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Simulation of evolution implemented in the mutualistic symbioses towards enhancing their ecological efficiency, functional integrity and genotypic specificity

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ABSTRACT

We created the mathematical model for the evolution of the Efficiency of Mutualistic Symbioses (EMS) which was estimated as the microsymbiont impacts on the host's reproductive potential. Using the example of rhizobia–legume interaction, the relationships were studied between EMS and Functional Integrity of Symbiosis (FIS) which is represented as a measure for concordance of changes in the partners' genotypic frequencies under the environmental fluctuations represented by the minor deviations of the systemic model parameters. The FIS indices correlate positively with EMS values suggesting an enhancement of FIS *via* the natural selection operating in the partners' populations in favor of high EMS. Due to this selection, nodular habitats may be closed for colonization by the non-beneficial bacterial strains and the Genotypic Specificity of Mutualism (GSM) in partners' interactions is enhanced: the selective advantage of host-specific *vs* non-host-specific mutualists is increasing. The novelty of our model is to suggest a selective background for macroevolutionary events reorganizing the structure and functions of symbiotic systems and to present its evolution as a result of shifting the equilibrium between different types of mutualists under the impacts of the symbiosis-stipulated modes of natural selection.

0. Introduction

Mutualistic symbioses represent the fundamental strategy for the cooperative adaptation of organisms towards the unfavorable environment which is often based on the functional, structural and genetic integration of partners resulted in developing the novel phenotypic traits (Tikhonovich and Provorov, 2009). Analysis of the broad spectrum of symbioses demonstrated that the enhancement of the ecological efficiency of mutualism (its impacts on the partners' fitness; Douglas, 1994) depends on the two basic properties of symbiotic systems: their structural/functional integrity and genotypic specificity. For example, evolution of N_2-fixing legume–rhizobia nodular symbiosis involved a marked increase of partners' integrity expressed as: (i) transition from inter-cellular to intra-cellular location of N_2-fixing rhizobia cells (development of mechanisms for endocytosis and formation of stable intra-cellular organelles, symbiosomes; in some legumes it is followed by differentiation of rhizobia into the non-viable bacteroids); (ii) complication of the pathways for root infection by rhizobia (from a "primitive" epidermal crack entry to the more "advanced" uptake of bacteria *via* the specially deformed root hairs); (iii) increased efficiency for assimilation of fixed N, e.g., *via* its incorporation into ureids (instead of amides) reducing the C cost of symbiosis (Sprent, 2001, 2007; Provorov and Vorobyov, 2010). A coordinated increase of the symbiosis integrity and efficiency of partners' interactions may be traced also in the evolution of insect-microbe symbioses which involved transitions from the inter- to intra-cellular location of microbial cells (implementing the synthesis of essential metabolites: amino acids, vitamins, etc.) resulted in enhancing the mutual partners' dependency and the host's adaptive potential (Ishikawa, 1989; Douglas, 1989; Baumann and Moran, 1997).

The other important evolutionary trend followed by mutualistic symbiosis is represented by increasing specificity in the partners' interactions—a dependency of symbiotic traits expression on the particular combinations of the host and microsymbiont genotypes. Phylogenetic and population-genetic analyses of the legume–rhizobia systems suggest that the enhancement of N_2 fixation and of the specificity of partners' genotypic interactions occur in parallel (Dorosinsky and Lazareva, 1968; Provorov and Vorobyov, 2010). The highest N_2-fixing activities were revealed in the "Galegoid" legumes (e.g., in *Medicago sativa* and *Galega orientalis*) which represent the narrow cross-inoculation groups separated strictly from the other legumes in their symbiotic affinities (Provorov, 1994). Two-factor analysis of variance for the mutualism indices in different strain-cultivar

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combinations (which ensures a dissection of genetic variation of symbiotic traits into additive/genotype-non-specific and non-additive/genotype-specific components; Mytton et al., 1984; Provorov and Tikhonovich, 2003) suggests that the average symbiotic efficiency correlates strongly with the non-additive genotypic impacts of legumes and rhizobia (which represent the specificity of mutualistic interactions), not with the additive impacts of the partners' genotypes.

The genetic control and phenotypic expression of the legume–rhizobia symbiotic specificity is studied comprehensively at the level of nodulation which is dependent on the signaling interactions; the majority of *nod*/*nol*/*noe* genes involved in these interactions (encoding the principle symbiotic signal, lipo-chito-oligosaccharide Nod factor) are restricted to the particular rhizobia species and have the well defined host-specific manifestations (Ovtsyna and Staehelin, 2005). In contrast, the control of specificity at the level of N_2-fixing activity is studied poorly since the majority of *nif* /*fix* genes responsible for the nitrogenase synthesis/operation are common to the diverse rhizobia species and are characterized by a non-host-specific manifestation. Curiously, in spite of the well defined phenotypic expression of host specificity of N_2 fixation, few examples of *hsf* genetic factors are available (Wilson et al., 1987; Chun et al., 1994; Oh et al., 2001; Miller et al., 2007) suggesting some important differences in the genetic control and evolutionary mechanisms for the nodulation and N_2 fixation specificities.

The development of the quantitative (mathematical) descriptions for the symbioses integrity and specificity is important to reconstruct the partners' co-evolution for a high efficiency of mutualism. In this paper, we suggest the indices for the Functional Integrity of Symbiosis (FIS) which represent the measure for concordance of deviations in partners' genotypic frequencies under the environmental fluctuations. For the newly developed FIS indices, we demonstrated the positive correlations with the Efficiency of Mutualistic Symbiosis (EMS) assessed as an impact of bacterial inoculation on the seed production by the host. We demonstrate that the natural selection responsible for a high EMS may lead to increasing the structural/functional integrity of mutualistic symbioses and the Genotypic Specificity of Mutualism (GSM) which is measured as a selective advantage of host-specific *vs* non-host-specific mutualists in the microsymbiont populations. This approach enables us to correlate the microevolutionary processes (resulted in the EMS and GSM changes) to the macroevolutionary ones (represented by FIS changes) providing a novel approach to analyze the trade-off between adaptive (increased partners' fitness) and progressive (improved structural/functional complexity of symbiosis) components in the evolution of mutualistic interactions.

1. Simulation of partners' co-evolution

Developing the previously suggested approaches (Provorov and Vorobyov, 2000, 2006, 2008a,b), we simulated the microevolution in rhizobia–legume symbiosis as a cyclic process, wherein each (t-th) cycle involves: (i) generation of novel (mutant) bacterial genotypes with the altered symbiotic traits occurring in the soil habitat; (ii) bacterial inoculation of the symbiotic (rhizospheric, nodular) habitats offered by plants (*via* the bacterial migration from soil into rhizospheric habitats followed by a migration into nodular habitats); (iii) bacterial colonization of the inoculated habitats (*via* multiplication of bacterial inoculants) accompanied by the formation of seeds by plants; (iv) release of bacteria from rhizosphere and nodules followed by the differential extinction of strains in soil resulted in the novel free-living population structure being initial for the next, $t+1$-th cycle (Appendix, Tables A.1–A.5; Fig. 1A).

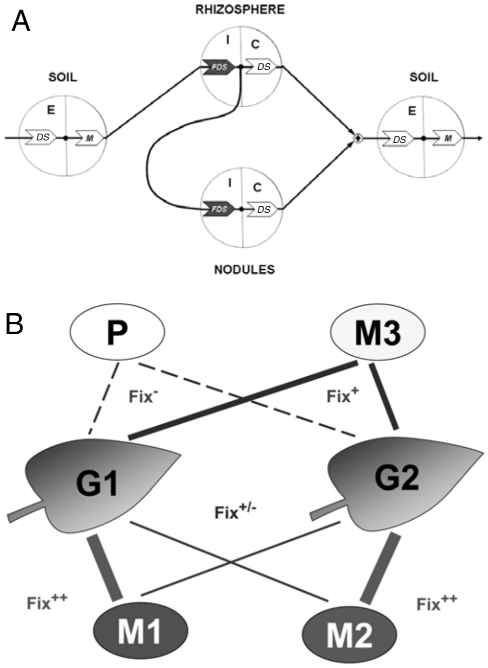

Fig. 1. Structure of the simulated symbiotic system. A. Schematic representation of microevolutionary processes. The population dynamics of rhizobia in the habitats available within the "plant–soil" system is represented by the inoculation (I), colonization (C) and extinction (E) events during which the shifts in genetic structures occur due to Darwinian selection (DS), frequency-dependent selection (FDS) or mutation (M) pressures; ⊕ represents the summation of the bacterial numbers after release of rhizobia from symbiotic (rhizospheric, nodular) habitats into soil where the population wave is formed (mathematical description of relevant processes is given in Appendix, Tables A.1–A.5). B. Genotypic plant-microbial interactions. The system is composed of dimorphic plant population (genotypes G1, G2) and low-polymorphic bacterial population: parental "cheating" strain P forming non-N_2-fixing (Fix^-) nodules with both plant genotypes and its three N_2-fixing mutants—M1, M2, M3. The partners' genotypic combinations involving the mutant genotypes were classified into three types according to N_2-fixing activity: (1) specific—with maximal activity, Fix^{++} (G1/M1, G2/M2); (2) non-specific—with intermediate activity, Fix^+ (G1/M3, G2/M3); (3) anti-specific—with minimal activity, $Fix^{+/-}$ (G1/M2, G2/M1). Ratio of N_2-fixing activities in the genotypic combinations Fix^{++} : Fix^+ : $Fix^{+/-}$ is 3:2:1 (Appendix, Table A.6).

With respect to the previously published models (Provorov and Vorobyov, 2000, 2006, 2008a), the novel model contains a set of parameters and formulae which enables us to give a comprehensive description of: (a) partners' metabolic feedbacks responsible for evolution of mutualistic traits in the *in planta* rhizobia population (b_{ki} parameters for the specific N_2-fixing activities, Appendix: Tables A.4 and A.6); (b) availability of nodular habitat for colonization of particular rhizobia strains (c0, ci parameters: Table A.3) required to present the macroevolutionary events in the plants. The latter are quantified using the newly introduced indices for the Functional Integrity of Symbiosis (FIS) represented as a measure of concordance for the changes in partners' genotypic frequencies induced by the fluctuations of systemic parameters. The suggested model innovations enabled us to address the trade-off between the progressive and adaptive evolution of the symbiotic system which represents the major point of novelty in this paper.

1.1. Genetic structure and ecological efficiency of symbiosis

We constructed the system (Fig. 1B) composed of a dimorphic plant population (genotypes G1, G2) and a low-polymorphic bacterial population which consists of the non-N_2-fixing parental strain (P) and its three N_2-fixing mutants representing different types of the Genotypic Specificity of Mutualism (GSM). M1