastids in tissue, which is eaten by ants that furiously attack anything that touches the tree.

We here discuss the quantitative aspects of an increasingly tight coupling between two interacting free-living populations. We show quantitatively how a two-species system can reduce its degrees of freedom step-wise on an incremental basis, and eventually become fully interlocked. We will focus on the implications of metabolic interactions and refrain from discussions of mutualistic aspects, i.e. whether or not a particular interaction is 'beneficial' for both species. The judgement beneficial involves the identification of an explicit optimization criterion. Apart from the problem that such a criterion, which usually directly or indirectly relates to some fitness measure, cannot be defined uniquely in a useful way (Metz, Nisbet & Geritz, 1992), nature is full of examples that cross the boundary from beneficial to adverse. The oxpecker *Buphagus*, for instance, feeds on insects that are attracted to wounds on giraffes, antelopes and other bovids. The removal of insects stimulates the healing of the wound and reduces the risk of infections and of attack by fly-borne parasites. At first sight this is a classic example of a mutualistic relationship. However, oxpeckers also try to keep wounds attractive to insects by using their beaks to keep them open.

Two very different time scales are involved in our discussion of the process of coupling between partners: the population dynamical time scale of several generations, and the evolutionary time scale of multi-million years. The fast process will be followed through changes of state variables (amounts of biomass of the partners), the slow process through changes of parameter values. The evolutionary pattern can only be tested experimentally in an indirect way. We will discuss the pattern that, we hypothesize, leads to full coupling

using examples of present-day metabolic relationships. We are aware that this approach inherently involves an anecdotal element in our reasoning. Our hypothesis that strong coupling evolved from weak coupling is not meant to imply that any particular weak coupling is in the process of evolution towards strong coupling.

II. QUANTITATIVE FRAMEWORK

Before we start the discussion of steps that lead to endosymbiosis, we sketch the general quantitative framework of the Dynamic Energy Budget (DEB) theory (Kooijman, 2000, 2001; Nisbet *et al.*, 2000), which we will use to understand the steps quantitatively. This theory partitions biomass into reserve (a set of compounds of a more temporary nature) and structure (a set of compounds with a more permanent nature). Variations in the ratio of reserve and structure, called the reserve density, allow for variation in the chemical composition of biomass; the chemical composition of both reserve and structure is assumed to be fixed, a property called strong homeostasis and applies to reserve and structure separately. So, reserve and structure are both treated as generalized compounds: mixtures of chemical compounds (carbohydrates, lipids, proteins, etc.) that can vary in amounts, but not in composition. The basic DEB formulation delineates three life stages: embryos (which do not feed), juveniles (which do not reproduce) and adults. Unicellular organisms that propagate by division are conceived as juveniles because they feed and do not reproduce.

Substrate uptake (in juveniles and adults) is proportional to surface area; we here assume that surface area is (approximately) proportional to the structure's volume, this is generally not true, but gives a good simple approximation for unicellular organisms that divide into two parts, i.e. their range of volumes covers a factor of two. Substrate use for maintenance is proportional to structural mass. Substrates are converted into intracellular reserve. Reserves are used at a rate that is a first-order process on the basis of reserve density, for the purposes of maintenance and growth.

The significance of the reserve is to allow for changes in the chemical composition of biomass and for a rich dynamic repertoire of product formation, which plays a key role in symbiogenesis as we will argue. In the case of a single substrate, product formation can be written as a weighted sum of three basic fluxes (Kooijman, Kooi & Hallam, 1999): assimilation, maintenance and growth; faeces is an example of a product

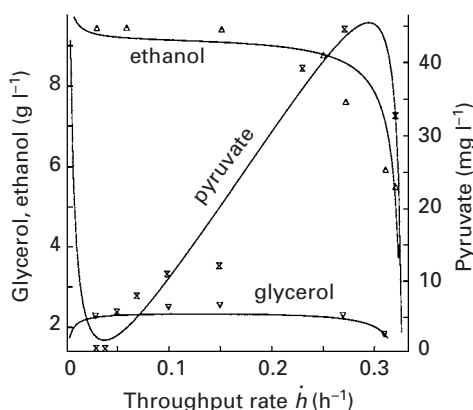


Fig. 1. Steady-state densities of products (ethanol, Δ , glycerol, ∇ , pyruvate, $\otimes$) excreted by the glucose-fermenting yeast *Saccharomyces cerevisiae* as functions of the throughput rate of a chemostat; the throughput rate equals the specific growth rate at steady-state. Data from Schatzmann (1975). The curves are based on expectations of the dynamic energy budget model (Hanegraaf, 1997). Assimilation, maintenance and growth all contribute to product formation, but pyruvate is consumed, rather than produced in association with growth. This explains the deviating shape of curve for pyruvate concentrations.

associated with assimilation only, carbon dioxide and ammonia are examples of products with contributions from all three fluxes. Fig. 1 illustrates product formation as a weighted sum of three basic fluxes. Without the reserve, we would have had just two degrees of freedom rather than three, since the third flux would follow from the mass balance equation. This reduced repertoire is known as the Leudeking–Pirt kinetics for product formation (Leudeking & Pirt, 1959), and is linked to the Marr–Pirt model (Marr, Painter & Nilson, 1969; Pirt, 1975) for uptake and growth. The Leudeking–Pirt model for product formation cannot fit the data presented in Fig. 1; in other words, we need reserves to understand product formation. If we also refrain from the introduction of maintenance, we are left with a single degree of freedom for product formation, which is linked to Monod’s model (Monod, 1942). The Marr–Pirt and Monod models are indeed special cases of the DEB model, as is Droop’s model (Droop, 1983) (which has a reserve, but no maintenance. Droop actually modelled (nutrient) reserve (which equals Droop’s cell quota minus subsistence quota) as a function of growth rate at steady-state; the DEB model can be considered as a dynamic reconstruction of Droop’s model).

The reserve dynamics follows from two important assumptions (Kooijman, 2000): biomass is weakly homeostatic (i.e. its composition remains constant

during growth at constant environmental conditions), and the reserve density dynamics is partitionable (i.e. the reserve can be partitioned into two chemical classes, such as lipids and non-lipids, with identical dynamics, without affecting reserve dynamics). A fixed fraction of the utilized reserve is allocated to somatic maintenance plus growth, the remaining fraction to maturity maintenance plus maturation in embryos and juveniles or reproduction in adults.

For simplicity, we start by assuming that one species lives on a substrate that is present in the environment, and forms a product that the second species can use. The second species lives on another substrate, and forms a product that the first species can use. So each species, therefore, lives on two compounds: one substrate and one product. These compounds can be substitutable, supplementary or a mixture of both. We implicitly assume that all other nutrients that both species might need are not growth-limiting.

The interaction between substrate and product in the assimilation is described by an elaboration of the theory on synthesizing units (Kooijman, 1998, 2000; Muller *et al.*, 2001; Brandt, 2002). This theory is based on classic enzyme kinetics with two modifications: the dissociation rate between enzyme and substrate is taken to be negligibly small, and the production rates are taken to be a function of the arrival rates of substrate to the enzyme, rather than concentrations of substrate in the environment. The zero dissociation rates between substrate and enzyme allow a substantial simplification of the resulting dynamics, and feasible applications in more complex settings, such as mixtures between supplementary and substitutable interactions. The latter application is crucial herein.

We assume that one species (the future host) has a body size that is large in comparison with the other (the future endosymbiont). This is to simplify spatial arguments that we will use; we will not make use of expected differences in parameter values between the species that are implied by the body size scaling arguments of the DEB theory (Kooijman, 1986, 2000).

Our aim is to focus on conceptual aspects, and we present a summary of precise quantitative formulations in Appendix B. The models are coded in the package DEBtool, which can be downloaded freely from the electronic DEB laboratory at <http://www.bio.vu.nl/thb/deb/>. The package allows time integrations and steady-state analyses for all steps that we will delineate. The user can evaluate the effects of changing the parameter values. Mass and energy conservation laws and the second law of thermodynamics imply constraints on parameter values, which are listed in Appendix D.

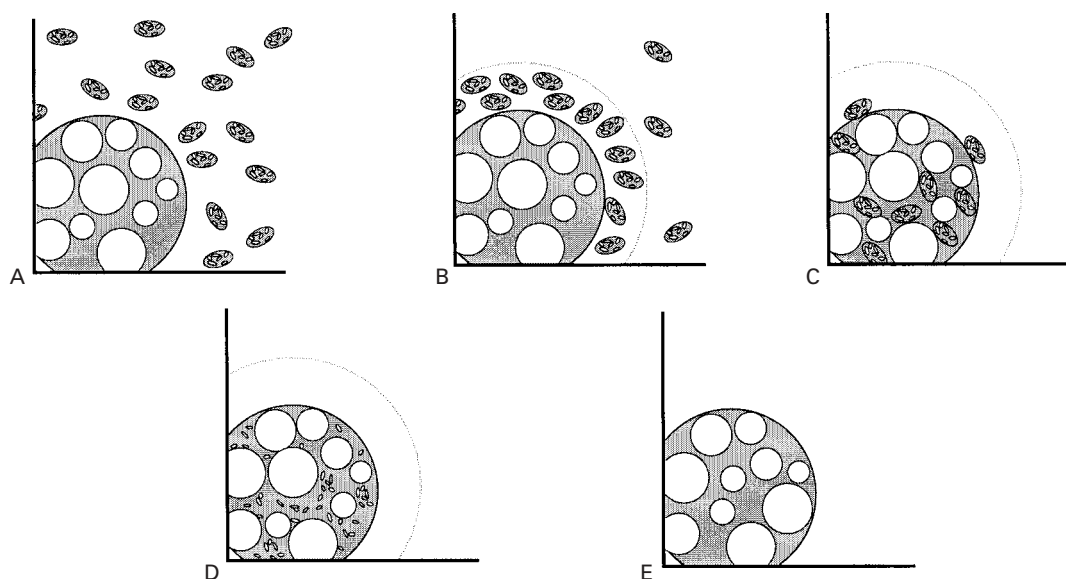


Fig. 2. Future host (a single individual is indicated) and future symbionts originally live independently (A). When they start exchanging products, an accumulation of symbionts in the hosts' mantle space occurs (B), followed by an internalization (C). A merging of structures then can occur (D), followed by a merging of reserves (E). A new entity then exists. The light areas in the hosts and symbionts indicate reserves, the dark regions indicate structure; reserves integrate after structures. The mantle space around the hosts' body is indicated where hosts' product accumulates and it stimulates symbionts' growth. The text describes these and other steps quantitatively.

III. EIGHT STEPS TO ENDOSYMBIOSIS

Starting from two free-living populations in the same environment, eight steps of reductions of degrees of freedom can be delineated to arrive at a fully integrated endosymbiotic system that can be treated as a single population for all practical purposes. All those steps in the asymptotic behaviour of the populations can be made on an incremental basis, i.e. by a continuous and incremental change in (some) parameter values. The general idea is that the parameter values are under evolutionary control. Fig. 2 illustrates some of the steps.

(1) Interacting populations

Originally two species possibly coexist by living on a different substrate each, so they initially might have little interaction and just happen to live in the same environment. Each species excretes products in a well-mixed environment. A weak form of interaction starts when the products are used by the other species as a substitutable compound for their own substrate, a situation that we can call reciprocal syntrophy. Gradually the nutritional nature of the product changes with respect to the substrate from substitutable to supplementary, and the two species become involved in an obligatory symbiotic relationship; they can no

longer live independently of each other (see Fig. 3). The mechanism can be that the partners' product is a metabolite of an organism's own substrate; eventually the metabolic pathway for that metabolite becomes suppressed and later deleted (Stouthamer & Kooijman, 1993). A well-known example is the human inability to synthesize vitamin C, which is generally interpreted as an adaptation to fruit eating; the genes for coding vitamin C synthesis are still present in the human genome, but they are not expressed. The theory behind the uptake of compounds that make a gradual transition from being substitutable to supplementary is discussed in (Brandt, 2002), together with tests against experimental data on co-metabolism.

Fig. 4 summarizes our numerical studies. It gives the steady-state amounts of structures of hosts and symbionts as functions of the throughput rate of the chemostat for the various steps in symbiogenesis. The throughput rate equals the specific growth rate at steady-state. The maximum throughput rate is less than the potential maximum growth rate; equality only holds for infinitely high concentrations of substrate in the inflowing medium. The amounts of structures are zero at a throughput rate of zero because of maintenance; the curves for the Monod and Droop models would decrease monotonically. At step 0, where substrates are substitutable to products, the introduction of the partner enhances growth. This is clearly visible in

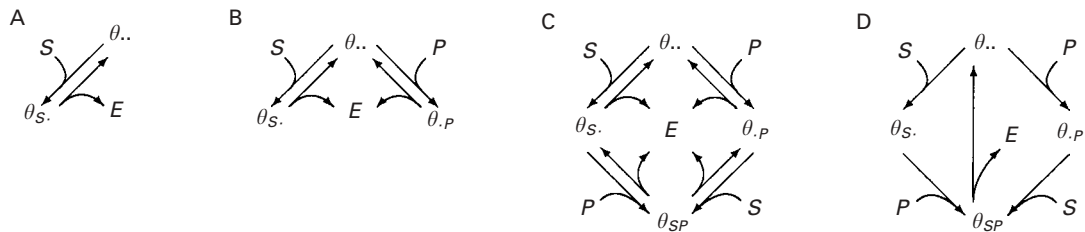


Fig. 3. The evolution of the transformation from substrate S , and later also from product P , into reserve E . The interaction of substrate and product in the transformation to reserve evolved from sequential-substitutable (B), via parallel-substitutable (C), to parallel-supplementary (D). The symbol θ represents a synthesizing unit (SU) that is free $\theta_{..}$, bound to the substrate $\theta_{S..}$, to the product $\theta_{P..}$, or to both θ_{SP} .

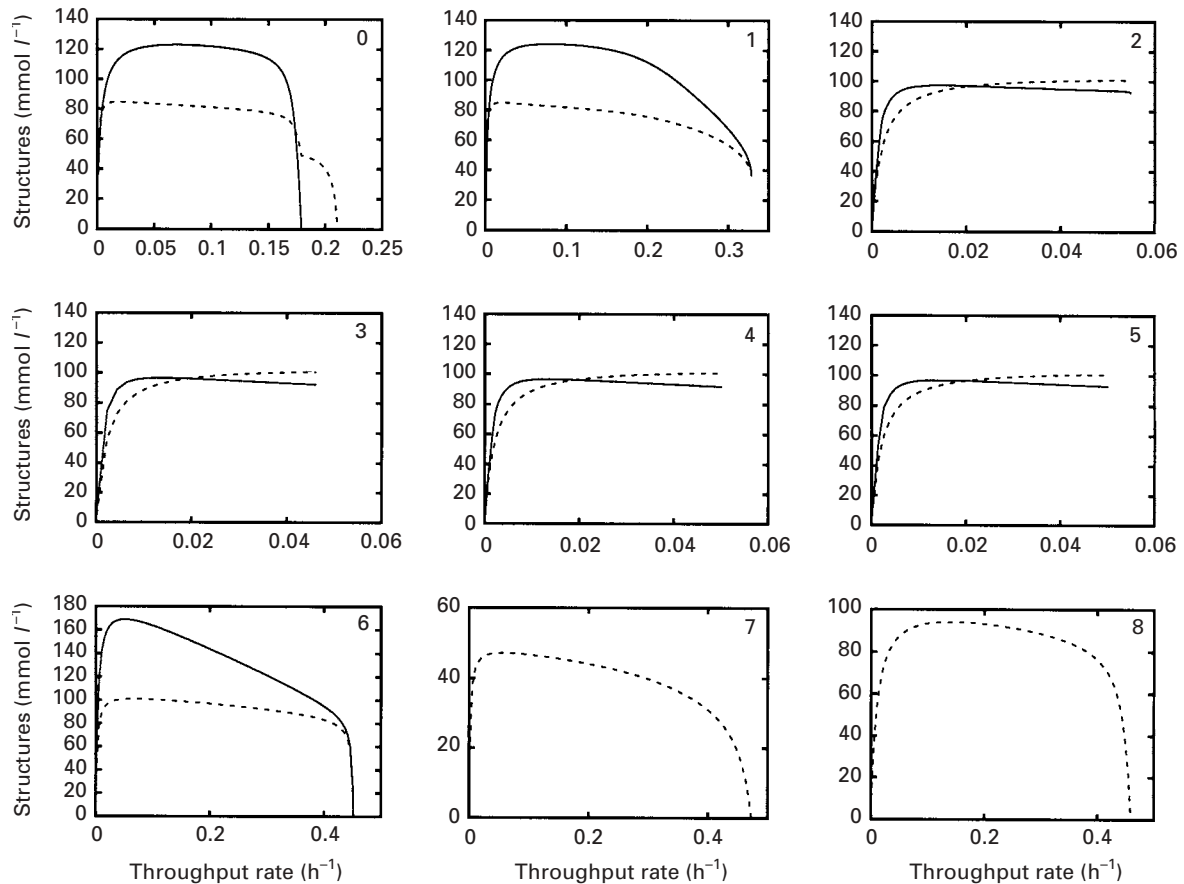


Fig. 4. Steady-state values of the amounts of structure of hosts (dashed curves) and symbionts (drawn curves) as functions of the throughput rate of the chemostat for the different steps in symbiogenesis. If substrates and products are substitutable (0) symbiosis is facultative and the host can live independently of the symbiont. With this parameter setting (see Appendix D) the hosts' maximum growth rate is higher. If substrates and products make their transition to become supplementary (1), and especially if they are supplementary (2), symbiosis is obligatory. The environment is homogeneous in steps 0–2 and 5–8, but in step 3 symbionts can live in the free space, in the hosts' mantle space, as well as within the host. The parameter settings are such that the internal population of symbionts outcompetes the mantle and free-living populations; the curve for the symbionts in step 3 corresponds to the internal population; other values for the transport parameters allow the coexistence of all three populations, or any selection from these three. Step 4 has internal symbionts only, but the mantle space can differ from the free space in concentrations of substrates and products. These differences disappear in step 5 (by increasing the transport rates between both spaces). Product transfer is on flux basis in step 6, rather than on concentration basis (steps 0–5); the symbioses can grow much faster and the amounts of structure are again zero at the maximum throughput rate. The transition from step 5 to 6 can be smooth if the host can reduce the leaking of product. The merging of both structures (7) and reserves (8) does not have substantial effect at steady-state. The single structure in steps 7 and 8 can handle two substrates; each structure in steps 0–6 handles only one.

the curve for the host around the maximum throughput rate for the symbiont. Growth stimulation also occurs for the symbiont, of course, but this is less visible in the figure since the host is always present when the symbiont is present with this parameter setting (see Appendix D); without the host, the biomass and the maximum throughput rate of the symbiont would be less. The maximum throughput rates for hosts and symbionts can differ as long as substrate and products are substitutable, but not if they are supplementary (steps 1–6). The amounts of structures then cannot decrease gradually to zero for increasingly high growth rates (steps 1–5), because product formation, and thus product concentration, will then also decrease gradually to zero, while the equally rapid growing partner needs a lot of product. Fig. 4 also shows that the transition from substitutable to supplementary products (from step 1 to 2) comes with a substantial reduction in the maximum growth rate if no other mechanism ensures an easy access to the products; the concentration of products in a well-mixed environment is very low. It is therefore likely that this transition is simultaneous with, rather than prior to, subsequent steps in symbiogenesis (i.e. spatial clustering, so spatial structure). We will return to this point later.

Examples of product exchange with little spatial clustering can be found among micro-organisms in animal guts. The species originally describes as *Methanobacillus omelianskii* turned out to consist of a chemotrophic ‘S-organism’, which consumes ethanol, and produces di-hydrogen, and a methanogen, which consumes di-hydrogen and carbon dioxide. *Ruminococcus albus* ferments glucose to acetate, ethanol and di-hydrogen, but in the presence of fumarate-fermenting *Vibrio succinogenes*, *R. albus* produces acetate, and not the energetically expensive ethanol; this is only possible when *V. succinogenes* removes di-hydrogen (Fenchel & Finlay, 1995). This exchange pattern is typical for methanogen–partner interactions.

Transitions from syntrophy to competition and parasitism occur. The dung beetle (family Scarabaeidae), which feeds on mammalian faeces, is an example of the use of a product that is associated with hosts’ assimilation. A transition to competition is found in shark-suckers *Echeneis*, which feed on fish fragments derived from the shark’s meals. Antbirds of the family Formicariidae feed on well-camouflaged locusts that jump to escape from an advancing front of army ants (sub-family Dorylinae); syntrophy here completes the transition to direct food competition.

We have discussed the use of products in ways that do not affect the production process directly. The use of products can be associated, however, with a change

of product flux in complex ways, which we will not analyse in depth. The honey guider *Indicator* guides mammals (e.g. badgers and humans) to bees’ nests, itself feeding on the wax that is left over after the nest has been opened by the guided animal. The birds’ activity might increase its average feeding rate as well as that of the guided animal. The house dust mite *Glycyphagus domesticus* enhances human skin flake production by inducing allergic reactions in humans, and so increases its food supply. The moth *Hypochrosis* increases tear production of big mammals, such as Asian elephants, by poking its tongue into mammals’ eye. Both mites and moths ‘milk’ their hosts, so increasing the hosts’ product formation, which is here associated with the hosts’ maintenance process. Syntrophy involves a transition to biotrophy in the sucking of mammalian blood by mosquitos or in the extraction of plant saps by mistletoes or aphids. A transition to predation or parasitism is found in young pearl fish *Carapus*, which feed on the gonad tissue of sea cucumbers.

The concentration of products in the environment and the biomass ratios of the species can vary substantially in time. In the unlikely case that substrates, products or biota all remain in a given local homogeneous environment, it initially takes an amount of substrate to build up product concentrations, but once these concentrations and the populations settle to a constant value, the environment no longer acts as a sink and the situation is very similar to a direct transfer of product from one species to the other during steady-state. The inefficiency of product transfer in well-mixed environments becomes clear during transient states and if the product decays away (chemically, by physical transport or biologically mediated). A lot of product will not reach the partner, and population levels will be low.

(2) Spatial clustering

Exchange of compounds between the species is enhanced by spatial clustering; most individuals of the small-bodied species live in a narrow mantle around an individual of the large-bodied species. Although the real mantle will not have a sharp boundary with the outer environment, we treat it as a distinct and homogeneous environment that can exchange substrate and products with the outer environment and with the volume inside the host. The individuals of the large-bodied species secrete all products into their mantle; both products and substrates can leave or enter the mantle with certain specific rates according to a generalized diffusion process. We take the mantle’s volume (at the population level) just proportional to the structural mass of the large-bodied species (which seems

reasonable at the population level). Diffusion and related transport processes mean that the mantle is actually not homogeneous; concentration gradients will build up, as discussed in Kooijman (2000). This level of detail is not required, however, for our present aim.

The emigration rate of the small-bodied species to and from the mantle space will be relatively small. Since the growth rate will be much higher in the mantle, the small-bodied species accumulates in the mantle space of the large-bodied one, depending on the transport rates for substrates and products to the other environment, and on intraspecific competition. Under rather general conditions for the maximum specific assimilation rate, the specific maintenance and growth costs, and the hazard rate (i.e. the instantaneous death rate), the population inside the mantle space even outcompetes that outside. This spatial clustering does not involve optimization arguments.

Many species create a special environment to grow their symbionts. Examples are animals intestines, which harbour the gut flora, or pits in the leaves of the floating fern *Azolla* and *Gunnera*, which harbour blue-green bacteria (*Anabaena*) that fix di-nitrogen; some flowering plants, such as *Alnus*, leguminosae, *Hippophaë*, have special structures in the roots for harvesting the bacteria *Rhizobium* for the same purpose. Cockroaches lose their gut flora at moulting, and inoculate from mother's faecal supplies. The water flea *Daphnia magna* is a popular species used for ecotoxicity testing (Kooijman & Bedaux, 1996). This led to the discovery that repeated media refreshment reduces daphnids' condition, which is probably caused by a wash-out of the gut flora; daphnids natural schooling behaviour thus may be related to re-inoculation of the gut flora.

(3) Physical contact: epibionts

The exchanging products now hardly accumulate in the environment, and compound exchange is direct on the basis of fluxes. The exchange is further optimized if the partners live in direct physical contact with each other, since losses will be even smaller. Such situations frequently occur in nature. The surface of the euglenoid *Postgaardi* is completely covered with bacteria (Fig. 5). Product exchange is probably the reason that many micro-organisms live in flocks, rather than in free suspension (Brandt & Kooijman, 2000). Ascomycetes make physical contact with green algae in lichens. Basidiomycetes do so with vascular plants in ectomycorrhizas. Cyanobacteria, *Prochloron*, live on the outer surface of didemnids.

If the products decay away, the two species can still improve product exchange by internalization, where

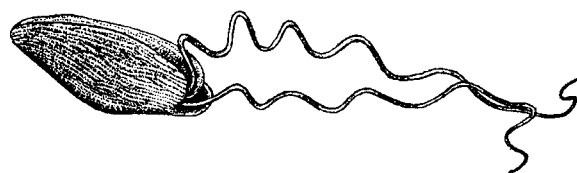


Fig. 5. The cell surface of the colourless amitochondriate euglenoid *Postgaardi mariagerensis* is fully covered with elongated rod-shaped heterotrophic bacteria. Based on an electronmicrograph in Simpson *et al.* (1996); the length of the cell is 60 μm .

the small-bodied species (endosymbionts) lives inside the large-bodied one (host). This requires phagocytosis, which is absent in prokaryotes, but widely spread among eukaryotes. During this internalization process, the host acquires the product of its symbiont both from the mantle space, as well as from inside its own body. The natural description of the uptake process of product in the mantle space is on the basis of the concentration of product, in combination with a generalized diffusion process that transports the product to the hosts' product-carriers in the outer membrane. While the symbionts' access to the hosts' product is enhanced by internalization, its access to the substrate can be reduced because that substrate now has to pass through the hosts' outer membrane. From outside the host the endosymbiont is no longer visible and it appears as if the host is now feeding on two substrates, rather than one. The argument becomes subtle if the host transforms the symbionts' substrate before it reaches the symbiont.

The internal population eventually outcompetes the population in the mantle space, and the endosymbiont can lose its capacity to live freely due to adaptation to its cytoplasmic environment, which is under the hosts' homeostatic control. It is curious to note that the chloroplasts of chromista (which include diatoms, brown algae and many other 'algal' groups), live inside the hosts' endoplasmic reticulum, while they live outside it in other groups (Cavalier-Smith, 1998). The passage of the extra membrane during the internalization process is obviously conserved during evolution, which suggests that the internalization process is rare and reveals the evolutionary relationships between these protist taxa.

Our numerical studies did not account for transport mechanisms that enhance the intracellular accumulation of products. A much higher maximum growth rate can be obtained by decreasing the parameters that control the leaking of products and substrates from the cell, for example. The low maximum growth rate shown in Fig. 4, steps 2–5, suggests that the control of

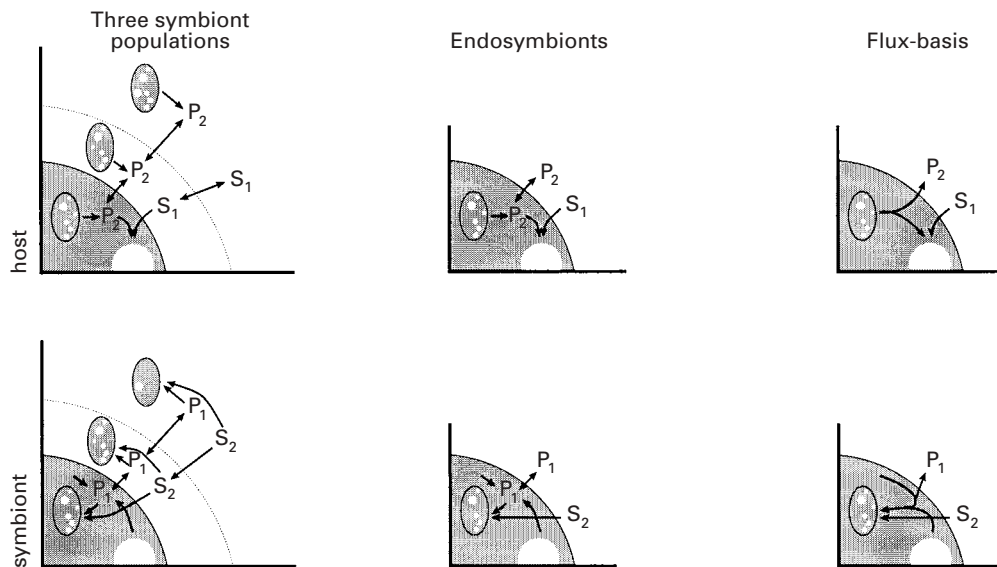


Fig. 6. The feeding process of host (top) and symbiont (bottom) on substrate (S_1 and S_2) from the environment and product (P_1 and P_2) that is produced by the partner. The first column delineates three populations of symbionts: free, epi- and endosymbionts. The second column only delineates endosymbionts, but still uses intracellular concentrations of products to quantify feeding. With the degradation of intracellular product from an ecological to a physiological variable, feeding is specified in terms of fluxes (third column).

transport may be a rather essential feature of symbiogenesis. These transport parameters also determine the fate of the three populations of symbionts (free-living, epibiotic and internal). They can all co-exist or each of them can outcompete the others, depending on these parameter values. The competitive exclusion principle, which states that the number of species of competitors cannot exceed the number of types of resources at steady-state, only holds for homogeneous environments. The values that are used in Fig. 4, step 3, lead to extinction of the epibiotic and free-living populations. The mantle space still can differ in concentrations of substrates and products in step 4, while in step 5 the transport rates of these compounds from the mantle to the free space and *vice versa* are so large that the environment is homogeneous again. It is clear that these differences hardly affect the dynamics with this choice of parameter values, the curves in steps 3–5 are very similar; the differences are clear for smaller transport rates.

The uptake of product evolves from concentration-based to flux-based (see Fig. 6); this comes with an increase of the maximum throughput rate and a qualitative change in behaviour of the steady-state amounts of structure around this growth rate: the symbiosis becomes independent of the extracellular product concentration. The role of the products partly degrades from an ecological to a physiological one. Fig. 4 illustrates this in step 6, where the steady-state amounts of structure at the maximum throughput rate is (again)

zero, while the maximum throughput rate is substantially increased compared with that for step 5.

Many examples are known for endosymbioses. The nitrogen-fixing cyanobacterial symbionts of the diatoms *Rhizosolenia* and *Hemiaulus* live between the cell wall and the cell membrane (Villareal, 1987, 1991). Pogonophorans (annelids) and *Xyloplax* (echinoderms (Baker, Rowe & Clark, 1986)) do not possess guts, but harbour chemoautotrophic bacteria inside their tissues. The parabasal flagellate *Caduceia theobromae* bears two species of ectosymbiotic and one species of endosymbiotic bacteria, which assist wood digestion in termite guts. Cyanobacteria live inside the fungus *Geosiphon* and the diatoms *Richelia*, *Hemiaulus* and *Rhopalodia*. Several species of heterotrophic bacteria live endosymbiotically in *Amoeba proteus*.

The location of mitochondria inside cells further testifies to the optimization of transport by spatial clustering. Mitochondria cluster close to blood capillaries in mammalian muscle cells and form interdigitating rows with myofibrils to enable peak performance during contraction (Bereiter-Hahn, 1990). The association of mitochondria with the nuclear envelope is thought to relate to the demand of ATP for synthesis in the nucleus, and to the reduction of damage to DNA by reactive oxygen species. The association of mitochondria with the rough endoplasmic reticulum is not so well understood but might be related to the movement of mitochondria within cells.

(4) Weak homeostasis for structure

The ratio of the amounts of structure of the partners varies within a range that becomes increasingly narrow. At constant substrate levels the population sizes grow exponentially, but their ratio becomes constant, a condition that we term 'weak homeostasis' for structure. The ratio might still depend on the substrate levels at steady-state. The importance of products taken up from the environment becomes small; almost all products are exchanged within the body of the host. If the products are fully supplementary to the substrates, some excess product might still leak from host's body, due to stoichiometric restrictions in its use. If part of the product is still substitutable for the substrate, such a leak is unlikely.

Many photosymbionts seem to have a rather constant density in hosts' tissues, although knowledge about digestion of symbionts by hosts as part of a density regulation system is frequently lacking. Our analysis shows that product exchange is such a strong regulation mechanism, that other regulation mechanisms are not necessary to explain a relatively constant population density of endosymbionts. This does not exclude the existence of regulation mechanisms, of course. Section (5) suggests that regulation mechanisms might affect the coupling of parameters that control product formation.

(5) Strong homeostasis for structure

The ratio of the amounts of structure of the partners becomes fixed and independent of the substrate concentrations at steady-state. We suggest that the mechanism of tuning the weight coefficients for product formation depends on assimilation, maintenance and growth.

If the endosymbiont is limited in its growth by hosts' product(s), simple constraints apply to ensure that the endosymbiont and the host have equal specific growth rates, and that the ratio of their structures is independent of the hosts' substrate concentration at steady-state. The constraints are that (1) their reserve turnover times are equal, (2) endosymbionts' assimilation capacity is large with respect to the realized assimilation, (3) no products are produced in association with growth, (4) some products are produced in association with assimilation, and (5) the ratio of the weight coefficients for product formation in association with maintenance and growth is the following function of maintenance rate coefficients:

$$\frac{y_{PM}^i}{y_{PE}^i} = y_{EV}^i \frac{\dot{k}_M^j / \dot{k}_M^i - 1}{\dot{k}_M^j / \dot{k}_E + 1}, \quad (1)$$

where y_{PM}^i is hosts' yield of product on reserve in the maintenance flux, y_{PE}^i is hosts' yield of product on reserve in the assimilation flux, y_{EV}^i is the hosts' yield of structure on reserve, $\dot{k}_E$ the reserve turnover rate, $\dot{k}_M$ the maintenance rate coefficient of the host, $\dot{k}_M^i$, and of the endosymbiont, $\dot{k}_M^j$. All yield coefficients are expressed in terms of C-mol per C-mol; a C-mol is a mol of C-atoms, so 1 mol glucose equals 6 C-mol glucose. Hosts' specific product formation is given by $j_P^i = y_{PE}^i j_E^i + y_{PM}^i \dot{k}_M^i$, where j_E^i is the specific assimilation flux, i.e. the synthesis rate of hosts' reserve from substrate and product in terms of C-mol of reserve per C-mol of structure per unit time.

The large assimilation capacity of the endosymbiont (condition 2) is necessary to avoid wastage of hosts' products. If the conditions are not exactly fulfilled, strong homeostasis is not strict and (small) changes in biomass composition remain possible. Strict homeostasis probably only exists in the human mind, but this does not devalue the usefulness of the concept. It has an intimate relationship with stoichiometric restrictions on growth; the time-varying resources have to be incorporated into fixed ratios.

The conditions for strong homeostasis are independent of the details of hosts' assimilation process. The host might be product as well as substrate limited. The product and the substrate might also be substitutable compounds, such as in the case of algal symbionts of heterotrophs, where the algal carbohydrates serve as an alternative energy source for the host. The significance of these carbohydrates in the hosts' diet might be complex, while the strong homeostasis condition still holds true. If prey is abundant, and the host's maximum assimilation capacity is reached, the extra carbohydrates contribute little to the hosts' assimilation. This dynamics is consistent with the rules for sequential processing of substitutable substrates by synthesizing units (Kooijman, 2000) and explains why symbiotic and aposymbiotic hosts grow equally fast at high substrate levels, as has been observed in ciliates and hydras (Karakashian, 1963; Muscatine & Lenhoff, 1965). At low prey abundance (the typical situation in the oligotrophic waters around coral reefs), the extra carbohydrates do contribute to the hosts' diet and propagation.

The fact that the conditions for strong homeostasis are independent of the hosts' assimilation process also implies the independence of details of the endosymbionts' product formation. This simplifies matters considerably, because the excretion of carbohydrates by algal symbionts is not a process covered by fixed associations with assimilation, maintenance and/or growth. It is an active excretion due to stoichiometric

constraints of carbohydrates and ammonia (from the host) to form new algal biomass. This excretion process is discussed in the next step of integration: the single structure–two reserves case, where the algae have a carbon and a nitrogen reserve.

If the host is limited in its growth by endosymbionts' products, similar constraints apply to ensure a constant ratio of structures that is independent of endosymbionts' substrate; the role of host and endosymbionts are just interchanged in this situation. In the case of limitation by substrate of both partners, more stringent constraints apply on energy parameters.

The constraint of small actual assimilation rates might help to explain why symbioses are most frequently found in oligotrophic environments; regulation of relative abundances is more difficult under non-limiting environmental conditions.

Eventually the ratio of amounts of host and symbiont structures also remains constant during transient states, a condition that we term strong homeostasis for structure. We can now replace endosymbionts' and hosts' structure by a single combined structure, with two reserves. The reduction is possible without loss of generality on the basis of the concept of a generalized compound that we introduced earlier. This step is in reality usually accompanied by a transfer of (part of) the endosymbionts' DNA to that of host (Martin *et al.*, 1998); endosymbionts are now called organelles.

The numerical effect of the merging of the structures is small with our parameter choice (Fig. 4, from step 6 to step 7). The technical details of the merging are discussed in Appendix B.

Examples of endosymbioses that approach strong homeostasis are mitochondria, hydrogenosomes, chloroplasts and other plastids and peroxysomes. The number of these organelles per host cell depends very much on the species. Diatoms frequently have just a single chloroplast, which implies that the growth and division cycle of the chloroplast must be tightly linked to that of the cell.

(6) Coupling of assimilation pathways

The assimilation routes for the organic substrate(s) become coupled, especially in situations where substrate levels co-vary in time. The reason for the co-variation can be purely physical when the substrates originate from a common source (for example another organism with a rather constant chemical composition, or some erosion process of rocks which extracts minerals in fixed ratios). An alternative possibility is that specialization on a single substrate occurs. Details of product exchange are no longer visible in the dynamics

of the integrated system; products now have a strict physiological role, where they still determine the relative importance of the different sub-structures, and so the substrate uptake capacity. We no longer need to know of their existence to predict how the population responds to environmental factors. If substrates co-vary in fixed ratios in the environment, the range of the ratio of amounts of reserves becomes increasingly narrow.

In situations where substrate abundances do not co-vary in time, coupling of the assimilation processes will not occur, and the host will maintain two reserves (see below). Another pattern of development is then likely: part of the unused reserve that is allocated to growth is returned to that reserve, with the result that each reserve can accumulate and reach very high levels when the other reserve limits growth. The logic is, when the substrate ratio in the environment changes, the reserve density will (eventually) change, and so also the ratio of fluxes of used reserves. After paying the maintenance costs, the reduced flux arrives at the synthesizing units for the growth of structure. The chemical composition of structure is constant, however, so there are stoichiometric restrictions on the reserve fluxes that can be used, and part of one of the fluxes will remain unused and will be partly excreted into the environment. This is how algae excrete carbohydrates under nitrogen limitation, or nitrogen-containing toxicants under, e.g. silica limitation. Quantitative details are discussed in Kooijman (2000) and Kooijman, Andersen & Kooi (2001). An important implication is that the density of the growth-limiting reserve always increases with the growth rate, but the density of the non-limiting reserve can increase as well as decrease with the growth rate; spectacular accumulation of reserves can only occur for the non-limiting reserves at low growth rates.

The functionality of this storage mechanism can be illustrated with algal growth in the sea, where carbohydrate reserves are boosted at the nutrient-poor surface layers where light is plentiful, and the nutrient reserves are filled in the dark bottom layers of the photic zone, which are usually rich in nutrients (Kooijman, Dijkstra & Kooi, 2002). Owing to the uncoupled reserves the alga is able to grow in an environment that would otherwise hardly allow growth. It is the wind, rather than light or nutrients, that is in proximate control of algal growth (Fig. 7).

(7) Coupling of reserve dynamics

At constant substrate levels, the reserve ratio of the growing populations settles at a constant value, but

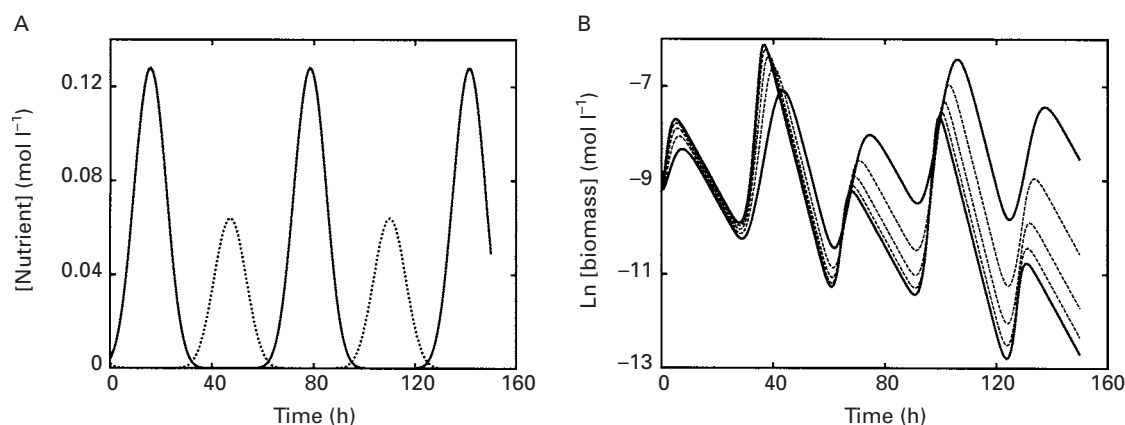


Fig. 7. (A) A cyclic pattern of two nutrient concentrations, as experienced for example by algae. (B) The expected biomass concentrations in the case of a single structure, and two reserves. The curves correspond to increasing reserve turnover times from top to bottom. The two curves with extreme turnover values are shown with solid lines; the larger this turnover time, the lower the intracellular storage capacity for nutrients. The graphs show that the mean growth rate increases with the storage capacity under these conditions, but that the nutrients must be stored independently.

the value of this ratio can still depend on the substrate levels. This condition has been called weak homeostasis for reserves. The chemical composition of biomass can still depend on the growth conditions in complex ways. If the assimilatory pathways are coupled, reserve dynamics are coupled almost automatically, because the coupling of structures already requires that the reserve turnover rates are equal. Data on reserve dynamics in algae have shown that the reserve turnover rates are equal even when the assimilatory pathways are not coupled (Kooijman, 2000). This can be understood from the machinery that is used to mobilize reserves and use them for growth. Ribosomal RNA (rRNA) is an important component of this machinery, and most of the cellular pool of rRNA is directly related to the amount of reserve (Kooijman, 2000). This explains why the rRNA content of cells and organisms depends on the growth rate; the coupling is so strict that the rRNA content is sometimes used to measure growth (Buckley, 1984; Hovekamp, 1991).

The numerical effect of the merging of the reserves is small using the present parameter values (Fig. 4, from step 7 to step 8). The merging only requires that the reserve turnover rates are equal, and the ratio of concentrations of substrate remains constant.

Examples of fully coupled single reserve systems can be found in carnivores, where the rather constant chemical composition of the prey provides the mechanism for the coupling of assimilation of the various nutrients that are required by the carnivore. Parasites also experience a rather constant chemical environment inside their host. Other examples can be found

in heterotrophs in eutrophic environments, where a single resource is often limiting, all other resources being available in excess.

(8) Strong homeostasis for reserves

The ratio of intrahost reserves becomes fixed and independent of the substrate levels at steady-state. Eventually, the ratio remains fixed during transient states and we arrive at a strong homeostasis for reserves. We can now replace the two reserves by a single combined one. Notice that the reserve ratios no longer depend on growth conditions, but the reserve levels still do. So the chemical composition of biomass also still depends on growth conditions, but in a less complex way.

The increasingly tight coupling of the dynamics of several types of reserves relates to the situation where maintenance and growth drain reserves in fixed and equal ratios. It is the reason why the metabolic performance of cats can be understood using a single reserve, while that of algae cannot. When a carnivore changes its diet over an evolutionary time scale to become a herbivore, using food with a less constant chemical composition, it frequently continues to be less flexible in its metabolism. This is why the metabolic performance of cows can still be understood using a single reserve. The conversion of grass into cow biomass (reserves) is poor from an energy point of view; the conversion efficiency can be greatly improved using protein-rich food supplements. The one reserve-one structure system is obviously a highly simplified model that can only approximate the gross behaviour of real-world physiological systems at best.

IV. CYCLIC ENDOSYMBIOSIS BY SPECIALIZATION

Symbiogenesis, as described here, allows the host to use substrates, which it could not use without the symbionts. The opposite process is specialization on a single substrate, where the endosymbiont is no longer functional. This has occurred for instance in aerobic mitochondriate species that invaded anaerobic environments. In some species the mitochondria evolved into hydrogenosomes, but in others the mitochondria were lost. The loss of plastids has occurred in at least some species of all major groups of organisms, while in some parasitic groups, such as the kinetoplastids, they assume other functions. Specialization on metabolic substrates seems to be linked directly to the loss of genes (Stouthamer & Kooijman, 1993). The loss of non-functional machinery increases the growth rate (Stouthamer & Kooijman, 1993); the reduction of the amount of DNA causes a reduction in the time required to copy it, which reduces mean cell size. This size reduction increases the surface area to volume ratio, and substrate uptake is proportional to surface area. Some properties of the symbiont might be retained, however, as testified by mitochondrion-derived genes in species that have lost their mitochondria.

Generally, (endo)symbiosis might be considered to be a process by which metabolic properties are acquired several orders of magnitude faster than by the Darwinian route of mutation and selection (Margulis & Fester, 1991). Darwin's mutation/selection route can be particularly cumbersome, because all intermediary stages have to be vital enough to continue the acquisition with incremental steps; almost all metabolic pathways involve several, or even many, enzymes. This provides constraints on the type of properties any particular organism can acquire along the Darwinian route. Such constraints do not apply to acquisition of metabolic traits by endosymbiosis.

Specialization with its accompanying losses of organelles completes the endosymbiotic cycle, which has been repeated many times in the evolutionary history of life, and has created a bewildering biodiversity.

V. DISCUSSION

We have here discussed the situation of a single substrate and a single product for each species. Although this will give more chemical realism than is usual for population dynamical models, it is too simple to be realistic. It is even too simple to deal with photo-autotrophic endosymbionts of heterotrophs. Such an

analysis should elaborate on multiple nutrient limitation, and must involve more than one type of reserve. We also assumed that surface area is proportional to volume, which can be an appropriate approximation for micro-organisms, but is not so for animals and plants. Other choices must be made here, and for plants we also need more than one structure (e.g. roots and shoots). Such extensions of the DEB theory have been developed (Kooijman, 2000), but obviously complicate the argument. Here we concentrate on first principles. Some of the phenomena have complex details; the acoel *Convoluta roscoffensis*, for instance, harbours its prasinophyte symbiont *Tetraselmis* during its adult stage only (Smith & Douglas, 1987). It needs this endosymbiont to complete its life cycle, which complicates theoretical predictions for population dynamics.

The natural framework to capture the population dynamics of endosymbionts in hosts is an individual-based structured population dynamical one, rather than an unstructured one as is used here. We again decided to use the simplest approach first, leaving the more natural, but also much more complex, structured approach as a future exercise.

Although we did not make use of an explicit optimization criterion, we did incorporate an implicit optimization, where the endosymbionts outcompete the epi- and free-living symbionts. An essential difference with typical optimization criteria is that we did not optimize properties of individuals in a constant environment, but include the effects that properties of individuals have on their local environment (by setting substrate and product concentrations). This type of reasoning is consistent with the theory of adaptive dynamics (Geritz *et al.*, 1997, 1998), which studies the evolutionary bifurcation of parameter values that quantify properties of individuals while they interact with their environment. In this context it is not necessary to delineate an optimization criterion, because selection is already implicit in the system that consists of organisms plus their environment. We presently use the theory for adaptive dynamics to improve our understanding of the conditions under which symbiogenesis and specialization (loss of symbionts) can occur.

Competition and prey-predator relationships have traditionally received more attention in population biology than symbioses, with the competitive exclusion principle as one of the favourite topics. This principle says that the number of species of competitors that can coexist eventually cannot exceed the number of resource types if the system has a point attractor. This principle does not apply if the competing species are preyed upon (Kooij & Kooijman, 2000; Domingos, 2000), or if the system has a cyclic or chaotic attractor

(Huisman & Weissing, 1999). The latter conclusion is from numerical studies only; such numerical studies have limited value and the competitive exclusion principle is extremely robust. Numerical studies also indicate that the parameter volume that allows 'long-term' coexistence of a larger number of competing species is rather small. This indicates that the occurrence of cycles and chaotic behaviour probably does not explain the seemingly frequent violation of the exclusion principle in nature.

We suggest that the explanation for the frequent violation of the competitive exclusion principle is, apart from spatial heterogeneities (Hutchinson, 1961; Eddison & Ollason, 1978; Chesson & Huntly, 1997; Fenchel, Esteban & Finlay, 1997; Kassen *et al.*, 2000), in the chemical details of the resource types. Each nutrient usually comes in many chemical species, so the number of resource types is actually much larger than is generally taken into account for simplicity's sake. Apart from that, the excretion of products that serve as supplementary substrates for other species, as discussed here, is a widespread phenomenon. The larger the number of species, the larger is the number of different products, since most products are species-specific. This allows the long-term coexistence of a large number of species that compete for a small number of primary resources, even in the case of a point attractor. Point attractors themselves are not realistic; real-world populations always vary in numbers, although not necessarily chaotically. Our suggestion rests on the property that this substrate mechanism is not at all sensitive to parameter values, contrary to coexistence mechanisms by predation or oscillatory or chaotic behaviour of models.

The tuning of parameter values between symbiotic partners is an essential feature of our derivation. We did not discuss the machinery that organisms use for tuning these values. From toxicity studies, we know that parameter values can be affected by chemical compounds (Kooijman & Bedaux, 1996). We suggest that organisms use chemical compounds for this purpose that they synthesize themselves (such as hormones). These regulation systems may be (partly) under genetic control.

The DEB model is thought to apply to all types of organisms (microbes, plants, animals). Since these species diversified in the course of their evolutionary history, and endosymbiosis is widespread, we identified a very important property of the model, which should apply to all models that claim not to be species-specific: the DEB model is closed under endosymbiosis. By this we mean that when two species that follow the DEB rules become engaged in an endosymbiotic relationship and integrate fully, the new single species

again follows the DEB rules. This property poses strong constraints on the structure of the model. In our case reserve dynamics is crucial, which follows from the partitionability and weak homeostasis requirements. We here demonstrated that the partitionability argument can be reversed and converted to a merging argument. It is still too early to delineate the complete class of models with this property, but we would not be surprised to learn that this class is very small.

VI. CONCLUSIONS

(1) We hypothesized that the exchange of metabolic products between partners is basic to symbiogenesis and that strong interactions developed gradually from weak ones. These products are substitutable for substrates in weak interactions, and supplementary in strong ones. Our study confirms earlier findings that substrates (including nutrients and light) and derived products play a key role in the interaction between endosymbionts and hosts (Dubinsky & Jokiel, 1994). We here give a full quantitative specification of the essential steps within the framework of the DEB theory.

(2) Spatial clustering and internalization developed because it facilitates the exchange of products and so enhances propagation. The transport rates of products and substrates between the free, mantle and intra-organismal spaces are crucial for the development of clustering and internalization.

(3) The smooth reductions of the degrees of freedom in the dynamics of two interacting species during symbiogenesis are directly linked to the evolution of weak homeostasis. Weak homeostasis implies constraints on the dynamics of the sub-systems. Strong homeostasis developed from weak homeostasis. It is a property of the integrated system, and is basic to the concept of 'generalized compound' that is frequently used (implicitly) in the modelling of biological systems. Strong homeostasis implies strict stoichiometric constraints on growth.

(4) Our numerical results indicate that symbiogenesis develops through a phase where both partners must be (locally) abundant to meet their partners' nutritional requirements. Chemostats' steady-state biomass levels at throughput rates just below the maximum rate that allows the existence of the symbiosis are high during these intermediary stages of symbiogenesis, while they are low in the early and late stages. The chemostat here only served to illustrate an important dynamic property of symbiogenesis, which can be difficult to recognize in more natural environmental settings, but nevertheless applies.

(5) Structures (i.e. compounds with a more permanent nature) of hosts and symbionts merge prior to reserves (i.e. compounds with a more temporary nature). This preserves the metabolic flexibility of the partly merged system and allows it to grow in transient environments where local conditions do not favour growth at any particular moment in time. We derived conditions that lead to the merging of structures, and so to the evolution of homeostasis.

(6) Specialization on particular substrates can lead to the loss of (endo)symbionts or genes from hosts and/or symbionts. This gives a cyclic character to symbiogenesis. Symbiogenesis results in a recombination of metabolic properties across species at a rate that is several orders of magnitude faster than the development of metabolic properties through Darwinian evolution; this has accelerated evolution considerably after a phase where Darwinian evolution created basic metabolic diversity.

(7) We suggest that the use of metabolites from other species together with spatial structure explains the seemingly frequent violation of the principle of competitive exclusion.

(8) We showed that two independent species that follow the DEB rules can integrate fully and form a single new species that again follows the DEB rules through a series of incremental changes of parameter values.

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IX. APPENDIX A: NOTATION

The symbols are explained in Table 1. For variables, we append subscript 1 for the host, subscript 2 for the symbiont, and we suppress the subscript for the integrated system. Alternatively, we use subscript i for 1 or 2, and subscript $j=3-i$. For parameter values and auxiliary variables, rather than system variables, we use superscripts instead, to indicate the species; so $y_{PE}^j S_j j_P^i$ should be read as $y_{PE}^{12} S_2 j_P^1$ and $y_{PE}^{21} S_1 j_P^2$. The quantities θ_{SP} and θ_{SP}^i , for example, basically mean the same thing except that θ_{SP}^i refers explicitly to species i . Notice that product i is produced by species i and consumed by species j , while substrate i is consumed by species i . Therefore, j_P^i denotes the specific production rate of product i by species i , while j_P^j denotes the specific consumption of product j by species i . We similarly use subscript and superscript m to denote the mantle space, and c the cytoplasm of species 1 (which is the host); so S_i , S_i^m and S_i^c are the amounts of substrate type i in the free space, in the mantle space and in the cytoplasm, totalled for all host individuals; the cytoplasm of species 1 is separated from the free space by the mantle space, so they do not have direct contact.

Dots above symbols indicate rates (i.e. per unit time), and have nothing to do with differentiation. We use superscript $\circ$, $\circ$ and $\circ\circ$ to denote quantities with a similar interpretation, but a different value; so for example $\dot{k}_S^i$ and $\dot{k}_S^{\circ i}$ are both specific rates of substrate S of type i . We use superscript $*$ to indicate steady-state values, so e.g. j_E^* is the value of j_E^i at steady-state, and m_i^* is the value of m_i at steady-state.

The reserve density m_i is defined as the ratio of the reserve E_i and the structure V_i , so $m_i = E_i V_i^{-1}$ and $m_i^{-1}(d/dt)m_i = E_i^{-1}(d/dt)E_i - V_i^{-1}(d/dt)V_i$. The total biomass of species i in C-molar is the sum of its reserve and structure, so $V_i(1 + m_i)$. We need the yields y_{PM}^i and y_{PG}^i , where P is the product, M the maintenance-product and G the growth-product. These are generalized compounds and hence are mixtures of chemical compounds with fixed compositions. The maintenance-product is excreted. Structure V is part of the growth-product; the other parts of the growth-product (including carbon dioxide for instance) are excreted in direct link with the overhead of the growth process.

The export and death processes are parameterized by hazard rates; the hazard rate multiplied by a time is the probability of disappearance during that time increment, given that the item (organism or compound) is present (alive). If all hazard rates are equal to some value $\dot{h}$, and the supply rate of substrate i equals $\dot{j}_{Sr}^i = \dot{h} S_r^i$, where S_r^i denotes the concentration of substrate i in the inflowing medium, we have the situation

of a chemostat with specific throughput rate $\dot{h}$. The (specific) throughput rate equals the specific growth rate (of hosts and symbionts) at steady-state, so $\dot{h} = \dot{r}^*$. We choose the reactor to be of unit volume, and eliminate notational distinctions between amounts and concentrations.

X. APPENDIX B: DYNAMICS

This appendix specifies a mathematical model within the DEB framework for each of the eight steps of symbiogenesis; we start with independent populations for future hosts and symbionts, and end with a fully integrated symbiosis that operates just like the original host or symbiont. The models have the property that they can be converted into each other by appropriate incremental changes of one or more parameter values; these changes are indicated. This appendix also discusses constraints on parameter values that lead to homeostasis.

(1) Independent populations

The species produce products already, but the products are not (yet) consumed by the other species. They do not interact at all and just happen to live in the same environment. The substrate supply rates to the system, $\dot{j}_{Sr}^1$ and $\dot{j}_{Sr}^2$, are here taken to be constants for simplicity, but are in reality a function of time, and are controlled by other variables.

The products P_1 and P_2 are present but do not (yet) affect the system. The changes of the concentration of substrate (S_i), product (P_i) and structure (V_i) and of reserve density (m_i) are for $i=1, 2$,

$$\frac{d}{dt} S_i = \dot{j}_{Sr}^i - \dot{h}_S^i S_i - j_S^i V_i \quad \text{with} \quad j_S^i = \dot{k}_S^i / (1 + K_S^i / S_i), \quad (\text{B1})$$

$$\frac{d}{dt} P_i = j_P^i V_i - \dot{h}_P^i P_i \quad \text{with} \quad j_P^i = y_{PS}^i j_S^i + y_{PM}^i \dot{k}_M^i + y_{PG}^i \dot{r}^i, \quad (\text{B2})$$

$$\frac{d}{dt} V_i = (\dot{r}^i - \dot{h}_V^i) V_i \quad \text{with} \quad \dot{r}^i = \frac{\dot{k}_E^i m_i - \dot{k}_M^i y_{EV}^i}{m_i + y_{EV}^i}, \quad (\text{B3})$$

$$\frac{d}{dt} m_i = j_E^i - \dot{k}_E^i m_i. \quad (\text{B4})$$

The flux $\dot{j}_{Sr}^i$ of substrate i in equation (B1) enters the reactor; the hazard rates $\dot{h}_S^i$, $\dot{h}_P^i$, $\dot{h}_V^i$ for substrate, product and structure quantify the specific rates at

Table 1. List of symbols used in the text. Subscripts or superscripts i and $j = 3 - i$ in the symbols denote the type or species, and can assume values 1 and 2. Subscripts $*_1$ and $*_2$ are ‘wild cards’ for any compound: substrate (S), product (P), reserve (E), structure (V), maintenance-product (M), growth-product (G). Dimension symbols: t, time; m, C-molar mass; l, length; e, energy

Symbol	Dimension	Description
S_r^i	ml^{-3}	Concentration of substrate in the inflowing medium
S_b, P_b, V_b, E_b, E_i	ml^{-3}	Concentration of substrate, product, structure, reserve
S_i^m, P_i^m, V_2^m	ml^{-3}	Conc. of substrate, product, structure in the mantle of species 1
S_2^c, P_i^c, V_2^c	ml^{-3}	Conc. of substrate, product, structure in the cytoplasm of species 1
K_S^i, K^i, K	ml^{-3}	Half saturation concentration (for substrate i)
m, m_i	mm^{-1}	Reserve density ($m_i = E_i / V_i$)
$\mathcal{Y}_{*1*2}^i, \mathcal{Y}_{*1*2}^i, \mathcal{Y}_{*1*2}^i, \mathcal{Y}_{*1*2}^{\bar{i}}$	mm^{-1}	Yield coefficient of $*_1$ on $*_2$
t	t	Time
$\dot{r}, \dot{r}^i$	t^{-1}	Specific growth rate, of species i
$\dot{h}_V, \dot{h}_V^i, \dot{h}_P, \dot{h}_S$	t^{-1}	Hazard rates for structure, product and substrate
$\dot{h}_{\max}^i$	t^{-1}	Maximum throughput rate of the chemostat
$\dot{k}_E, \dot{k}_E^i$	t^{-1}	Reserve turnover rate
$\dot{k}_M, \dot{k}_M^i$	t^{-1}	Specific maintenance costs
$\dot{k}_S^i, \dot{k}_S^i, \dot{k}_P^i, \dot{k}_P^i, \dot{k}$	t^{-1}	Specific reserve production rates from substrate and product
$\dot{k}_1^i, \dot{k}_2^i, \dot{k}_+$	t^{-1}	Compound rate parameters
α	—	Help variable to shorten notation
v	—	Ratio of densities of structures of symbionts and hosts
ρ_P^i	—	Binding probability of arriving product j in species i
θ	—	Fraction, introduced to shorten notation
$\theta^i, \theta_S^i, \theta_P^i, \theta_{SP}^i$	—	Fractions of unbounded SU, and SU bound to substrate, product or both for species i
$\Theta^i, \Theta_S, \Theta_P, \Theta_{SP}$	$\text{m}^3 \text{m}^{-3} \text{t}^{-3}$	Not-normalized SU-fractions θ summing to Θ_+ : $\theta_{*1*2} = \Theta_{*1*2} / \Theta_+$
$\dot{J}_{Sr}^i, \dot{J}_{Sr}$	$\text{mt}^{-1} \text{l}^{-3}$	Substrate supply rate
$\dot{J}_E^i$	$\text{mm}^{-1} \text{t}^{-1}$	Specific assimilation of reserve
$\dot{J}_P^i, \dot{J}_P^{\circ i}, \dot{J}_P^{\circ \circ i}$	$\text{mm}^{-1} \text{t}^{-1}$	Specific product production
$\dot{J}_S^i, \dot{J}_S^{\circ i}, \dot{J}_S^{\circ \circ i}$	$\text{mm}^{-1} \text{t}^{-1}$	Specific consumption of substrate i by species i
$\dot{J}_{Sm}^i, \dot{J}_{Pm}^i$	$\text{mm}^{-1} \text{t}^{-1}$	Specific consumption of substrate, production of product in mantle pop.
$\dot{J}_{Sc}^i, \dot{J}_{Pc}^i$	$\text{mm}^{-1} \text{t}^{-1}$	Specific consumption of substrate, production of product in internal pop.
$\dot{J}_P^{\bar{i}}, \dot{J}_{Pm}^{\bar{i}}, \dot{J}_{Pc}^{\bar{i}}$	$\text{mm}^{-1} \text{t}^{-1}$	Specific consumption of product j in free space, mantle and cytoplasm
$\dot{J}_+^i, \dot{J}_+^{\circ i}$	$\text{mm}^{-1} \text{t}^{-1}$	Specific consumption of combinations of substrate i and product j
$\dot{b}_S^i, \dot{b}_S^i, \dot{b}_S^i, \dot{b}_P^i, \dot{b}_P^i, \dot{b}_P^i$	$\text{m}^{-1} \text{t}^{-1} \text{l}^3$	Specific binding rates for substrate, product
$\dot{b}_{SP}^i, \dot{b}_{PS}^i$	$\text{m}^{-1} \text{t}^{-1} \text{l}^3$	Specific binding rates for combinations of substrate and product
$\dot{b}_{Sm}^i, \dot{b}_{mS}^i, \dot{b}_{Pm}^i, \dot{b}_{mP}^i$	$\text{m}^{-1} \text{t}^{-1} \text{l}^3$	Transport rates to and from mantle for substrate, product
$\dot{b}_{Sc}^i, \dot{b}_{cS}^i, \dot{b}_{Pc}^i, \dot{b}_{cP}^i$	$\text{m}^{-1} \text{t}^{-1} \text{l}^3$	Transport rates to and from cytoplasm for substrate, product
μ_{*1}^i	em^{-1}	Chemical potential of compound $*_1$

which these compounds leave the reactor. The half-saturation concentration $K_S^i = \dot{k}_S^i / \dot{b}_S^i$ in equation (B1) can be written as the ratio of dissociation rate $\dot{k}_S^i$ of the SU–substrate complex, and the arrival rate $\dot{b}_S^i$ of substrate to the assimilation SU. (We need this for the next step.) The maximum of the specific substrate consumption $\dot{J}_S^i$ equals the dissociation rate $\dot{k}_S^i$. The specific production of product $\dot{J}_P^i$ in equation (B2) is a weighted

sum of the specific substrate consumption $\dot{J}_S^i$, the maintenance rate coefficient $\dot{k}_M^i$, and the specific growth rate $\dot{r}^i$; the three weight coefficients ($\mathcal{Y}_{PS}^i, \mathcal{Y}_{PM}^i$ and $\mathcal{Y}_{PC}^i$) have the interpretation of yield coefficients. The specific growth rate $\dot{r}^i$ is given in equation (B3) as the ratio of the reserve allocated to growth (which is the flux $\dot{k}_E^i m_i$ released from the reserve minus the flux $\dot{k}_M^i \mathcal{Y}_{EV}^i$ allocated to maintenance) and the costs for growth

(which is the sum of the costs for structure j_{EV}^i and the reserve density m_i). The flux $j_E^i = j_S^i j_{ES}^i$ in equation (B4) is the specific reserve assimilation flux (i.e. the flux that is synthesized from substrate and added to the reserve); it is proportional to the specific substrate consumption j_S^i . The reserve turnover rate k_E^i determines to what extent reserves can accumulate; the higher its value, the lower is the maximum reserve capacity.

(2) Interacting populations: free-living symbionts

The species are now consuming the other species' product, which also gives an extra degree of freedom for product formation. We delineate five synthesizing units (SU)-mediated transformations from substrate S and/or product P to reserve E :

- (1) $SU + S \xrightarrow{\dot{b}_S} SU-S \xrightarrow{\dot{k}_S} SU + j_{ES}E$;
- (2) $SU-P + S \xrightarrow{\dot{b}_{SP}} SU-P-S \xrightarrow{\dot{k}'_S} SU-P + j_{ES}E$;
- (3) $SU + P \xrightarrow{\dot{b}_P} SU-P \xrightarrow{\dot{k}_P} SU + j_{EP}E$;
- (4) $SU-S + P \xrightarrow{\dot{b}_{PS}} SU-S-P \xrightarrow{\dot{k}'_P} SU-S + j_{EP}E$;
- (5) $SU + j_{S+}S + j_{P+}P \xrightarrow{\dot{b}_S, \dot{b}_P} SU-S-P \xrightarrow{\dot{k}} SU + j_{E+}E$.

The transformation rates $\dot{b}_S$, $\dot{b}_{SP}$, $\dot{b}_P$ and $\dot{b}_{PS}$ have the dimension per concentration per time, while the transformation rates $\dot{k}_S$, $\dot{k}'_S$, $\dot{k}_P$, $\dot{k}'_P$, $\dot{k}$ have dimension per time; the primes only serve to allow a difference between, e.g. $\dot{k}_P$ and $\dot{k}'_P$. The stoichiometric coefficients j_{ES} , j_{EP} , j_{S+} , j_{P+} and j_{E+} have the interpretation of yield coefficients; subscript $+$ only serves to allow a difference between, e.g. j_{SE} and j_{S+} , and refers to the transformation in which S as well as P are involved.

In the case of non-interacting populations, we only had transformation 1. Then transformation 3 develops (from $\dot{b}_P = 0$ to $\dot{b}_P > 0$), followed by 2 and 4 (from $\dot{b}_{SP} = \dot{b}_{PS} = 0$ to $\dot{b}_{SP}, \dot{b}_{PS} > 0$); eventually transformation 5 dominates, and 1–4 become impossible ($\dot{k}_S, \dot{k}_P, \dot{k}'_S, \dot{k}'_P \rightarrow 0$).

Equations (B3) and (B4) still apply for interacting populations; the fluxes of reserves, substrate and products are

$$j_E^i = j_{ES}^i \dot{k}_S^i \theta_{S.}^{i*} + j_{EP}^i \dot{k}_P^i \theta_{P.}^{i*} + (j_{ES}^i \dot{k}'_S^i + j_{EP}^i \dot{k}'_P^i + j_{E+}^i \dot{k}^i) \theta_{SP.}^{i*}, \quad (B5)$$

$$j_S^i = \dot{k}_S^i \theta_{S.}^{i*} + (\dot{k}'_S^i + j_{S+}^i \dot{k}^i) \theta_{SP.}^{i*}, \quad (B6)$$

$$j_P^i = \dot{k}_P^i \theta_{P.}^{i*} + (\dot{k}'_P^i + j_{P+}^i \dot{k}^i) \theta_{SP.}^{i*}, \quad (B7)$$

$$\frac{d}{dt} S_i = \dot{j}_{Sr}^i - \dot{k}_S^i S_i - j_S^i V_i, \quad (B8)$$

$$\frac{d}{dt} P_i = j_P^i V_i - j_P^i V_j - \dot{k}_P^i P_i \quad \text{with} \quad j_P^i = j_{PS}^i j_S^i + j_{PP}^i j_P^i + j_{PM}^i \dot{k}_M^i + j_{PG}^i \dot{r}^i, \quad (B9)$$

where $\theta_{S.}^{i*}$, $\theta_{P.}^{i*}$ and $\theta_{SP.}^{i*}$ are the steady-state fractions of SU bound to S , P or both, and will be specified below. The specific product formation j_P^i in equation (B9) has the extra term $j_{PP}^i j_P^i$ in comparison with equation (B2), which quantifies the production of product i by species i in association with the uptake j_P^i of product j formed by species j .

The fractions θ^* are the steady-state solutions of the change in fractions of variously bound SUs (with $1 = \theta_{..} + \theta_{S.} + \theta_{P.} + \theta_{SP.}$):

$$\frac{d}{dt} \theta_{..} = -(\dot{j}_S^\circ + j_P^\circ) \theta_{..} + \dot{k}_S \theta_{S.} + \dot{k}_P \theta_{P.} + \dot{k} \theta_{SP.}, \quad (B10)$$

$$\frac{d}{dt} \theta_{S.} = \dot{j}_S^\circ \theta_{..} - (\dot{k}_S + j_P^\circ) \theta_{S.} + \dot{k}'_P \theta_{SP.}, \quad (B11)$$

$$\frac{d}{dt} \theta_{P.} = j_P^\circ \theta_{..} - (\dot{k}_P + j_S^\circ) \theta_{P.} + \dot{k}'_S \theta_{SP.}, \quad (B12)$$

$$\frac{d}{dt} \theta_{SP.} = j_P^\circ \theta_{S.} + j_S^\circ \theta_{P.} - (\dot{k}'_S + \dot{k}'_P + \dot{k}) \theta_{SP.}. \quad (B13)$$

Explicit formulae for the steady-state fractions θ^* are lengthy, because of the normalization ensuring that the sum of all fractions equals one. To simplify the expressions for the θ^* 's, we introduce the quantities Θ , which are the not yet normalized θ^* 's. Suppressing index i we thus have $\theta_{..}^* = \Theta_{..} / \Theta_+$, $\theta_{S.}^* = \Theta_{S.} / \Theta_+$, $\theta_{P.}^* = \Theta_{P.} / \Theta_+$ and $\theta_{SP.}^* = \Theta_{SP.} / \Theta_+$ with $\Theta_+ = \Theta_{..} + \Theta_{S.} + \Theta_{P.} + \Theta_{SP.}$ and

$$\Theta_{..} = j_S^\circ j_P^\circ \dot{k} + j_S^\circ \dot{k}_S (\dot{k} + \dot{k}'_P) + j_P^\circ \dot{k}_P (\dot{k} + \dot{k}'_S) + \dot{k}_S \dot{k}_P \dot{k}_+, \quad (B14)$$

$$\Theta_{S.} = j_S^\circ (j_+^\circ \dot{k}'_P + j_S^\circ \dot{k}) + j_S^\circ \dot{k}_P \dot{k}_+, \quad (B15)$$

$$\Theta_{P.} = j_P^\circ (j_+^\circ \dot{k}'_S + j_P^\circ \dot{k}) + j_P^\circ \dot{k}_S \dot{k}_+, \quad (B16)$$

$$\Theta_{SP.} = j_S^\circ j_P^\circ j_+^\circ + j_S^\circ j_P^\circ \dot{k}_S + j_P^\circ j_S^\circ \dot{k}_P, \quad (B17)$$

with $\dot{k}_+ = \dot{k} + \dot{k}'_S + \dot{k}'_P$ and $j_S^\circ = S \dot{b}_S$, $j_P^\circ = P \dot{b}_P$, $j_+^\circ = j_S^\circ + j_P^\circ$, $j_S^\circ = S \dot{b}_{SP}$, $j_P^\circ = P \dot{b}_{PS}$. Superscripts $\circ$ and $\circ\circ$ are used to allow for differences between, e.g. j_S° , $j_S^\circ\circ$ and j_S while they all are specific fluxes of substrate.

The next two subsections show how the system evolves by incremental parameter changes within this

general expression for five simultaneous assimilation routes from a facultative to an obligate symbiosis of free-living partners in an homogeneous environment. Fig. 4, step 1, gives V_1^* and V_2^* as functions of $\dot{h} = \dot{h}_V^i = \dot{h}_P^i = \dot{h}_S^i$ for the parameter values given in Appendix D.

(a) *Products substitutable to substrates*

Products may initially be substitutable to substrates, and processed sequentially (transformations 1 and 3 only)

$$j_E^i = \frac{\mathcal{Y}_{ES}^i \dot{b}_S^i S_i + \mathcal{Y}_{EP}^i \dot{b}_P^i P_j}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}, \quad (\text{B18})$$

$$j_S^i = \frac{\dot{b}_S^i S_i}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}, \quad (\text{B19})$$

$$j_P^{\ddot{i}} = \frac{\dot{b}_P^i S_i}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}. \quad (\text{B20})$$

The derivation is as follows for $\dot{k}^i = \dot{k}_S^i = \dot{k}_P^i = 0$, $\dot{b}_{PS}^i = \dot{b}_{SP}^i = 0$, so $j_S^{\circ i} = j_P^{\circ i} = 0$. We catenate superscript i in the expressions (B14–B17) for the SU-fractions θ^* and arrive at

$$\theta_{..}^{i*} = \frac{\dot{k}_S^i \dot{k}_P^i}{\Theta_+^i / \dot{k}^i}; \quad \theta_{S.}^{i*} = \frac{j_S^{\circ i} \dot{k}_P^i}{\Theta_+^i / \dot{k}^i}; \quad \theta_{.P}^{i*} = \frac{j_P^{\circ i} \dot{k}_S^i}{\Theta_+^i / \dot{k}^i};$$

$$\frac{\Theta_+^i}{\dot{k}^i} = \dot{k}_S^i \dot{k}_P^i + j_S^{\circ i} \dot{k}_P^i + j_P^{\circ i} \dot{k}_S^i; \quad (\text{B21})$$

$$j_E^i = \mathcal{Y}_{ES}^i \dot{k}_S^i \theta_{S.}^{i*} + \mathcal{Y}_{EP}^i \dot{k}_P^i \theta_{.P}^{i*} = \frac{\mathcal{Y}_{ES}^i j_S^{\circ i} + \mathcal{Y}_{EP}^i j_P^{\circ i}}{1 + j_S^{\circ i} / \dot{k}_S^i + j_P^{\circ i} / \dot{k}_P^i}$$

$$= \frac{\mathcal{Y}_{ES}^i S_i \dot{b}_S^i + \mathcal{Y}_{EP}^i P_j \dot{b}_P^i}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}, \quad (\text{B22})$$

$$j_S^i = \dot{k}_S^i \theta_{S.}^{i*} + \frac{S_i \dot{b}_S^i}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}, \quad (\text{B23})$$

$$j_P^{\ddot{i}} = \dot{k}_P^i \theta_{.P}^{i*} + \frac{P_j \dot{b}_P^i}{1 + S_i \dot{b}_S^i / \dot{k}_S^i + P_j \dot{b}_P^i / \dot{k}_P^i}. \quad (\text{B24})$$

For $\dot{b}_P^i \rightarrow 0$, the system reduces to the independent case.

Depending on species parameters and reactor controls (S_r^1 , and S_r^2), one species can outgrow the other. Suppose that species i can grow faster than species j . At the maximum throughput rate for species i , $\dot{h}_{\max}^i$, we have $S_i^* = S_r^i$, $S_j^* = S_r^j$, $P_i^* = P_j^* = 0$, $V_i^* \simeq 0$, $V_j^* = 0$ and $j_E^* = \mathcal{Y}_{ES}^i \dot{b}_S^i S_r^i / (1 + S_r^i \dot{b}_S^i / \dot{k}_S^i)$. So, for $m_i^* = j_E^* / \dot{k}_E^i$, the maximum throughput rate for i is $\dot{h}_{\max}^{i*} = (\dot{k}_E^i m_i^* - \dot{k}_E^i \mathcal{Y}_{EV}^i) / (m_i^* + \mathcal{Y}_{EV}^i)$.

At the maximum throughput rate for species j we have $S_j^* = S_r^j$, $P_j^* = 0$, $V_j^* \simeq 0$, $V_i^* = (S_r^i - S_i^*) \dot{h}_{\max}^j / j_S^{i*}$, $S_i^* = j_E^* (\dot{b}_S^i (\mathcal{Y}_{ES}^i - j_E^* / \dot{k}_S^i))^{-1}$, $P_i^* = (S_r^i - S_i^*) j_P^* / j_S^{i*}$, $j_E^* = (\mathcal{Y}_{ES}^i \dot{b}_S^i S_r^i + \mathcal{Y}_{EP}^i \dot{b}_P^i P_j^*) / (1 + S_r^i \dot{b}_S^i / \dot{k}_S^i + P_j^* \dot{b}_P^i / \dot{k}_P^i)$. We find $\dot{h}_{\max}^j = \dot{k}_E^j (j_E^* - \dot{k}_M^j \mathcal{Y}_{EV}^j) / (j_E^* + \dot{k}_E^j \mathcal{Y}_{EV}^j)$. Notice that the consumption of product only increases the growth rate as long as $\mathcal{Y}_{EP}^j > j_E^* / \dot{k}_P^j$, otherwise time allocated to product consumption does not balance the additional synthesis of reserves.

Fig. 4, step 0, gives V_1^* and V_2^* as functions of $\dot{h}$ for the parameter values given in Appendix D.

(b) *Products supplementary to substrates*

Eventually products become supplementary to substrates, and they are processed in a parallel way (transformation 5 only)

$$j_E^i = ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i)^{-1} + (\dot{b}_P^i P_j)^{-1} - (\dot{b}_S^i S_i + \dot{b}_P^i P_j)^{-1})^{-1}, \quad (\text{B25})$$

$$j_S^i = j_E^i / \mathcal{Y}_{ES}^i, \quad (\text{B26})$$

$$j_P^{\ddot{i}} = j_E^i / \mathcal{Y}_{EP}^i, \quad (\text{B27})$$

with $\dot{k}^i = \dot{k}_{E+}^i$, $\dot{b}_S^i = \dot{b}_{S+}^i$, $\dot{b}_P^i = \dot{b}_{P+}^i$, $\mathcal{Y}_{ES}^i = \mathcal{Y}_{E+}^i / \mathcal{Y}_{S+}^i$ and $\mathcal{Y}_{EP}^i = \mathcal{Y}_{E+}^i / \mathcal{Y}_{P+}^i$.

The derivation is as follows for $\dot{k}_S^i = \dot{k}_P^i = \dot{k}_S^i = \dot{k}_P^i = 0$, $\dot{b}_{SP}^i = \dot{b}_S^i$, $\dot{b}_{PS}^i = \dot{b}_P^i$, so $j_S^{\circ i} = j_S^{\circ}$ and $j_P^{\circ i} = j_P^{\circ}$.

$$\Theta_{..} = j_S^{\circ} j_P^{\circ} \dot{k}; \quad \Theta_{S.} = j_S^{\circ} j_S^{\circ} \dot{k};$$

$$\Theta_{.P} = j_P^{\circ} j_P^{\circ} \dot{k}; \quad \Theta_{SP} = j_S^{\circ} j_P^{\circ} j_P^{\circ}, \quad (\text{B28})$$

$$j_E^i = \mathcal{Y}_{E+}^i \dot{k}^i \theta_{SP}^{i*} = \mathcal{Y}_{E+}^i \left(\frac{1}{\dot{k}^i} + \frac{1}{j_+^{\circ i}} + \frac{j_S^{\circ i}}{j_P^{\circ i} j_+^{\circ i}} + \frac{j_P^{\circ i}}{j_S^{\circ i} j_+^{\circ i}} \right)^{-1}$$

$$= \mathcal{Y}_{E+}^i \left(\frac{1}{\dot{k}^i} + \frac{1}{j_S^{\circ i}} + \frac{1}{j_P^{\circ i}} - \frac{1}{j_+^{\circ i}} \right)^{-1}, \quad (\text{B29})$$

$$j_S^i = \mathcal{Y}_{S+}^i \dot{k}^i \theta_{SP}^{i*} = j_E^i \mathcal{Y}_{S+}^i / \mathcal{Y}_{E+}^i, \quad (\text{B30})$$

$$j_P^{\ddot{i}} = \mathcal{Y}_{P+}^i \dot{k}^i \theta_{SP}^{i*} = j_E^i \mathcal{Y}_{P+}^i / \mathcal{Y}_{E+}^i. \quad (\text{B31})$$

Notice that $j_E^i \rightarrow 0$ if $j_P^i \rightarrow 0$; so one species can no longer exist without the other. Since the uptake of substrate in equation (B26) is now proportional to that of product in equation (B27), due to stoichiometric constraints, the extra degree of freedom in product formation in association with assimilation equation (B9) is lost, and product formation is again described by equation (B2).

Fig. 4, step 2, gives V_1^* and V_2^* as functions of $\dot{h}$ for the parameter values given in Appendix D.

(3) Spatial clustering

We now delineate a mantle around each individual of species 1, and indicate with superscript m the concentrations of substrate, product, and species 2 in this mantle. For simplicity's sake we exclude migration of species 2 to and from the mantle; differences in feeding conditions inside and outside the mantle cause differences in reserve, which becomes very complex if we allow for migration. We also assume that substrate 1 exchanges rapidly with the mantle, so its concentration in the mantle is the same as that outside. Due to the difference in population density of species 2, this does not hold for substrate 2 and the products, and we thus introduce an intra-mantle concentration.

The changes of the concentration of substrate and product outside the mantle (S_i, P_i) and in the mantle (S_2^m, P_1^m) for $i = 1, 2$ are

$$\frac{d}{dt} S_1 = \dot{J}_{S_1}^1 - \dot{h}_S^1 S_1 - j_S^1 V_1, \quad (\text{B32})$$

$$\frac{d}{dt} S_2 = \dot{J}_{S_2}^2 - \dot{h}_S^2 S_2 - j_S^2 V_2 - \dot{b}_{S_m}^2 V_1 S_2 - \dot{b}_{mS}^2 V_1 S_2^m, \quad (\text{B33})$$

$$\frac{d}{dt} S_2^m = -\dot{h}_S^2 S_2^m - j_{S_m}^2 V_2^m + \dot{b}_{S_m}^2 V_1 S_2 - \dot{b}_{mS}^2 V_1 S_2^m, \quad (\text{B34})$$

$$\frac{d}{dt} P_1 = -j_P^{21} V_2 - \dot{h}_P^1 P_1 + \dot{b}_{mP}^1 V_1 P_1^m - \dot{b}_{P_m}^1 V_1 P_1, \quad (\text{B35})$$

$$\frac{d}{dt} P_1^m = j_{P_m}^1 V_1 - j_{P_m}^{21} V_2^m - \dot{h}_P^1 P_1^m - \dot{b}_{mP}^1 V_1 P_1^m + \dot{b}_{P_m}^1 V_1 P_1, \quad (\text{B36})$$

$$\frac{d}{dt} P_2 = j_P^2 V_2 - \dot{h}_P^2 P_2 + \dot{b}_{mP}^2 V_1 P_2^m - \dot{b}_{P_m}^2 V_1 P_2, \quad (\text{B37})$$

$$\frac{d}{dt} P_2^m = j_{P_m}^2 V_2^m - j_P^{12} V_1 - \dot{h}_P^2 P_2^m - \dot{b}_{mP}^2 V_1 P_2^m + \dot{b}_{P_m}^2 V_1 P_2, \quad (\text{B38})$$

where subscript and superscript m refers to the mantle. The specific exchange rate of substrates and products between the free and the mantle space, $\dot{b}_{S_m}^i V_1$, $\dot{b}_{mS}^i V_1$, $\dot{b}_{P_m}^i V_1$ and $\dot{b}_{mP}^i V_1$, are proportional to V_1 because the surface area of the interface between these spaces is proportional to V_1 .

Equations for j_P^i in (B2), and (B3), (B4), (B25), (B26) and (B27) still apply, but we have now two environments for species 2.

The specific assimilation rates outside the mantle (j_E^i) and in the mantle (j_{Em}^i) are (cf. equation (B25), we

here drop the primes for convenience)

$$j_E^1 = ((\dot{k}^1)^{-1} + (\dot{b}_S^1 S_1)^{-1} + (\dot{b}_P^1 P_2^m)^{-1} - (\dot{b}_S^1 S_1 + \dot{b}_P^1 P_2^m)^{-1})^{-1}, \quad (\text{B39})$$

$$j_E^2 = ((\dot{k}^2)^{-1} + (\dot{b}_S^2 S_2)^{-1} + (\dot{b}_P^2 P_1)^{-1} - (\dot{b}_S^2 S_2 + \dot{b}_P^2 P_1)^{-1})^{-1}, \quad (\text{B40})$$

$$j_{Em}^2 = ((\dot{k}^2)^{-1} + (\dot{b}_S^2 S_2^m)^{-1} + (\dot{b}_P^2 P_1^m)^{-1} - (\dot{b}_S^2 S_2^m + \dot{b}_P^2 P_1^m)^{-1})^{-1}. \quad (\text{B41})$$

The population of species 2 outside the mantle is out-competed by the population in the mantle space and goes extinct (so $V_2 \rightarrow 0$) if one of the following conditions is satisfied:

$$k_E^2 \leq \frac{\dot{k}^2 \dot{h}_V^2}{\dot{k}^2 - (\dot{h}_V^2 + \dot{k}_M^2) \gamma_{EV}^2} \quad (\text{death rate exceeds maximum growth rate}); \quad (\text{B42})$$

$$\dot{k}^2 \leq (\dot{k}_M^2 + \dot{h}_V^2) \gamma_{EV}^2. \quad (\text{B43})$$

(4) Internalization

We now suppose that some individuals invade the bodies (i.e. the cytoplasm) of species 1, and build up a population which will eventually outcompete the mantle population. We call the internal population V_2^c , and the mantle population still V_2^m . We also need to delineate internal substrate and product concentrations, S_2^c and P_1^c .

The host now acquires the symbionts' product from the mantle space, as well as from its own cytoplasm, so

$$j_E^1 = ((\dot{k}^1)^{-1} + (\dot{b}_S^1 S_1)^{-1} + (\dot{b}_P^1 P_2^m + \dot{b}_P^{1c} P_2^c)^{-1} - (\dot{b}_S^1 S_1 + \dot{b}_P^1 P_2^m + \dot{b}_P^{1c} P_2^c)^{-1})^{-1}, \quad (\text{B44})$$

$$j_{Ec}^2 = ((\dot{k}^2)^{-1} + (\dot{b}_S^{2c} S_2^c)^{-1} + (\dot{b}_P^{2c} P_1^c)^{-1} - (\dot{b}_S^{2c} S_2^c + \dot{b}_P^{2c} P_1^c)^{-1})^{-1}, \quad (\text{B45})$$

where $\dot{b}_P^{ic}$ and $\dot{b}_S^{1c}$ are the binding rates of products and substrate 1 in the cytoplasm (the affinity depends on the environment because it involves a transport process). Product 2 flux $j_P^{12} = j_E^{12} / \gamma_{EP}$ that is taken up by species 1 can be partitioned into a flux from the mantle and from inside the body as

$$j_{Pm}^{12} = j_P^{12} \theta; \quad j_{Pe}^{12} = j_P^{12} (1 - \theta); \quad \theta = \dot{b}_P^{2m} / (\dot{b}_P^{2m} + \dot{b}_P^{1c} P_2^c), \quad (\text{B46})$$

where the fraction θ weighs the contribution from the mantle, relative to that from the cytoplasm. The flux of product 2 that is excreted from the bodies of species 1

into the mantle is the difference between the production by internal species 2 and the consumption by species 1 of that flux, so $j_{Pc}^{12} V_2^c - j_{Pc}^{12} V_1$, which must be non-negative.

The change in the concentrations in the mantle (S_2^m, P_1^m) and the cytoplasm (S_2^c, P_2^c) of substrates and products are

$$\frac{d}{dt} S_2^m = -\dot{k}_S^2 S_2^m - j_{Sm}^2 V_2^m + \dot{b}_{Sm}^2 V_1 S_2 - \dot{b}_{mS}^2 V_1 S_2^m + \dot{b}_{cS}^2 V_1 S_2^c - \dot{b}_{Sc}^2 V_1 S_2^m, \quad (B47)$$

$$\frac{d}{dt} S_2^c = -\dot{k}_S^2 S_2^c - j_{Sc}^2 V_2^c - \dot{b}_{cS}^2 V_1 S_2^c + \dot{b}_{Sc}^2 V_1 S_2^m, \quad (B48)$$

$$\frac{d}{dt} P_1^m = -\dot{h}_P^1 P_1^m + j_{Pm}^1 V_1 - j_{Pm}^{21} V_2^m - \dot{b}_{mP}^1 V_1 P_1^m + \dot{b}_{Pm}^1 V_1 P_1 - \dot{b}_{Pc}^1 V_1 P_1^m + \dot{b}_{cP}^1 V_1 P_1^c, \quad (B49)$$

$$\frac{d}{dt} P_1^c = -\dot{h}_P^1 P_1^c + j_P^1 V_1 - j_{Pc}^{21} V_2^c + \dot{b}_{Pc}^1 V_1 P_1^m - \dot{b}_{cP}^1 V_1 P_1^c, \quad (B50)$$

$$\frac{d}{dt} P_2^m = -\dot{h}_P^2 P_2^m + j_{Pm}^2 V_2^m + j_{Pc}^2 V_2^c - j_P^{12} V_1 - \dot{b}_{mP}^2 V_1 P_2^m + \dot{b}_{Pm}^2 V_1 P_2 - \dot{b}_{Pc}^2 V_1 P_2^m + \dot{b}_{cP}^2 V_1 P_2^c, \quad (B51)$$

$$\frac{d}{dt} P_2^c = -\dot{h}_P^2 P_2^c + j_{Pc}^2 V_2^c + j_{Pc}^{12} V_1 + \dot{b}_{Pc}^2 V_1 P_2^m - \dot{b}_{cP}^2 V_1 P_2^c, \quad (B52)$$

where $j_P^{12} = j_{Pm}^{12} + j_{Pc}^{12}$. Note that j_{Pc}^i, j_{Pc}^{ji} and j_{Sc}^2 in the cytoplasm are similar to j_{Pm}^i, j_{Pm}^{ji} and j_{Sm}^2 in the mantle. Substrate 1 is taken up by the host at the outer membrane, so S_1^c is non-existent; product 2 is also taken up by the host at its outer membrane from the mantle space (on the basis of concentration at specific rate $\dot{b}_{cP}^2 P_2^c$) as well as internally (on flux basis at specific rate j_{Pc}^{12}). The specific transport rates $\dot{b}_{Sc}^i V_1, \dot{b}_{cS}^i V_1, \dot{b}_{Pc}^i V_1$ and $\dot{b}_{cP}^i V_1$ of substrates and products across the interface between the mantle space and the cytoplasm are again proportional to V_1 because the surface area of this interface is proportional to V_1 .

Fig. 4, step 3, gives V_1^*, V_2^*, V_2^{m*} and V_2^{c*} as functions of $\dot{h}$ for the parameter values given in Appendix D. Fig. 4, step 4, gives V_1^* and V_2^{c*} , assuming that $V_2 = V_2^m = 0$.

(5) Product fluxes rather than concentrations

We now assume that the mantle and free-living populations no longer matter. The exchange of substrates and products from the mantle to the free space is

sufficiently large to equalize the concentrations in these spaces, so $\dot{b}_{Sm}^i, \dot{b}_{mS}^i, \dot{b}_{Pm}^i, \dot{b}_{mP}^i \rightarrow \infty$, and $S_i^m \simeq S_i$ and $P_i^m \simeq P_i$. The internal product dynamics reduces to

$$\frac{d}{dt} P_1^c = -\dot{h}_P^1 P_1^c + j_P^1 V_1 - j_{Pc}^{21} V_2^c + \dot{b}_{Pc}^1 V_1 P_1 - \dot{b}_{cP}^1 V_1 P_1^c, \quad (B53)$$

$$\frac{d}{dt} P_2^c = -\dot{h}_P^2 P_2^c + j_{Pc}^2 V_2^c - j_{Pc}^{12} V_1 + \dot{b}_{Pc}^2 V_1 P_2 - \dot{b}_{cP}^2 V_1 P_2^c. \quad (B54)$$

The contribution of the external products in the assimilation reduces, $\dot{b}_{Pc}^1 V_1 P_1, \dot{b}_{Pc}^2 V_1 P_2 \rightarrow 0$, so $\theta \rightarrow 0$ and $j_{Pc}^{12} \rightarrow j_P^{12}$ and $j_{Pc}^{21} \rightarrow j_P^{21}$. Fig. 4, step 5, gives V_1^* and V_2^{c*} as functions of $\dot{h}$ for the parameter values given in Appendix D.

Assimilation becomes product-limited, $\dot{b}_S^{ic} S_i^c \gg \dot{b}_P^{ic} P_i^c$, and the assimilation capacity greatly exceeds the actual rate, $\dot{k}^i \gg j_{Ec}^i$. The exchange of products is now directly on the basis of fluxes, so we replace $\dot{b}_P^{2c} P_1^c$ by $\rho_P^2 j_P^2 V_1 / V_2^c$ and $\dot{b}_P^{1c} P_2^c$ by $\rho_P^1 j_P^1 V_2^c / V_1$ in the assimilation process, where ρ_P^i represents the binding probability of product j to the receptors of species i . The affinities for products $\dot{b}_P^{ic}$ become large; the internal product concentrations P_i^c become small and their dynamics no longer affect the population dynamics. We now have

$$j_E^1 = ((\dot{k}^1)^{-1} + (\dot{b}_S^1 S_1)^{-1} + (\rho_P^1 j_P^2 V_2^c / V_1)^{-1} - (\dot{b}_S^1 S_1 + \rho_P^1 j_P^2 V_2^c / V_1)^{-1}), \quad (B55)$$

$$j_E^2 = ((\dot{k}^2)^{-1} + (\dot{b}_S^2 S_2^c)^{-1} + (\rho_P^2 j_P^1 V_1 / V_2^c)^{-1} - (\dot{b}_S^2 S_2^c + \rho_P^2 j_P^1 V_1 / V_2^c)^{-1}). \quad (B56)$$

Equations for j_P^i in (B2), and equations (B3), (B4), (B26) and (B27) still apply. Notice that these equations define j_E^i implicitly only, because these quantities also appear in j_P^i .

The difference between the fluxes of produced and consumed product is excreted into the environment,

$$\frac{d}{dt} S_1 = \dot{J}_{Sr}^1 - \dot{h}_S^1 S_1 - j_S^1 V_1, \quad (B57)$$

$$\frac{d}{dt} S_2 = \dot{J}_{Sr}^2 - \dot{h}_S^2 S_2 + \dot{b}_{Sc}^2 S_2 V_1 - \dot{b}_{cS}^2 S_2 V_1, \quad (B58)$$

$$\frac{d}{dt} S_2^c = -\dot{b}_{Sc}^2 S_2^c V_1 + \dot{b}_{cS}^2 S_2 V_1 - j_S^2 V_2^c - \dot{h}_S^2 S_2^c, \quad (B59)$$

$$\frac{d}{dt} P_1 = j_P^1 V_1 - j_P^{12} V_2^c - \dot{h}_P^1 P_1, \quad (B60)$$

$$\frac{d}{dt} P_2 = j_P^2 V_2^c - j_P^{21} V_1 - \dot{h}_P^2 P_2. \quad (B61)$$

At steady-state we have $m_i^* \dot{k}_E^i = j_E^{i*}$, and $\dot{r}^{i*} = (j_E^{i*} - \dot{k}_M^i y_{EV}^i) / (j_E^{i*} / \dot{k}_E^i + y_{EV}^i)$. Weak homeostasis requires $\dot{r}^{1*} = \dot{r}^{2*}$.

(6) Free-living endosymbionts

If the exchange rates $\dot{b}_{S_c}^2$ and $\dot{b}_{cS}^2$ are large enough, the variable S_2^c becomes redundant because $S_2^c = S_2$, and equation (B1) applies again. This completes the internalization process. The system variables are reduced again to those with which we started: $S_1, S_2, V_1, m_1, V_2^c, m_2$, while the product concentrations P_1 and P_2 still present, but no longer affect the system. The products still have an important role in regulating the growth of species 2 relative to that of species 1 *via* j_E^i . Fig. 4, step 6, gives V_1^* and V_2^* as functions of $\dot{h}$ for the parameter values given in Appendix D.

This stage of obligate endosymbionts living inside their hosts represents a natural resting point in the derivation. Later steps will deal with the situation where the endosymbionts lose their metabolic independence, and eventually become fully integrated. We list the full system while suppressing superscript c in V_2^c , while $i = 1, 2$ and $j = 3 - i$. The dynamic system is perfectly mirror-symmetrical in i and j , so the notation does not reveal which species lives inside which.

$$\frac{d}{dt} S_i = \dot{J}_{Sr}^i - j_S^i V_i - \dot{h}_S^i S_i \quad \text{with} \quad j_S^i = y_{SE}^i j_E^i, \quad (\text{B62})$$

$$\frac{d}{dt} P_i = j_P^i V_i - j_P^j V_j - \dot{h}_P^i P_i \quad \text{with} \quad j_P^j = y_{PE}^j j_E^j, \quad (\text{B63})$$

$$j_P^i = y_{PE}^i j_E^i + y_{PM}^i \dot{k}_M^i + y_{PG}^i \dot{r}^i, \quad (\text{B64})$$

$$\frac{d}{dt} V_i = (\dot{r}^i - \dot{h}_V^i) V_i \quad \text{with} \quad \dot{r}^i = \frac{\dot{k}_M^i m_i - \dot{k}_M^i y_{EV}^i}{m_i + y_{EV}^i}, \quad (\text{B65})$$

$$\frac{d}{dt} m_i = j_E^i - \dot{k}_E^i m_i, \quad (\text{B66})$$

$$j_E^i = ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i)^{-1} + (\rho_P^i j_P^j V_j / V_i)^{-1} - (\dot{b}_S^i S_i + \rho_P^i j_P^j V_j / V_i)^{-1})^{-1}, \quad (\text{B67})$$

where $y_{PE}^i = y_{PS}^i y_{SE}^i$. Notice that assimilation fluxes (j_E^1, j_E^2) are given implicitly only. The next step in the derivation shifts this implicitness from assimilation to growth $\dot{r}^i$. The product concentrations P_i and the parameters $\dot{h}_P^i$ and y_{PE}^j are of interest in relation to mass balance problems, but are no longer of importance to the dynamical properties of the system. Thus we consider six state variables and 24 parameters.

An alternative route is that the affinity for substrate 2, $\dot{b}_{S_c}^2$, becomes large and the symbionts' uptake is

limited by the hosts' uptake of that substrate. In this case the amount of the symbionts' biomass in the uptake is replaced by the hosts' biomass, and the equations are no longer skew-symmetric in 1 and 2; we will not explore this route in detail.

We now consider the ratio of the structures at steady-state, and obtain constraints that make this ratio independent of the growth conditions. This is a preparation for the merging of structures, which is the next step in the evolution of homeostasis.

From equation (B25), and $j_E^{i*} = \dot{k}_E^i m_i^*$ at steady-state, we know that

$$\begin{aligned} \frac{V_j^*}{V_i^*} &= \frac{2\rho_P^i j_P^j (1/\dot{k}_2^i - 1/\dot{k}_1^i)}{1 - \dot{k}_1^i / \dot{k}_2^i + \sqrt{(\dot{k}_1^i / \dot{k}_2^i - 1)(\dot{k}_1^i / \dot{k}_2^i + 3)}} \\ &= \frac{1 - \dot{k}_1^i / \dot{k}_2^i + \sqrt{(\dot{k}_1^j / \dot{k}_2^j - 1)(\dot{k}_1^j / \dot{k}_2^j + 3)}}{2\rho_P^j j_P^i (1/\dot{k}_2^j - 1/\dot{k}_1^j)} \end{aligned} \quad (\text{B68})$$

with $\dot{k}_1^i = \dot{b}_S^i S_i^*$ and $1/\dot{k}_2^i = 1/j_E^{i*} - 1/\dot{k}^i$ for $i = 1, 2$.

Under chemostat conditions, where $\dot{h}_V^i = \dot{h}_S^i = \dot{h}_P^i = \dot{h}$ and $\dot{J}_{Sr}^i = \dot{h} S_r^i$ for $i = 1, 2$, we have at steady-state $\dot{r}^{i*} = \dot{h}$ and for $i = 1, 2$,

$$m_i^* = y_{EV}^i \frac{\dot{k}_M^i + \dot{h}}{\dot{k}_E^i - \dot{h}} \quad \text{with} \quad \frac{d}{d\dot{h}} m_i^* = y_{EV}^i \frac{\dot{k}_E^i + \dot{k}_M^i}{(\dot{k}_E^i - \dot{h})^2}, \quad (\text{B69})$$

$$j_E^{i*} = \dot{k}_E^i m_i^* \quad \text{with} \quad \frac{d}{d\dot{h}} j_E^{i*} = \dot{k}_E^i \frac{d}{d\dot{h}} m_i^*, \quad (\text{B70})$$

$$\begin{aligned} j_P^i &= y_{PE}^i j_E^{i*} + y_{PM}^i \dot{k}_M^i + y_{PG}^i \dot{h} \quad \text{with} \\ \frac{d}{d\dot{h}} j_P^i &= y_{PE}^i \dot{k}_E^i \frac{d}{d\dot{h}} m_i^* + y_{PG}^i, \end{aligned} \quad (\text{B71})$$

$$\begin{aligned} j_E^{i*} &= ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i^*)^{-1} + (\rho_P^i j_P^j V_j^* / V_i^*)^{-1} - \\ &(\dot{b}_S^i S_i^* + \rho_P^i j_P^j V_j^* / V_i^*)^{-1})^{-1}, \end{aligned} \quad (\text{B72})$$

$$S_i^* = S_r^i - y_{SE}^i j_E^{i*} V_i^* / \dot{h}. \quad (\text{B73})$$

This set of equations is implicit in S_i^* and V_i^* for $i = 1, 2$. We simplify the analysis by making use of the observation that the flux ranges for which substrate and product co-limit growth of the endosymbiont are really narrow, so j_E^{i*} reduces to either $j_E^{i*} = ((\dot{k}_i^i)^{-1} + (\rho_P^i j_P^j V_j^* / V_i^*)^{-1})^{-1}$ or $j_E^{i*} = ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i^*)^{-1})^{-1}$ for most of the ranges of the product and substrate fluxes. Below we first address product limitation, then discuss substrate limitation.

(a) Species j is product-limited

We first analyse the situation where species j is not limited by substrate, e.g. by $y_{SE}^j \rightarrow 0$, while $y_{SE}^i > 0$

(otherwise we would have a thermodynamic miracle). Alternatively we can take b_s^j or $\dot{f}_{S_r}^j \rightarrow \infty$. The assumption here is that $\rho_{PJ}^j V_i^* / V_j^* \ll b_s^j S_j^*$, so substrate S_j is no longer of relevance to the dynamic system in the neighbourhood of the steady-state. The parameters $\dot{f}_{S_r}^j$, $\dot{h}_s^j$, b_s^j are also no longer relevant, which leaves a system with five state variables and 20 parameters. We now have

$$j_E^j = ((\dot{k}^j)^{-1} + (\rho_{PJ}^j V_i / V_j)^{-1})^{-1}. \quad (B74)$$

We ask under what constraints does the ratio of the structures not depend on growth conditions, and so on $\dot{h}$, i.e. under what constraints do we have $(d/dh)(V_j/V_i)=0$? The derivation of the constraints to achieve a ratio of structures that is independent of growth conditions at steady-state does not involve hosts' assimilation directly. The host, species i , might, therefore, be substrate as well as product limited.

The derivation starts at a fixed but tunable specific growth rate $\dot{h}$, from which a reserve density follows, and, therefore an assimilation rate, and a substrate concentration.

From equation (B74) we know that the ratio of the structures equals

$$\frac{V_j}{V_i} = \rho_{PJ}^j \left(\frac{1}{j_E^j} - \frac{1}{\dot{k}^j} \right). \quad (B75)$$

For a structure ratio that is independent of $\dot{h}$, we must have that

$$\left(1 - \frac{j_E^j}{\dot{k}^j} \right) \frac{d}{dh} \ln j_P^j = \frac{d}{dh} \ln j_E^j. \quad (B76)$$

Substitution of the steady-state relationships into the constraint (B76) results in

$$0 = \left(y_{PE}^i \dot{k}_E^i \frac{d}{dh} m_i^* + y_{PG}^i \right) (1 - m_j^* \dot{k}_E^j / \dot{k}^j) m_j^* - (y_{PE}^b \dot{k}_E^i m_i^* + y_{PM}^i \dot{k}_M^i + y_{PG}^i h) \frac{d}{dh} m_j^*. \quad (B77)$$

for $\dot{k}_E^i = \dot{k}_E^j = \dot{k}_E$, and multiplication by $(\dot{k}_E - \dot{h})^4$, this constraint reduces to a fourth-degree polynomial in the throughput rate being equal to zero, $0 = \sum_{s=0}^4 a_s \dot{h}^s$ with fixed coefficients a_s ; this only holds true if $a_s = 0$ for all s . From $a_4 = -y_{PG}^j (1 + y_{EV}^j \dot{k}_E / \dot{k}^j) = 0$ we must have that $y_{PG}^j = 0$, and the constraint reduces for $y_{PE}^j y_{EV}^j = y_{PV}^j$ to

$$0 = (1 - m_j^* \dot{k}_E / \dot{k}^j) m_j^* y_{PE}^i \dot{k}_E \frac{d}{dh} m_i^* - (y_{PE}^i \dot{k}_E m_i^* + y_{PM}^i \dot{k}_M^i) \frac{d}{dh} m_j^* \quad (B78)$$

or

$$0 = (1 - m_j^* \dot{k}_E / \dot{k}^j) m_j^* \alpha - y_{PE}^i m_i^* - y_{PM}^i \dot{k}_M^i / \dot{k}_E \quad \text{with} \quad \alpha = \frac{\dot{k}_E + \dot{k}_M^i}{\dot{k}_E + \dot{k}_M^j} \frac{y_{PV}^i}{y_{EV}^j}. \quad (B79)$$

After multiplication with $(\dot{k}_E - \dot{h})^2$, this equation has the form $0 = \sum_{s=0}^2 a_s \dot{h}^s$. From $a_2 = 0$, $a_1 = 0$ and $a_0 = 0$ we must have $y_{PE}^j > 0$ and

$$\left(1 + y_{EV}^j \frac{\dot{k}_E}{\dot{k}^j} \right) \frac{\dot{k}_E + \dot{k}_M^i}{\dot{k}_E + \dot{k}_M^j} = 1 - \frac{y_{PM}^i}{y_{PV}^i} \frac{\dot{k}_M^i}{\dot{k}_E}, \quad (B80)$$

$$\left(\dot{k}_E - \dot{k}_M^j - 2y_{EV}^j \dot{k}_E \frac{\dot{k}_M^j}{\dot{k}^j} \right) \frac{\dot{k}_E + \dot{k}_M^i}{\dot{k}_E + \dot{k}_M^j} = \dot{k}_E - \dot{k}_M^i - 2\dot{k}_M^i \frac{y_{PM}^i}{y_{PV}^i}, \quad (B81)$$

$$\left(1 - y_{EV}^j \frac{\dot{k}_M^j}{\dot{k}^j} \right) \frac{\dot{k}_E + \dot{k}_M^i}{\dot{k}_E + \dot{k}_M^j} = \left(1 + \frac{y_{PM}^i}{y_{PV}^i} \right) \frac{\dot{k}_M^i}{\dot{k}_M^j}. \quad (B82)$$

These three constraints are mutually dependent, and reduce to the constraints that $\dot{k}^j \rightarrow \infty$ (i.e. $y_{EV}^j / \dot{k}^j = 0$, but y_{EV}^j cannot approach to zero for thermodynamic reasons) and $(y_{PM}^i / y_{PV}^i) = (\dot{k}_E / \dot{k}_M^i) (\dot{k}_M^j - \dot{k}_M^i) / (\dot{k}_E + \dot{k}_M^j)$, which were derived in Kooijman (2000, p. 303). The host must, therefore, not be able to supersaturate the endosymbiont with its product, which can always be achieved by reducing the binding probability ρ_P^j for product by the endosymbiont (which can be under hosts' control). We also used the constraints of equal reserve turnover rates, $\dot{k}_E^i = \dot{k}_E^j = \dot{k}_E$, of an absence of product formation in association with growth, $y_{PG}^j = 0$, and of a positive product formation in association with assimilation, $y_{PE}^j > 0$.

Notice that these are constraints at steady-state to achieve a ratio of structures that is independent of growth conditions. Under those conditions we can allow product consumption (rather than production) in association with maintenance, so a negative value for y_{PM}^i . This cannot apply to transient conditions, however, since situations can occur in which product is consumed that is not formed (assimilation stops if substrate is temporarily absent, while maintenance continues). In practice, this means that y_{PM}^i should be non-negative or $\dot{k}_M^j \geq \dot{k}_M^i$.

The ratio of the structures settles at

$$\frac{V_j}{V_i} = \rho_P^j \frac{y_{PV}^i + y_{PM}^i}{y_{EV}^j \dot{k}_M^j / \dot{k}_M^i}. \quad (B83)$$

Notice that the substrate supply rates $\dot{J}_{Sr}^i$ and hosts' assimilation are not involved in the derivation of the constraints for a constant ratio of structures at steady-state.

(i) *Species i is product limited*

If the host and symbiont are both product limited ($\dot{b}_S^1, \dot{b}_S^2 \rightarrow \infty$, or $\dot{J}_{Sr}^1, \dot{J}_{Sr}^2 \rightarrow \infty$), we have an autonomous system, with no feedback to its environment:

$$\frac{d}{dt} V_i = (\dot{r}^i - \dot{k}_V^i) V_i \quad \text{with} \quad \dot{r}^i = \frac{\dot{k}_E^i m_i - \dot{k}_M^i \mathcal{Y}_{EV}^i}{m_i + \mathcal{Y}_{EV}^i}, \quad (\text{B84})$$

$$\frac{d}{dt} m_i = \dot{j}_E^i - \dot{k}_E^i m_i \quad \text{with} \quad \dot{j}_E^i = ((\dot{k}^i)^{-1} + (\rho_{PJ}^i V_j / V_i)^{-1})^{-1}, \quad (\text{B85})$$

$$\dot{j}_P^i = \mathcal{Y}_{PE}^i \dot{j}_E^i + \mathcal{Y}_{PM}^i \dot{k}_M^i + \mathcal{Y}_{PG}^i \dot{r}^i. \quad (\text{B86})$$

This theoretical system has curious properties. Numerical studies suggest that the ratio of specific growth rates always evolves to a constant value, for shrinking as well as growing populations and all physically meaningful parameter values. This value has been found to be very close to 1 for a broad range of parameter values, which means that an almost constant ratio of structures is a basic property of the model. The ratio of specific growth rates has to be exactly equal to 1 for an asymptotically constant ratio of structures. This perfection is perhaps more of mathematical than of biological interest.

With the reserves at steady-state, $m_i^* = \dot{j}_E^i / \dot{k}_E^i$, and equal reserve turnover rates, $\dot{k}_E^1 = \dot{k}_E^2 = \dot{k}_E$, and specific death rates, $\dot{h}_V^1 = \dot{h}_V^2$, and no product formation in association with growth, $\mathcal{Y}_{PG}^1 = \mathcal{Y}_{PG}^2 = 0$, we have that the ratio $v = V_j / V_i$ changes as

$$\frac{d}{dt} \ln v = \dot{r}^2 - \dot{r}^1 = \frac{\dot{j}_E^2 - \dot{k}_M^2 \mathcal{Y}_{EV}^2}{\dot{j}_E^2 / \dot{k}_E^2 + \mathcal{Y}_{EV}^2} - \frac{\dot{j}_E^1 - \dot{k}_M^1 \mathcal{Y}_{EV}^1}{\dot{j}_E^1 / \dot{k}_E^1 + \mathcal{Y}_{EV}^1}, \quad (\text{B87})$$

$$\begin{aligned} \dot{j}_E^1 &= ((\dot{k}^1)^{-1} + (\rho_{PJ}^1 v)^{-1})^{-1} \quad \text{with} \\ \dot{j}_P^2 &= \mathcal{Y}_{PE}^2 \dot{j}_E^2 + \mathcal{Y}_{PM}^2 \dot{k}_M^2, \end{aligned} \quad (\text{B88})$$

$$\begin{aligned} \dot{j}_E^2 &= ((\dot{k}^2)^{-1} + (\rho_{PJ}^2 / v)^{-1})^{-1} \quad \text{with} \\ \dot{j}_P^1 &= \mathcal{Y}_{PE}^1 \dot{j}_E^1 + \mathcal{Y}_{PM}^1 \dot{k}_M^1. \end{aligned} \quad (\text{B89})$$

The fluxes $\dot{j}_E^1$ and $\dot{j}_E^2$ can be solved explicitly, but the result is complicated. For $\dot{k}_M^1 = \dot{k}_M^2$ or $\mathcal{Y}_{PM}^1 = \mathcal{Y}_{PM}^2 = 0$, the result simplifies to

$$\dot{j}_E^1 = v \dot{k}^1 \frac{\rho_{PE}^1 \mathcal{Y}_{PE}^1 - (\rho_{PE}^2 \mathcal{Y}_{PE}^2)^{-1}}{v \rho_{PE}^1 \mathcal{Y}_{PE}^1 + \dot{k}^1 / \dot{k}^2}, \quad (\text{B90})$$

$$\dot{j}_E^2 = \dot{k}^2 \frac{\rho_{PE}^2 \mathcal{Y}_{PE}^2 - (\rho_{PE}^1 \mathcal{Y}_{PE}^1)^{-1}}{\rho_{PE}^2 \mathcal{Y}_{PE}^2 + v \dot{k}^2 / \dot{k}^1}. \quad (\text{B91})$$

(ii) *Species i is substrate limited*

If the host is substrate limited (small $\dot{b}_S^i$ or $\dot{J}_{Sr}^i$), and $\dot{j}_E^i = ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i)^{-1})^{-1}$ or $S_i = (\dot{b}_S^i / \dot{j}_E^i - \dot{b}_S^i / \dot{k}^i)^{-1}$, we find hosts' steady-state structure from $V_i^* = \dot{h} (S_i^* - S_i^*) / (\mathcal{Y}_{ES}^i \dot{j}_E^*)$, with $\dot{j}_E^* = \dot{k}_E \mathcal{Y}_{EV}^i (\dot{k}_M^i + \dot{h} / \dot{k}_E - \dot{h})$. Symbionts' steady-state structure (without application of the constraints for constant ratio of structures) is $V_j^* = \rho_P^j V_i^* (\dot{j}_P^* / \dot{j}_E^* - \dot{j}_P^* / \dot{k}^j)$, with $\dot{j}_E^* = \dot{k}_E \mathcal{Y}_{EV}^j (\dot{k}_M^j + \dot{h}) / (\dot{k}_E - \dot{h})$ and $\dot{j}_P^* = \mathcal{Y}_{PE}^j \dot{j}_E^* + \mathcal{Y}_{PM}^j \dot{k}_M^j + \mathcal{Y}_{PG}^j \dot{h}$. The maximum throughput rate without complete washout is $\dot{h}_{\max} = \dot{k}_E (1 - \mathcal{Y}_{EV}^j \dot{k}_M^j / \dot{j}_E^j) / (1 - \mathcal{Y}_{EV}^i \dot{k}_E / \dot{j}_E^i)$ with $\dot{j}_E^i = ((\dot{k}^i)^{-1} + (\dot{b}_S^i S_i^*)^{-1})^{-1}$, while $V_i^* = 0$ and $S_i^* = S_i^i$.

(iii) *Marr–Pirt and Monod models*

At this point in the reasoning, it is illuminating to reduce the argument to its bare essentials and investigate what happens if we remove the reserves by letting $\dot{k}_E^i \rightarrow \infty$, so $m_i = 0$. The DEB model for V1-morphs then reduces to the popular Marr–Pirt model, which includes the even more popular Monod model (where $\dot{k}_M^i = 0$).

The specific growth rate then equals $\dot{r}^i = \dot{j}_E^i / \mathcal{Y}_{EV}^i - \dot{k}_M^i$ and the specific product formation rate becomes $\dot{j}_P^i = \mathcal{Y}_{PE}^i \dot{j}_E^i + \mathcal{Y}_{PM}^i \dot{k}_M^i$. Notice that the term for product formation in association with growth becomes redundant, because growth is now linear in assimilation and maintenance. We now have for $\dot{r}^i = \dot{h}$ that $\dot{j}_E^i = \mathcal{Y}_{EV}^i (\dot{h} + \dot{k}_M^i)$, and $(d/dh) \dot{j}_E^i = \mathcal{Y}_{EV}^i$, for $i = 1, 2$.

From equation (B75), we find for that $\dot{j}_{PV}^i = \mathcal{Y}_{PE}^i \mathcal{Y}_{EV}^i$

$$\begin{aligned} \frac{d}{dh} \frac{V_j^*}{V_i^*} &= \rho_P^j \mathcal{Y}_{PV}^j \left(\frac{\mathcal{Y}_{VE}^j}{\dot{h} + \dot{k}_M^j} - \frac{1}{\dot{k}^j} \right) \\ &\quad - (\mathcal{Y}_{PV}^j (\dot{h} + \dot{k}_M^j) + \mathcal{Y}_{PM}^j \dot{k}_M^j) \frac{\rho_P^j \mathcal{Y}_{VE}^j}{(\dot{h} + \dot{k}_M^j)^2}. \end{aligned} \quad (\text{B92})$$

Equating this to zero to find constraints for ratios' independence of growth conditions, and multiplication with $(\dot{h} + \dot{k}_M^j)^2$ leads to

$$0 = -\rho_P^j \mathcal{Y}_{PV}^j (\dot{h} + \dot{k}_M^j)^2 / \dot{k}^j - \rho_P^j \mathcal{Y}_{PM}^j \dot{k}_M^j \mathcal{Y}_{VE}^j. \quad (\text{B93})$$

This has the form $\sum_{s=0}^2 a_s \dot{h}^s$, which only holds true for $a_0 = a_1 = a_2 = 0$. From $a_2 = 0$, it follows that $\dot{k}^j \rightarrow \infty$, which also follows from $a_1 = 0$. From $a_0 = 0$, it follows that $\dot{j}_{PM}^j = 0$. The ratio of the structures settles at

$$\frac{V_j^*}{V_i^*} = \rho_P^j \frac{\mathcal{Y}_{PV}^j \dot{k}_M^j}{\mathcal{Y}_{EV}^j \dot{k}_M^j}. \quad (\text{B94})$$

In the case of the Monod model, where $\dot{k}_M = 0$ for $i = 1, 2$, we have $V_j^*/V_i^* = \rho_P^j \gamma_{PV}^j / \gamma_{EV}^j$.

(b) *Species j is substrate limited*

We now analyse the situation where species j is not limited by product and

$$j_E^* = ((\dot{k}^j)^{-1} + (\dot{b}_S^j S_j^*)^{-1})^{-1} = \frac{\dot{k}^j S_j^*}{K^j + S_j^*} \quad \text{or} \quad S_j^* = (\dot{b}_S^j / j_E^* - \dot{b}_S^j / \dot{k}^j)^{-1}. \quad (\text{B95})$$

This equation for j_E^* does not fix the ratio of the structures (unlike the case of product limitation, where it did). Given $\dot{r}^{j*} = \dot{h}$, this fixes m_j^* , and so j_E^* , and S_j^* . We also have $V_j^* = \dot{h}(S_j^* - S_j^*) / (\gamma_{SE}^j j_E^*)$. We now consider the cases where species i is product or substrate limited.

(i) *Species i is product limited*

If species i is product limited, while j is substrate limited, we have

$$j_E^* = ((\dot{k}^i)^{-1} + (\rho_{PjP}^i V_j / V_i)^{-1})^{-1}. \quad (\text{B96})$$

The ratio of the structures is, similar to equation (B75), given by

$$\frac{V_j}{V_i} = \rho_{PjP}^i \left(\frac{1}{j_E^*} - \frac{1}{\dot{k}^i} \right). \quad (\text{B97})$$

The rest of the derivations again apply for interchanging indices i and j . Our conclusion is that if one of the species is substrate-limited, but not both species, simple constraints for homeostasis apply for the ratio of structures that are independent of growth conditions. These constraints concern values of energy parameters. The value of the ratio is a simple function of these energy parameters.

(ii) *Species i and j are substrate-limited*

We now assume that $\rho_{PjP}^j V_i^* / V_j^* \gg \dot{b}_S^j S_j^*$ for $i = 1, 2$ in the neighbourhood of the steady-state. If species i and j are both substrate-limited, and equation (B95) applies to both species, the ratio of the structures is given by

$$\frac{V_j^*}{V_i^*} = \frac{S_j^* - S_j^*}{\gamma_{SE}^j j_E^*} \frac{\gamma_{SE}^i j_E^*}{S_i^* - S_i^*} = \frac{\gamma_{SE}^i}{\gamma_{SE}^j} \frac{S_j^* / j_E^* - K^j / (\dot{k}^j - j_E^*)}{S_i^* / j_E^* - K^i / (\dot{k}^i - j_E^*)} \quad (\text{B98})$$

for $K^i = \dot{k}^i / \dot{b}_S^i$. The constraint $(d/d\dot{h})(V_j^* / V_i^*) = 0$ leads to

$$(S_r^j (j_E^*)^{-2} + K^j (\dot{k}^j - j_E^*)^{-2}) \frac{d}{d\dot{h}} j_E^* = (S_r^i (j_E^*)^{-2} + K^i (\dot{k}^i - j_E^*)^{-2}) \frac{V_j^*}{V_i^*} \frac{d}{d\dot{h}} j_E^*, \quad (\text{B99})$$

with $j_E^* = \dot{k}_E^i \gamma_{EV}^i (\dot{k}_M^i + \dot{h}) / (\dot{k}_E^i - \dot{h})$ and $(d/d\dot{h}) j_E^* = \dot{k}_E^i \gamma_{EV}^i (\dot{k}_E^i + \dot{k}_M^i) / (\dot{k}_E^i - \dot{h})^2$. For $\dot{k}_E^i = \dot{k}_E^j = \dot{k}_E$, this constraint reduces to

$$S_r^j (j_E^*)^{-2} + K^j (\dot{k}^j - j_E^*)^{-2} = \alpha S_r^i (j_E^*)^{-2} + \alpha K^i (\dot{k}^i - j_E^*)^{-2} \quad \text{with} \quad \alpha = \frac{V_j^* \gamma_{EV}^j}{V_i^* \gamma_{EV}^i} \frac{\dot{k}_E + \dot{k}_M^j}{\dot{k}_E + \dot{k}_M^i}. \quad (\text{B100})$$

For stringent substrate limitation, $\dot{k}^i \gg j_E^*$ for $i = 1, 2$, this constraint reduces to $j_E^* = j_E^* \sqrt{\alpha S_r^i / S_r^j}$. Independence from $\dot{h}$ is achieved for $\dot{k}_M^i = \dot{k}_M^j = \dot{k}_M$, and the constraint takes the form $(\gamma_{EV}^j / \gamma_{EV}^i) = (S_r^i / S_r^j) (V_j^* / V_i^*)$, while from equation (B98) we know that $(V_j^* / V_i^*) = (\gamma_{SE}^i / \gamma_{SE}^j) (\gamma_{EV}^j / \gamma_{EV}^i) (S_r^j / S_r^i)$. The constraint boils down to $j_{SE}^i = j_{SE}^j$, thus $(V_j^* / V_i^*) = (\gamma_{EV}^j / \gamma_{EV}^i) (S_r^j / S_r^i)$.

In the case of substrate-limited growth by both species, the ratio of the structures at steady-state is independent of growth conditions, if the limitation is stringent ($\dot{k}^i \rightarrow \infty$ for $i = 1, 2$), the reserve turnover rates are equal ($\dot{k}_E^i = \dot{k}_E^j = \dot{k}_E$), the maintenance rate coefficients are equal ($\dot{k}_M^i = \dot{k}_M^j = \dot{k}_M$), and the yield of reserve on substrate are equal ($\gamma_{SE}^i = \gamma_{SE}^j = \gamma_{SE}$). Since growth is not limited by products in this case, details of product formation are here irrelevant. Although we obtained constraints to achieve a ratio of structures that is independent of the growth rate, the ratio still depends on the availability of substrate via S_r^i / S_r^j .

Notice that rather stringent limitation (of at least one partner) and equal reserve turnover rates are requirements for a constant ratio of structures in all cases.

(7) Single structure

Now we consider the process of merging of structures when both substrates are required. We assume that the constraints for constant ratio of the structures at steady-state conditions apply, and we take that ratio as constant also during transient states. This allows us to merge the structures V_1 and V_2 into a new structure V .

Let us denote $v = V_2 / V_1$ and $V = V_1 + V_2$, so $V_1 = V / (1 + v)$ and $V_2 = Vv / (1 + v)$. We also take $\dot{h}_V^1 = \dot{h}_V^2 = \dot{h}_V$, $\dot{k}_E^1 = \dot{k}_E^2 = \dot{k}_E$ and $\gamma_{PG}^1 = \gamma_{PG}^2 = 0$.

The changes of the system variables are

$$\frac{d}{dt} S_i = \dot{J}_{Sr}^i - \dot{h}_S^i S_i - j_S^i V \quad \text{with} \quad j_S^i = \dot{k}_S^i / (1 + K_S^i / S_i), \quad (\text{B101})$$

$$\frac{d}{dt} P_i = j_P^i V - j_P^i V - \dot{h}_P^i P_i \quad \text{with} \quad j_P^i = \gamma_{PS}^i j_S^i + \gamma_{PM}^i \dot{k}_M^i + \gamma_{PG}^i \dot{r}, \quad (\text{B102})$$

$$\frac{d}{dt} V = (\dot{r} - \dot{h}_V) V, \quad (\text{B103})$$

$$\dot{r} = \left(\sum_i \left(\frac{\dot{k}_E^i - \dot{r}}{y_{EV}^i/m_i} - \dot{k}_M^i \right)^{-1} - \left(\sum_i \frac{\dot{k}_E^i - \dot{r}}{y_{EV}^i/m_i} - \dot{k}_M^i \right)^{-1} \right)^{-1}, \quad (\text{B104})$$

$$\frac{d}{dt} m_i = j_E^i - m_i \dot{k}_E^i \quad \text{with} \quad j_E^i = j_S^i / y_{SE}^i. \quad (\text{B105})$$

with the half-saturation concentration $K_S^i = \dot{k}_S^i / \dot{b}_S^i$. Notice that the specific growth rate $\dot{r}$ is now given implicitly only. The steady-state values under chemostat conditions must be obtained numerically. The products now determine only the relative substrate uptake capacities.

Fig. 4, step 5, gives V^* as a function of $\dot{h}$ for the parameter values given in Appendix D.

(8) Single substrate, single product, single reserve

We now suppose that the substrates are coupled in abundance; if the substrate supply rates vary in time, they do so proportionally. This eliminates the need to take into account supplementarity explicitly. The merging of substrates, products and reserves is possible by considering them as generalized compounds. We have $S = S_1 + S_2$, $\dot{J}_{Sr} = \dot{J}_{Sr}^1 + \dot{J}_{Sr}^2$, $P = P_1 + P_2$, $\dot{h}_S = \dot{h}_S^1 + \dot{h}_S^2$, $\dot{h}_P = \dot{h}_P^1 + \dot{h}_P^2$, $y_{PS} = y_{PS}^1 + y_{PS}^2$, $y_{PM} = y_{PM}^1 + y_{PM}^2$, $y_{PG} = y_{PG}^1 + y_{PG}^2$, $\dot{k}_E^1 = \dot{k}_E^2$, $K^1 = K^2$, $\dot{J}_{Sr}^1 / \dot{J}_{Sr}^2 = \dot{k}_S^1 / \dot{k}_S^2$.

The changes of the system variables are

$$\frac{d}{dt} S = \dot{J}_{Sr}^i - \dot{h}_S S - j_S V \quad \text{with} \quad j_S = \dot{k}_S / (1 + K/S), \quad (\text{B106})$$

$$\frac{d}{dt} P = j_P V - \dot{h}_P P \quad \text{with} \quad j_P = y_{PS} j_S + y_{PM} \dot{k}_M + y_{PG} \dot{r}, \quad (\text{B107})$$

$$\frac{d}{dt} V = (\dot{r} - \dot{h}_V) V \quad \text{with} \quad \dot{r} = \frac{\dot{k}_E m - \dot{k}_M y_{EV}}{m + y_{EV}}, \quad (\text{B108})$$

$$\frac{d}{dt} m = j_E - \dot{k}_E m \quad \text{with} \quad j_E = y_{ES} j_S, \quad (\text{B109})$$

with the half-saturation concentration $K = \dot{k}_S / \dot{b}_S$. Notice that only in this very simple case we can formulate assimilation directly in terms of substrate uptake; in a more complex situation we have to deduce substrate uptake from reserve synthesis.

The steady-states under chemostat conditions are $\dot{r}^* = \dot{h}$, which gives $m^* = y_{EV}(\dot{k}_M + \dot{h}) / (\dot{k}_E - \dot{h})$; so $j_E^* = \dot{k}_E m^*$, or $f^* = (\dot{k}_E m^* / y_{ES} \dot{k}_S)$, or $S^* = K f^* / (1 - f^*)$; this gives $V^* = (S_r - S^*) \dot{h} / j_S^*$ with $j_S^* = \dot{k}_S f^*$. Finally, we have $P^* = V^* j_P^* / \dot{h}$, with $j_P^* = y_{PS} j_S^* + y_{PM} \dot{k}_M + y_{PG} \dot{h}$.

Comparison of equations (B1–B4) with (B106–B109) shows that this completes the merging of the two species into a single one that behaves similarly to the original ones. During the integration steps, the system lost considerable freedom in response to environmental conditions. This becomes visible if the substrates no longer co-vary in the environment, and limit growth independently. We then have the following situation.

The reserve flux, and the changes in substrates and products are

$$j_E = (\dot{k}^{-1} + (\dot{b}_S^1 S_1)^{-1} + (\dot{b}_S^2 S_2)^{-1} - (\dot{b}_S^1 S_1 + \dot{b}_S^2 S_2)^{-1})^{-1}, \quad (\text{B110})$$

$$\frac{d}{dt} S_i = \dot{J}_{Sr}^i - \dot{h}_S^i S_i - j_S^i V \quad \text{with} \quad j_S^i = y_{SE}^i j_E, \quad (\text{B111})$$

$$\frac{d}{dt} P_i = j_P^i V - \dot{h}_P^i P_i \quad \text{with} \quad j_P^i = y_{PS}^i j_S^i + y_{PM}^i \dot{k}_M + y_{PG}^i \dot{r}. \quad (\text{B112})$$

The growth potential of this system under varying substrate conditions is less than that in the case of two reserves. Fig. 4, step 8, gives V^* as a function of $\dot{h}$ for the parameter values given in Appendix D; it still includes two substrates and two products, but with one structure and one reserve.

XI. APPENDIX C: CONSTRAINTS ON DEB PARAMETER VALUES

Several constraints apply to the parameter values of the series of models. We here evaluate only those on energy aspects on the assumption that only (non-limiting) nutrients are required for growth, in addition to substrate and product, that do not serve as energy source. Let us denote the chemical potentials by $\mu_S^i, \mu_P^i, \mu_V^i, \mu_E^i$, for $i = 1$ or 2 .

If product and substrate are substitutable, we must have

$$\mu_P^j > \mu_E^i y_{EP}^i + \mu_P^i y_{PP}^i \quad \text{because} \quad P_j \rightarrow y_{EP}^i E_i + y_{PP}^i P_i, \quad (\text{C1})$$

$$\mu_S^i > \mu_E^i y_{ES}^i + \mu_P^i y_{PS}^i \quad \text{because} \quad S_i \rightarrow y_{ES}^i E_i + y_{PS}^i P_i, \quad (\text{C2})$$

$$\mu_E^i > \mu_{VE}^i \gamma_{VE}^i + \mu_{PG}^i \gamma_{PG}^i \quad \text{because} \quad E_i \rightarrow \gamma_{VE}^i V_i + \gamma_{PG}^i P_i, \quad (\text{C3})$$

$$\mu_E^i > \mu_{PM}^i \gamma_{PM}^i / \gamma_{EP}^i \quad \text{because} \quad E_i \rightarrow P_i \gamma_{PM}^i / \gamma_{EP}^i. \quad (\text{C4})$$

If the product and substrate are supplementary, the first two constraints must be replaced by

$$(\mu_P^i - \mu_{PP}^i) / \gamma_{EP}^i + \mu_S^i / \gamma_{ES}^i > \mu_E^i \quad \text{because} \\ S_i \gamma_{EP}^i / \gamma_{ES}^i + P_j \rightarrow \gamma_{EP}^i E_i + \gamma_{PP}^i P_i. \quad (\text{C5})$$

XII. APPENDIX D: PARAMETER VALUES

The following parameters have been used to illustrate symbiogenesis. The formulae show which parameters apply at any merging stage; the number of parameters per stage is much less than this list suggests. The following parameters have been used to illustrate symbiogenesis. The formulae show which parameters apply at any merging stage; the number of parameters per stage is much less than this list suggests

$\dot{k}_E^1 = 3.0$	$\dot{k}_E^2 = 2.5$	h^{-1}	$\gamma_{E+}^1 = 0.9$	$\gamma_{E+}^2 = 0.9$	mol mol^{-1}
$\dot{k}_M^1 = 0.002$	$\dot{k}_M^2 = 0.004$	h^{-1}	$\gamma_{S+}^1 = 0.7$	$\gamma_{S+}^2 = 0.7$	mol mol^{-1}
$\dot{k}_S^1 = 0.4$	$\dot{k}_S^2 = 0.3$	h^{-1}	$\gamma_{P+}^1 = 0.2$	$\gamma_{P+}^2 = 0.2$	mol mol^{-1}
$\dot{k}_P^1 = 1.7$	$\dot{k}_P^2 = 1.5$	h^{-1}	$\dot{b}_{Sm}^2 = 1$	$\dot{b}_{mS}^2 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\dot{b}_S^1 = 0.2$	$\dot{b}_S^2 = 0.1$	$\text{mol mol}^{-1} \text{h}^{-1}$	$\dot{b}_{Sc}^2 = 1$	$\dot{b}_{cS}^2 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\dot{b}_P^1 = 0.3$	$\dot{b}_P^2 = 0.2$	$\text{mol mol}^{-1} \text{h}^{-1}$	$\dot{b}_{Pm}^1 = 1$	$\dot{b}_{mP}^1 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\gamma_{EV}^1 = 1.2$	$\gamma_{EV}^2 = 1.2$	mol mol^{-1}	$\dot{b}_{Pm}^2 = 1$	$\dot{b}_{mP}^2 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\gamma_{ES}^1 = 0.7$	$\gamma_{ES}^2 = 0.7$	mol mol^{-1}	$\dot{b}_{Pc}^1 = 1$	$\dot{b}_{cP}^1 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\gamma_{EP}^1 = 0.7$	$\gamma_{EP}^2 = 0.7$	mol mol^{-1}	$\dot{b}_{Pc}^2 = 1$	$\dot{b}_{cP}^2 = 1$	$\text{mol mol}^{-1} \text{h}^{-1}$
$\gamma_{PS}^1 = 0.2$	$\gamma_{PS}^2 = 0.2$	mol mol^{-1}	$\dot{b}_P^{1c} = 1$		$\text{mol mol}^{-1} \text{h}^{-1}$
$\gamma_{PP}^1 = 0.2$	$\gamma_{PP}^2 = 0.2$	mol mol^{-1}	$\rho_P^1 = 0.8$	$\rho_P^2 = 0.9$	—
$\gamma_{PM}^1 = 0.6$	$\gamma_{PM}^2 = 0.6$	mol mol^{-1}	$\gamma_{PE}^1 = 2$	$\gamma_{PE}^2 = 2$	mol mol^{-1}
$\gamma_{PG}^1 = 0.0$	$\gamma_{PG}^2 = 0.0$	mol mol^{-1}	$\gamma_{PE}^{12} = 0.2$	$\gamma_{PE}^{21} = 0.2$	mol mol^{-1}
$\dot{k}^1 = 1.5$	$\dot{k}^2 = 1.7$	h^{-1}	$\gamma_{PE}^{12} = 0.2$	$\gamma_{PE}^{21} = 0.2$	mol mol^{-1}
$\dot{k}_S^{r1} = 1.5$	$\dot{k}_S^{r2} = 1.5$	h^{-1}	$\dot{k} = 0.7$	$\dot{k}_E = 3$	h^{-1}
$\dot{k}_P^{r1} = 0.5$	$\dot{k}_P^{r2} = 0.4$	h^{-1}	$\dot{k}_M = 0.01$		h^{-1}
$\dot{b}_{SP}^1 = 0.05$	$\dot{b}_{SP}^2 = 0.05$	$\text{mol mol}^{-1} \text{h}^{-1}$	$\gamma_{EV} = 1.2$		mol mol^{-1}
$\dot{b}_{PS}^1 = 0.06$	$\dot{b}_{PS}^2 = 0.09$	$\text{mol mol}^{-1} \text{h}^{-1}$	$S_r^1 = 100$	$S_r^2 = 200$	mM