On the Host-Symbiont-Cycle of a Leafhopper (*Euscelis plebejus*) Endosymbiosis

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Summary. In the host-symbiont-cycle of *Euscelis plebejus* and its bacterial symbionts each of both symbionts ('*a*' and '*t*') appears in an 'infection form' during the intraovarial transmission (adult female) as well as during the entrance into the mycetocytes (embryo) and in a 'vegetative form' during the remaining time of the cycle.

Half a century ago Buchner[1] published the first comprehensive paper on leafhopper endosymbiosis including 103 species. Until now 436 leafhopper species have been examined. Of these, in only 10 species, belonging to the subfamily Typhlocybinae, no intracellular symbionts were found. The majority of the remaining species were associated with 2 or 3 different types of endosymbiotic microorganisms, while in some rare cases even 4, 5 or 6 such obligate endosymbiotic microorganisms were found[2]. Except for the so-called 'Hefen' (yeasts) or '*H*-symbionts'[3], all leafhopper symbionts are probably aberrant forms of bacteria. The fact that so far leafhopper symbionts cannot be cultivated in vitro makes their identification very difficult. Furthermore, very little is known concerning the host-symbiont interactions.

The common leafhopper *Euscelis plebejus* Fall. has 2 types of symbionts, which according to the nomenclature of Müller[4], are called '*a*-symbiont' and '*t*-symbiont'. The *a*- and the *t*-symbiont of *Euscelis* are transmitted from one generation to the next by transovarial infection, which represents the typical way of transmission of leafhopper symbionts[1]: Transmission forms leave the symbiont organ, the so-called 'mycetome'[5], of the adult female and reach the ovaries via the hemolymph. There they enter into the posterior pole of the oocyte through distinct follicle cells.

Material and methods. Euscelis plebejus was reared as previously described[6,7]. The development of the mycetome was studied by histological standard techniques. For

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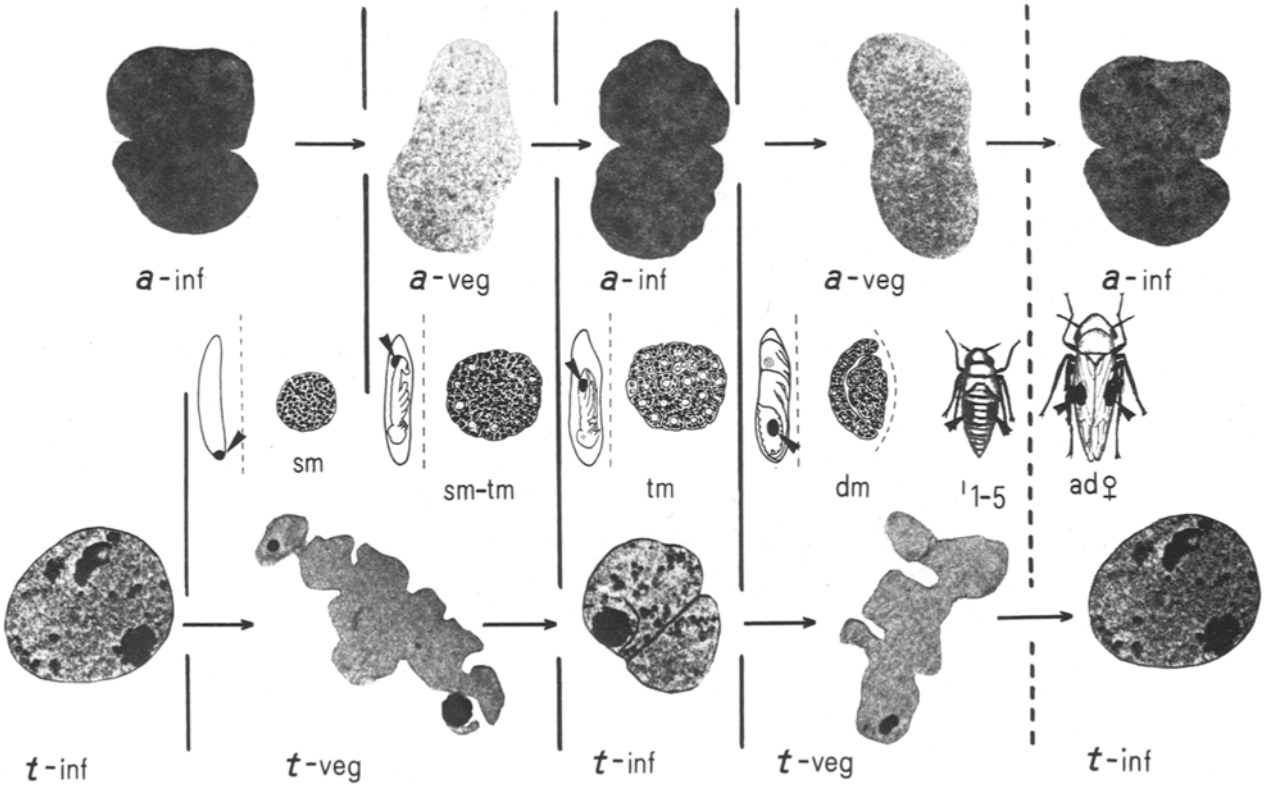

The diagrammatic host-symbiont-cycle of the leafhopper *Euscelis plebejus* Fall. shows the different forms of the *a*-symbiont (top) and of the *t*-symbiont (bottom) with regard to the corresponding developmental stages of the host and of the mycetome (centre). *a*- (or *t*-) inf, infection form of the *a*- (or *t*-) symbiont; *a*- (or *t*-) veg, vegetative form of the *a*- (or *t*-) symbiont; sm, symbiont mass; tm, transitory mycetome; dm, definitive mycetone; l_{1-5}, larval instars; ad ♀, adult female. For details see[7,8,12].

the ultrastructural studies, the embryo and the mycetomes of various stages were removed from the eggs and prepared for electron microscopy[8].

Results and discussion. During embryogenesis of the host, the paired symbiont organs develop in its abdomen. Following the basic investigations of the embryonic development of *Euscelis plebejus* by SANDER[6,9], the single steps between the infection mass of the symbionts and the mature mycetome could be studied[7]. The three main results of the above studies can be defined as follows: 1. Both types of symbionts are incorporated at specific times by distinct cells of the embryo. 2. Translocation experiments of the symbiont mass within the egg suggested that other cell types are not capable of incorporating the symbionts[10]. 3. When the symbionts were experimentally eliminated from the egg, embryogenesis as well as development of the (symbiont free) mycetome proceeded just as in the control eggs[11].

Electron microscopic examinations of the two symbionts during various stages of mycetome development show that each of both symbionts occurs in two forms which differ in their morphology. One of these forms, which appears during the entrance of the symbiont into the prospective mycetocytes, is called the 'infection form'[8]. The second form, called the 'vegetative form', is present during the remaining time of the host's embryogenesis.

The infection form of the *a*-symbiont often shows binary fission stages and appears extremely electron-dense. In contrast, the vegetative form of the *a*-symbiont exhibits lower electron density and is probably unable to divide. Enzymatic digestion experiments and the large number of ribosomes suggest that the infection form of the *a*-symbiont has an increased protein synthesis[8].

The corresponding forms of the *t*-symbiont, which show only slight variation in their electron density, can easily be distinguished by their characteristic morphology: the infection form of the *t*-symbiont appears more spherical, whereas the vegetative form has lobed contours[12].

BUCHNER[1] named the *a*- and *t*-symbionts which enter into the ovaries, 'transmission form' or 'infection form'. Infection forms and vegetative forms of the adult mycetome of the female correspond in their morphology to the infection forms and the vegetative forms in the embryonic mycetome. Thus, *a*- and *t*-symbionts develop a specific infection form, which serves not only for the transmission to the following host generations but also for invasion of the prospective mycetocytes of the embryonic mycetome. In analogy to a parasite-host-cycle, a simplified symbiont-host-cycle can be postulated in which a specific form of the symbiont is correlated with a certain developmental stage of the mycetome and of the host (Figure).

A similar pleomorphism to that mentioned above, has been described for numerous intracellular symbiotic bacteria. The symbionts of coleopterous families such as Nosodendridae, Chrysomelidae, Curculionidae, Silvanidae, Lyctidae, as well as of the Trypetidae (Diptera) for instance, develop an infection form designed to continue the symbiotic relationship[13]. In these groups investigations concerned with the behaviour of the symbionts during embryogenesis of the host are very rare up to now.

A remarkable feature of the *a*- and *t*-symbiont of *Euscelis plebejus* is that during the ovarial transmission, as well as during the development of the mycetome, the symbiotic microorganisms pass an extracellular stage before they are incorporated de novo by specialized host cells.

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In vitro Attachment of Trypanosomes to Plastic

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Summary. Description of an in vitro system for the study of the attachment of trypanosomes to polystyrene flasks by means of hemidesmosomes. This type of attachment, whose significance is so far unknown, reproduces a natural stage in the life cycle of medically important trypanosomatid flagellates in their vector.

Recently, several workers have studied the ultrastructure of trypanosomatid flagellates during their development in their insect hosts. The results so far have shown that all members of the Family Trypanosomatidae (of which many are of medical or veterinary importance) present developmental stages ('haptomonads') which are attached by their flagellar tips ot the cuticular lining of the gut wall by means of 'hemidesmosomes'. This general pattern has been found so far in the *Trypanosoma* subgenera *Herpetosoma*[2], *Duttonella*[3], *Trypanozoon*[4], *Megatrypanum*[5], in the insect flagellate genera *Crithidia*[6] and *Herpetomonas*[7] and in the genus *Leishmania*[8].

The molecular basis of the attachment, as well as the role played by such a mechanism in the life cycle of these parasites is still unknown, but these results suggest that attachment is an indispensable step for the establishment of infection and subsequent transmission. This communication reports the development of an in vitro model of the haptomonad attachment.

Material and methods. The in vitro culture of trypanosomes was carried out as previously described[9] in a 25 cm² Falcon flask in the presence of BHK-cells, an overlay of R.P.M.I. 1640 and 10% foetal bovine serum.

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