Chapter 3
Transovarial Transmission of Symbionts in Insects

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Abstract Many insects, on account of their unbalanced diet, live in obligate symbiotic associations with microorganisms (bacteria or yeast-like symbionts), which provide them with substances missing in the food they consume. In the body of host insect, symbiotic microorganisms may occur intracellularly (e.g., in specialized cells of mesodermal origin termed bacteriocytes, in fat body cells, in midgut epithelium) or extracellularly (e.g., in hemolymph, in midgut lumen). As a rule, symbionts are vertically transmitted to the next generation. In most insects, symbiotic microorganisms are transferred from mother to offspring transovarially within female germ cells. The results of numerous ultrastructural and molecular studies on symbiotic systems in different groups of insects have shown that they have a large diversity of symbiotic microorganisms and different strategies of their transmission from one generation to the next. This chapter reviews the modes of transovarial transmission of symbionts between generations in insects.

3.1 Introduction

Many animals are host to obligate symbiotic microorganisms (bacteria, fungi, protozoans), which may play various roles, e.g., supplement their unbalanced diet (in clams, tube worms living in deep-sea hydrothermal and cold vents, hematophagous insects and leeches, plant sap-sucking insects), digest complex carbohydrates (in termites), emit bioluminescence (in squids, fish), increase resistance against parasites and pathogens (in aphids, carpenter ants), and stimulate immune system (gut bacteria in mammals) (reviewed in Abt and Artis 2009; Baumann 1998; Buchner 1965; Dale and Moran 2006; Dasch et al. 1984; Kikuchi 2009). These microorganisms may be inherited in three ways: (1) vertically (maternally), i.e., from mother to offspring; (2) horizontally, i.e., between specimens of the same population; and (3) environmentally, i.e., through the acquisition of free-living microorganisms from the environment (Bright and Bulgheresi 2010; Kikuchi

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2009; Kikuchi and Fukatsu 2003). In most animals, the symbionts are transmitted to the next generation vertically; however, the mechanism of the vertical transmission in particular groups of animals may vary. In some heteropteran bugs, gut symbionts are transmitted to the progeny through the contamination of the egg surface with microorganisms, the deposition of capsules containing bacteria onto the egg surface, and feeding on mother's excrements by the newly hatched larva (Fukatsu and Hosokawa 2002; Hosokawa et al. 2005; Kikuchi et al. 2007). In most animals, which have obligate symbiotic microorganisms, they are transmitted to the next generation transovarially, i.e., through the infestation of female germ cells.

It is estimated that at least 15–20% (according to Ishikawa 2003, more than 70%) of species of insects harbor obligate bacteria and/or yeast-like symbionts (reviewed, e.g., in Baumann 2005; Buchner 1965; Douglas 1989, 1998; Ishikawa 1989; Kikuchi 2009; Moran and Baumann 2000). Results of recently conducted molecular studies have confirmed an earlier hypothesis based on morphological observations and experiments with sterile insects and eggs (Buchner 1965) that in most insects their symbionts are responsible for the synthesis of substances missing in the diet, required for the proper growth and reproduction of the host insect (Douglas 2009; McCutcheon and Moran 2007; McCutcheon et al. 2009; Wu et al. 2006). Microorganisms living in the body of hematophagous insects such as tsetse flies, triatomid bugs, lice, and bedbugs supply them with vitamin B (Dasch et al. 1984; Ishikawa 2003). Most plant sap-sucking hemipterans (aphids, scale insects, whiteflies, psyllids, cicadas, leafhoppers, treehoppers, spittlebugs, planthoppers) harbor symbiotic microorganisms, which provide them with the amino acids missing in the plant sap (Douglas 2009; Ishikawa 2003; Wilkinson and Ishikawa 2001). As a rule, symbiotic microorganisms are localized intracellularly in the insect body: in fat body cells, in the cells of the midgut epithelium, in the specialized giant cells of mesodermal origin termed bacteriocytes (the older term "mycetocytes"). Microorganisms living inside bacteriocytes are termed "mycetomic symbionts."

Paul Buchner, who is regarded as the father of studies on the symbiotic associations between insects and microorganisms, distinguished two types of symbionts: primary symbionts (later termed P-symbionts) and accessory symbionts (later termed secondary symbionts, S-symbionts, facultative symbionts) (Buchner 1965). Because the association between primary symbionts and insects is the result of a single, ancient infection of ancestor of this group of insects, these microorganisms are present in all individuals of the insect taxon (bacterium *Buchnera aphidicola* in aphids, bacterium *Carsonella ruddii* in psyllids, bacterium *Portiera aleyrodidarum* in whiteflies). The primary symbionts are, as a rule, harbored in bacteriocytes and are always transovarially transmitted between generations (Buchner 1965). The function of P-symbionts is to supplement the diet of the host insect with essential nutrients. In contrast to primary symbionts, secondary symbionts may occur in only some insect populations. They may be localized intracellularly in bacteriocytes (or in other cells, e.g., fat body cells) or extracellularly (e.g., in the hemolymph). These symbionts may be inherited both transovarially and horizontally (Fukatsu et al. 2000; Moran and Telang 1998;

Oliver et al. 2006, 2010). The results of studies on aphids have indicated that their S-symbionts belong to different taxa and may play various roles, such as increase of the resistance to heat stress or parasites. It is likely that they may also take over the function of the lost primary symbiont, bacterium *Buchnera aphidicola*, and provide nutrients (Burke et al. 2009; Lukasik et al. 2013: Koga et al. 2003; Montllor et al. 2002; Oliver et al. 2003; Scarborough et al. 2005; Vorburger et al. 2010). The above facts indicate that the presence of secondary symbionts in insects results from more recent, multiple, independent infections. A more complex situation has been found in hemipterans belonging to Auchenorrhyncha, which encompasses groups such cicadas, leafhoppers, treehoppers, planthoppers, and spittlebugs. As a rule, auchenorrhynchans are characterized by the occurrence of two types of symbiotic microorganisms (termed by Takiya et al. (2006) "coprimary symbionts"), both of which are engaged in the synthesis of amino acids. For example, in the glassy-winged sharpshooter, *Homalodisca vitripennis* (Cicadomorpha, Cicadellidae: Cicadellinae) one of the two symbionts synthesizes eight out of ten essential amino acids, whereas the second one is responsible for the production of the remaining two amino acids (McCutcheon and Moran 2007). The symbiotic systems of Auchenorrhyncha are unusual because, during the evolution of some lineages of this hemipteran group, ancient symbiotic microorganisms, such as Bacteroidetes bacterium *Sulcia muelleri* and betaproteobacterial symbionts, have been replaced by other bacteria or yeast-like symbionts (McCutcheon et al. 2009; Koga et al. 2013; Takiya et al. 2006).

Results of earlier histological observations (Buchner 1965; Ries 1931; Schneider 1940) and more recent ultrastructural studies (Cheng and Hou 2001; Cheung and Purcell 1999; Eberle and McLean 1982; Kobiałka et al. 2016; Koga et al. 2012; Michalik et al. 2014a, b, 2016; Pyka-Fosciak and Szklarzewicz 2008; Sacchi et al. 1988, 2008; Swiatoniowska et al. 2013; Szklarzewicz and Moskal 2001; Szklarzewicz et al. 2006, 2010, 2013, 2016; Żelazowska and Biliński 1999) have revealed that insects use a variety of mechanisms of transovarial transmission of mycetomic symbionts from mother to offspring; however, regardless of the method of the transmission, the beginning of the migration of symbionts from bacteriocytes to ovaries correlates with the stage of ovary development. This observation strongly supports the hypothesis presented by Eberle and McLean (1982), which postulates that the infestation of ovaries by symbiotic microorganisms is stimulated by a factor produced by the developing ovary.

3.2 Localization of Transovarially Transmitted Symbionts in the Insect Body

In most insects living in symbiotic association with microorganisms, the transovarially transmitted symbionts are harbored in the cytoplasm of giant, polyploid cells termed bacteriocytes, which are grouped in large organs termed

bacteriomes (Fig. 3.1a). Previously bacteriomes have been termed "mycetomes"; however, currently, the term "mycetome" designates only the organ containing yeast-like symbionts. In most insects, bacteriomes are localized between the body wall and the ovaries. In insects, which possess more than one type of symbiont (in aphids and scale insects with primary and secondary symbionts and in auchenorrhynchans with coprimary symbionts), these microorganisms are usually harbored in separate bacteriocytes (Fig. 3.1a). A few exceptions to this rule are (1) aphid *Aphis viburni* (Aphidomorpha, Aphidoidea) in which secondary symbionts occupy their own bacteriocytes and also coreside in bacteriocytes with the primary symbiont, bacterium *Buchnera aphidicola* (Fig. 3.1b) (Michalik et al. 2014a), (2) several species of adelgids (Aphidomorpha, Adelgoidea) in which betaproteobacterial and gammaproteobacterial symbionts occur in the same bacteriocytes (Toenshoff et al. 2012), and (3) whiteflies (Aleyrodomorpha) in which primary and secondary symbionts inhabit the same bacteriocytes (Costa et al. 1993; Gottlieb et al. 2008; Szklarzewicz and Moskal 2001).

In the scale insects *Icerya purchasi* and *Palaeococcus fuscipennis* (Monophlebidae) bacteriomes are composed of extremely large bacteriocytes surrounded by a single layer of small epithelial cells (Fig. 3.1c). They contain two types of symbiotic bacteria: large pleomorphic bacteria (primary symbionts) and small rod-shaped bacteria (secondary symbionts) (Niżnik and Szklarzewicz 2007; Szklarzewicz et al. 2006). The pleomorphic bacteria are harbored in the cytoplasm of bacteriocytes, whereas the rod-shaped bacteria are present in epithelial cells (Fig. 3.1c). Interestingly, in another genus of the family Monophlebidae, *Drosicha*, bacteriomes have different organization. They are composed of several lobes. The lobes' periphery contains smaller bacteriocytes with pleomorphic bacteria, whereas central syncytium contains enterobacterial symbionts (Matsuura et al. 2009). The syncytial organization of bacteriomes/mycetomes is also typical for psyllids (Kot et al. 2012, 2014) and planthoppers belonging to the families Flatidae and Delphacidae (Michalik et al. 2009). In psyllids, the bacteriomes consist of externally located bacteriocytes containing the primary symbiont (gammaproteobacterium *Carsonella ruddii*) and internal syncytium containing secondary symbionts (Fig. 3.1d), which belong to distinct clusters of Gammaproteobacteria (Fukatsu and Nikoh 1998; Kot et al. 2012, 2014; Spaulding and von Dohlen 1998; Thao et al. 2000a, b). In planthoppers from the Flatidae and Delphacidae families, the yeast-like symbionts are harbored in the syncytial mycetome, which is surrounded by a single layer of small epithelial cells (Cheng and Hou 2001; Michalik et al. 2009).

In 2001, von Dohlen and coworkers described in mealybugs (Pseudococcidae: Pseudococcinae) an unusual phenomenon (termed "nested symbiosis") of symbiotic bacteria within other bacteria. The ultrastructural, histochemical, and molecular methods showed that Pseudococcinae mealybugs are host to betaproteobacterial symbionts (named *Tremblaya princeps* by Thao et al. 2002), which in turn harbor gammaproteobacterial symbionts (Fig. 3.1e, f) (von Dohlen et al. 2001; Gatehouse et al. 2011; Husnik et al. 2013; Kono et al. 2008; McCutcheon and von Dohlen 2011; Thao et al. 2002). The molecular analyses have shown that both these
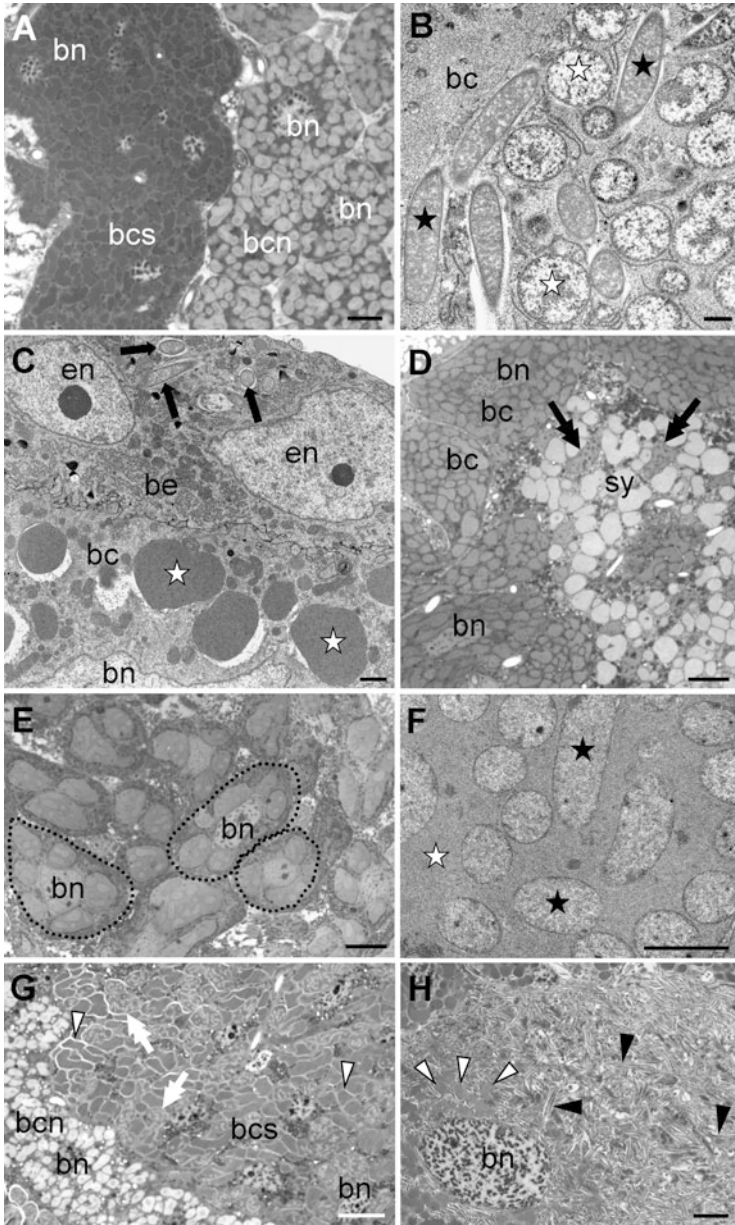
Fig. 3.1 Distribution of symbiotic microorganisms in the insect body (**a**) *Deltocephalus pulicaris* (Hemiptera, Cicadomorpha, Cicadellidae: Deltocephalinae). Fragment of the bacteriome composed of external bacteriocytes with *Sulcia muelleri* bacterium and internal bacteriocytes with the betaproteobacterial symbiont *Nasuia deltocephalinicola*. Methylene blue, scale bar = 20 µm. (**b**) *Aphis viburni* (Hemiptera, Aphidomorpha, Aphididae: Aphidinae). Primary symbiont *Buchnera aphidicola* (*white asterisks*) and secondary symbionts (*black asterisks*) in the same bacteriocyte. TEM, scale bar = 2 µm. (**c**) *Palaeococcus fuscipennis* (Hemiptera, Coccomorpha, Monophlebidae). Fragment of the bacteriocyte with pleomorphic bacteria (primary symbionts) (*white asterisks*). *Black arrows* indicate rod-shaped bacteria (secondary symbionts) in the

symbionts, like the coprimary symbionts of auchenorrhynchans (see above), contribute to the synthesis of amino acids for their host insect (Husnik et al. 2013; Husnik and McCutcheon 2016; McCutcheon and von Dohlen 2011). The similar phenomenon of "nested symbiosis" has recently been found in two species of Auchenorrhyncha: in *Macrosteles laevis* (Cicadomorpha, Cicadellidae: Deltocephalinae) and in *Cicadella viridis* (Cicadomorpha, Cicadellidae: Cicadellinae) (Kobiałka et al. 2016; Michalik et al. 2014b). In the bacteriocytes of these species, the bacteria contain other bacteria: gammaproteobacterium *Sodalis* inside of the bacterium *Sulcia* in *Cicadella viridis* and gammaproteobacterium *Arsenophonus* inside of the bacterium *Sulcia* in *Macrosteles laevis* (Fig. 3.1g) (Kobiałka et al. 2016; Michalik et al. 2014b). Moreover, in *Cicadella viridis,* before migration into the ovaries, the *Sodalis* bacteria enter the cells of *Sulcia* bacteria (Michalik et al. 2014b). This unusual phenomenon takes place in bacteriocytes containing both *Sulcia* and *Sodalis* bacteria (Fig. 3.1h). According to Michalik et al. (2014b), the symbiosis in *Cicadella viridis* represents the initial stage of the acquisition of bacterium *Sodalis*. These authors suggest that the occurrence of bacteria *Sodalis* inside of bacteria *Sulcia* may be associated with the high virulence of bacterium *Sodalis*. Thus, the "hiding" of *Sodalis* within *Sulcia* bacteria may protect them from the immune system of host insect.

In the heteropteran bug *Chilacis typhae* (Artheneidae) (Kuechler et al. 2011) and scale insect *Marchalina hellenica* (Buchner 1965), symbiotic bacteria do not occur in bacteriomes but in enlarged cells of the midgut epithelium. In both *Chilacis typhae* (Kuechler et al. 2011) and *Marchalina hellenica* (Buchner 1965; Szklarzewicz et al. 2013), symbionts leave the cells of the midgut epithelium and

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Fig. 3.1 (continued) epithelial cells. TEM, scale bar = 1 μm. (**d**) *Cacopsylla mali* (Hemiptera, Psyllomorpha, Psyllidae). Fragment of the bacteriome composed of external bacteriocytes with primary symbiont *Carsonella ruddii* and internal syncytium with secondary symbionts. *Double black arrows* indicate nuclei of the syncytium. Methylene blue, scale bar = 10 μm (courtesy of Marta Kot, Jagiellonian University, Kraków, Poland). (**e**) *Trionymus aberrans* (Hemiptera, Coccomorpha, Pseudococcidae: Pseudococcinae). Fragment of the bacteriome. Bacteriocytes (encircled by a *black dotted line*) are filled with large pleomorphic bacteria *Tremblaya princeps* which contain small, rod-shaped bacteria in the cytoplasm. Methylene blue, scale bar = 10 μm. (**f**) *Trionymus thulensis* (Hemiptera, Coccomorpha, Pseudococcidae: Pseudococcinae). Fragment of the pleomorphic bacterium *Tremblaya princeps* (*white asterisk*), which contains numerous rod-shaped bacteria (*black asterisks*). TEM, scale bar = 1 μm. (**g**) *Macrosteles laevis* (Hemiptera, Cicadomorpha, Cicadellidae: Deltocephalinae). Fragment of the bacteriome containing bacteriocytes with the bacterium *Nasuia deltocephalinicola* and bacteriocytes with the bacterium *Sulcia muelleri* (*white arrowheads*), as well as the bacterium *Sulcia muelleri* with the bacterium *Arsenophonus* inside its cells (*double white arrows*). Methylene blue, scale bar = 20 μm. (**h**) *Cicadella viridis* (Hemiptera, Cicadomorpha, Cicadellidae: Cicadellinae). Fragment of the bacteriocyte with the bacterium *Sulcia muelleri* (*white arrowheads*) and bacterium *Sodalis* (*black arrowheads*). Methylene blue, scale bar = 20 μm. *bc* bacteriocyte, *be* epithelium surrounding the bacteriome, *bcs* bacteriocyte with the bacterium *Sulcia muelleri*, *bcn* bacteriocyte with the bacterium *Nasuia deltocephalinicola*, *bn* bacteriocyte nucleus, *en* epithelial cell nucleus, *sy* syncytial region of the bacteriome containing the secondary symbionts

infest the ovaries. Because these symbionts are transovarially inherited, Kuechler et al. (2011) suggested that such a symbiosis might represent the intermediate state between the gut symbiosis and bacteriocyte symbiosis. On the other hand, in lice, the bacteriomes tightly adhere to the midgut epithelium (Buchner 1965; Eberle and McLean 1983; Ries 1931). In males, the bacteriocytes do not leave the bacteriomes, whereas in females, they migrate from the midgut bacteriome to the apical parts of lateral oviducts, where they form the large accumulations termed "ovarial ampullae" (named also "intraovarian bacteriomes") (Buchner 1965; Eberle and McLean 1983; Ries 1931; Żelazowska and Biliński 1999).

In several species of heteropteran bugs (belonging to the families Blissidae (*Ischnodemus sabuleti*), Lygaeidae (*Arocatus longiceps*, *Kleidocerys resedae*, *Nysius ericae*, and *Nithecus jacobaeae*), and Artheneidae (*Chilacis typhae*), bacteriocytes occur not only within bacteriomes/midgut epithelium but also within the ovarioles. In all these insects, the basal part of the tropharium containing early previtellogenic oocytes has a ring-shaped infection zone composed of several bacteriocytes (Fig. 3.2a, b, c) (Kuechler et al. 2010, 2011, 2012; Matsuura et al. 2012; Schneider 1940; Swiatoniowska et al. 2013).

In some insects, symbiotic microorganisms, e.g., yeast-like symbionts in leaf-hoppers (Hemiptera, Cicadomorpha, Cicadellidae) (Fig. 3.2d) and bacteria *Burkholderia* in scale insects *Acanthococcus aceris* and *Gossyparia spuria* (both Eriococcidae) (Fig. 3.2e), do not colonize specialized cells but are dispersed in the fat body (Kobiałka and Szklarzewicz, in preparation; Michalik et al. 2016). According to Michalik et al. (2016), the lack of bacteriocytes and the presence of symbiotic microorganisms in the fat body of scale insects indicate an initial (evolutionary young) stage of symbiosis.

3.3 Modes of Transovarial Transmission of Mycetomic Symbionts

3.3.1 Infestation of Embryos in Viviparous Aphids

Aphids represent widely distributed plant pests which are classified into the following three superfamilies: Adelgoidea, Phylloxeroidea, and Aphidoidea (Wojciechowski et al. 2015). All generations of Adelgoidea and Phylloxeroidea are oviparous, whereas Aphidoidea (termed "modern aphids") have a complex life cycle with parthenogenetic viviparous females and sexual oviparous females (Dixon 1985; Moran 1992).

The symbiotic systems of aphids belong to the most explored systems within insects. These studies showed that the representatives of Aphidoidea, as a rule, harbor a primary symbiont—gammaproteobacterium *Buchnera aphidicola*—as well as secondary symbionts belonging to different systematic groups (with the exception of some representatives of the subfamily Hormaphidinae which, instead
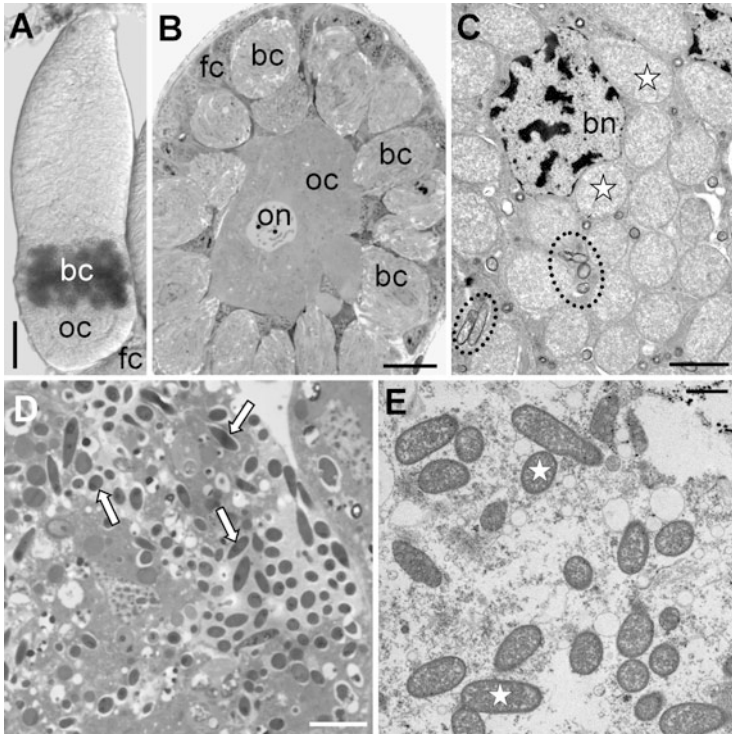
Fig. 3.2 Distribution of symbiotic microorganisms in the insect body (**a**) *Nysius ericae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the ovariole with the bacteriocytes in the ring-shaped infection zone. Differential interference contrast, scale bar = 100 μm. (**b**) *Nithecus jacobaeae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the ovariole, which contains the infection zone (cross section). Methylene blue, scale bar = 20 μm. (**c**) *Nysius ericae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the bacteriocyte residing in the infection zone filled with large, elongated bacteria (*white asterisks*) and small, rod-shaped bacteria (encircled by a *black dotted line*). TEM, scale bar = 2 μm. (**d**) *Graphocraerus ventralis* (Hemiptera, Cicadomorpha, Cicadellidae: Deltocephalinae). Fragment of the fat body with yeast-like symbionts (*white arrows*). Methylene blue, scale bar = 20 μm (courtesy of Michał Kobiałka, Jagiellonian University, Kraków, Poland). (**e**). *Gossyparia spuria* (Hemiptera, Coccomorpha, Eriococcidae). Bacteria *Burkholderia* (*white asterisks*) dispersed in the fat body cells. TEM, scale bar = 1 μm. *bc* bacteriocyte, *bn* bacteriocyte nucleus, *fc* follicular epithelium, *oc* oocyte, *on* oocyte nucleus

of bacteria, have yeast-like symbionts) (for further details see, e.g., Baumann et al. 1995; Buchner 1965; Douglas 1998; Fukatsu and Ishikawa 1992, 1996; Hongoh and Ishikawa 2000; Ishikawa 2003; Michalik et al. 2014a; Nováková et al. 2013; Oliver et al. 2010; Wegierek et al. 2017).

Viviparous females of Aphidoidea are characterized by an unusual developmental strategy termed "the telescoping of generations" (Kindlmann and Dixon 1989; Moran 1992). In the females of these aphids, embryos develop, inside of which the next generation of embryos forms (Fig. 3.3a). Bacteriocytes are present in both the
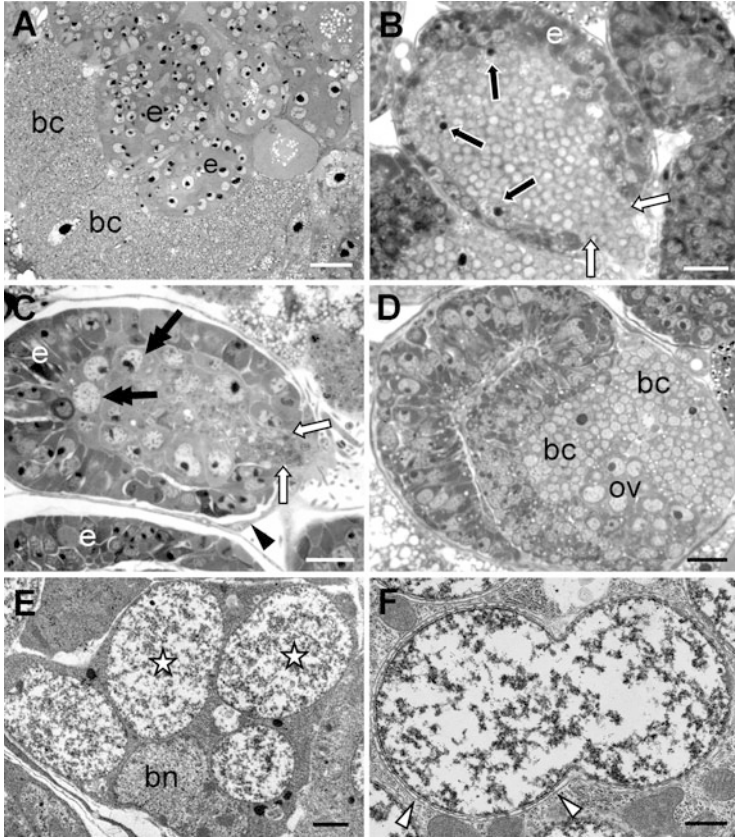
Fig. 3.3 The successive stages of transovarial transmission of symbionts in viviparous aphids (Hemiptera, Aphidomorpha, Aphidoidea, Aphididae) (**a**) *Aphis viburni* (Aphidinae). Fragment of the embryo. Bacteriocytes with bacterium *Buchnera aphidicola* (primary symbiont) are present in the vicinity of next-generation embryos. Methylene blue, scale bar = 20 μm. (**b**) *Cavariella theobaldi* (Aphidinae). Symbiotic bacteria infest the embryo at the blastula stage (longitudinal section). *White arrows* indicate the broad stream of bacteria entering the embryo. Note the nuclei of the presumptive bacteriocytes (*black arrows*). Methylene blue, scale bar = 10 μm. (**c**) *Aphis craccae* (Aphidinae). The embryo at late blastula stage (longitudinal section). Note the newly formed bacteriocytes (*double black arrows*) and the opening at the posterior pole of the embryo (*white arrows*). *Black arrowhead* indicates flattened follicular cells surrounding the embryo. Methylene blue, scale bar = 10 μm. (**d**) *Cavariella theobaldi* (Aphidinae). The embryo contains bacteriocytes, which are packed with symbiotic bacteria (longitudinal section). Methylene blue, scale bar = 10 μm. (**e**) *Hamamelistes betulinus* (Hormaphidinae). Newly formed bacteriocyte filled with few bacteria *Buchnera aphidicola* (*white asterisks*). TEM, scale bar = 1 μm. (**f**) *Stomaphis quercus* (Lachninae). The dividing bacterium *Buchnera aphidicola*. *White arrowheads* indicate the external most "perisymbiotic membrane," which is derived from the host insect. TEM, scale bar = 1 μm. *bc* bacteriocyte, *bn* bacteriocyte nucleus, *e* embryo, *ov* ovary

body of the female and their embryos (Fig. 3.3a). At the time the embryos are at the blastula stage, the symbionts commence the invasion of their posterior poles (Fig. 3.3b, c). The embryo is surrounded by a single layer of flattened follicular cells (Fig. 3.3c), and embryo's interior is filled with a syncytial mass of nuclei of presumptive bacteriocytes (Fig. 3.3b). As the released symbionts accumulate around the posterior pole of the embryo, the follicular cells, as well as cells of the embryo, separate from each other to form a wide opening that facilitates the entry of microorganisms (Fig. 3.3b, c). The bacteriocytes, as well as the ovaries, are one of the first cells/organs to differentiate during embryonic development (Fig. 3.3c, d) (Braendle et al. 2003; Buchner 1965; Koga et al. 2012; Miura et al. 2003; Pyka-Fosciak and Szklarzewicz 2008; Wegierek et al. 2017; Wilkinson et al. 2003). During further development, bacteriocytes undergo polyploidization and are gradually colonized by symbionts (Fig. 3.3d, e).

Because the symbiotic bacteria enter the cytoplasm of bacteriocytes, follicular cells, and oocytes using endocytic vesicle pathway, they remain surrounded by an additional, host vesicle-derived membrane, termed the perisymbiotic membrane (Fig. 3.3f).

3.3.2 Infestation of Undifferentiated Germ Cells (Cystocytes) and Young Oocytes

As a rule, symbionts invade the ovaries of older females containing full-grown oocytes (for the classification and characterization of insect ovaries, see Biliński 1998; Büning 1994); however, in several groups of insects (some scale insects, some planthoppers, some heteropterans, some hymenopterans, mallophagans, termites, cockroaches), the larval ovaries containing undifferentiated germ cells (cystocytes) or ovaries of young adult females with early previtellogenic oocytes become infested (Buchner 1965; Fukatsu et al. 2007; Kuechler et al. 2010, 2011; Kupper et al. 2016; Matsuura et al. 2012; Niżnik and Szklarzewicz 2007; Sacchi et al. 1988, 2000; Schneider 1940; Swiatoniowska et al. 2013). Interestingly, in some insects possessing two or more types of symbionts (e.g., in the cottony cushion scale, *Icerya purchasi*, or the planthopper *Cixius nervosus*), one of the symbionts invades the larval ovary, whereas the others infest the older (vitellogenic or choriogenic) oocytes in the ovary of the adult female (Niżnik and Szklarzewicz 2007). In the second larval instar of *Icerya purchasi*, the rod-shaped bacteria (i.e., the secondary symbionts occupying the bacteriocyte epithelium; see above) infect the cystocytes (Fig. 3.4a, b). During further ovary development, cystocytes differentiate into oocytes and trophocytes (nurse cells), and both contain the symbionts (Niżnik and Szklarzewicz 2007). Bacteria present in the trophocytes migrate through the trophic core and nutritive cord into developing oocytes (Fig. 3.4c). The primary symbionts of members of other families of scale insects such as *Puto*
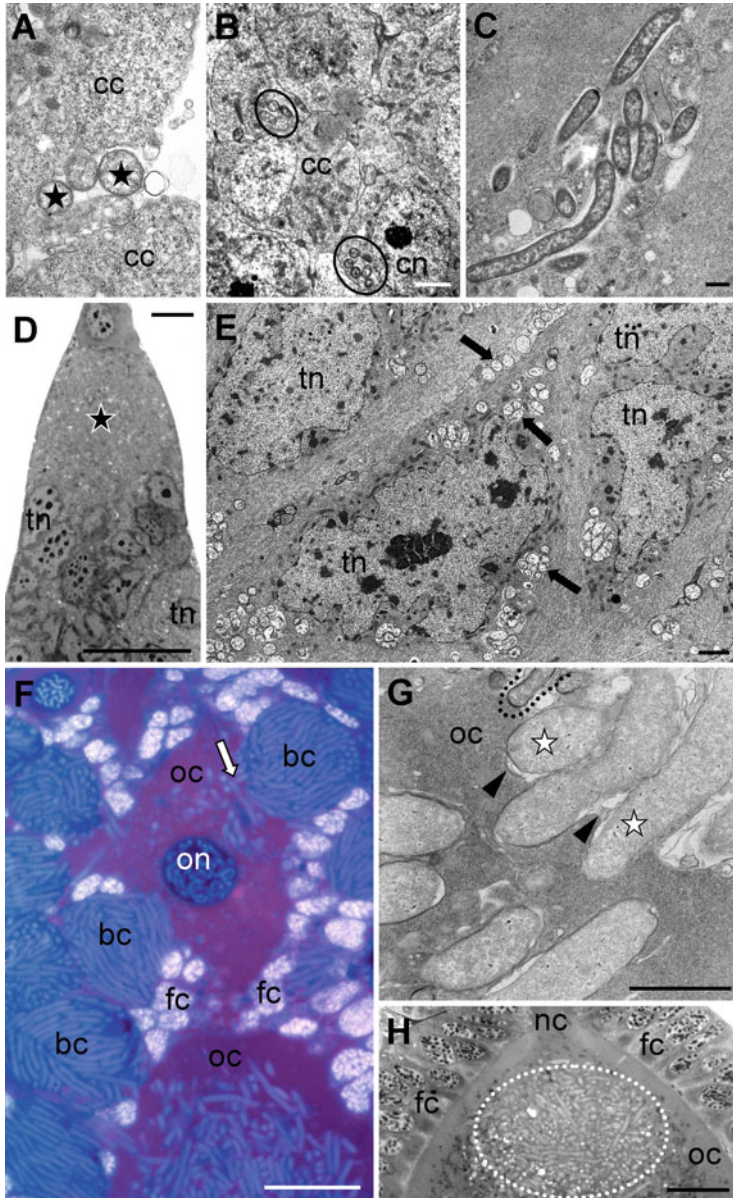
Fig. 3.4 Infestation of undifferentiated germ cells (cystocytes) and young oocytes by symbiotic microorganisms (**a**) *Icerya purchasi* (Hemiptera, Coccomorpha, Monophlebidae). Rod-shaped bacteria (*black asterisks*) invade the cystocyte. TEM, scale bar = 1 μm. (**b**) *Icerya purchasi* (Hemiptera, Coccomorpha, Monophlebidae). Fragment of the cystocyte cluster constituting the larval ovary. In the cystocyte cytoplasm, rod-shaped bacteria are present (encircled by a *black line*). TEM, scale bar = 1 μm. (**c**) *Marchalina hellenica* (Hemiptera, Coccomorpha, Marchalinidae). Bacteria migrating through the nutritive cord. TEM, scale bar = 1 μm. (**d**) *Cixius nervosus* (Hemiptera, Fulgoromorpha, Cixiidae). The anterior region of the tropharium (longitudinal section) filled with numerous rod-shaped bacteria (*black asterisk*). Methylene blue, scale bar = 20 μm. (**e**) *Cixius nervosus* (Hemiptera, Fulgoromorpha, Cixiidae). Fragment of the

albicans (Putoidae) (Szklarzewicz et al. 2010) and *Marchalina hellenica* (Marchalinidae) (Szklarzewicz et al. 2013) are also transported this way.

A similar situation to that of *Icerya purchasi* was observed in the bedbug *Cimex lectularius* (Heteroptera, Cimicidae) (Buchner 1965) and in the planthopper *Cixius nervosus* (Fulgoromorpha, Cixiidae) (Szklarzewicz et al. 2007). Ultrastructural observations have demonstrated that the tropharium of the telotrophic ovariole of *Cixius nervosus* is tightly packed with rod-shaped bacteria (Fig. 3.4d). These bacteria were also observed in nutritive cords and in arrested oocytes. The presence of symbionts in the tropharium (Fig. 3.4e), in the nutritive cords, and in the oocytes suggests that ovarioles of *Cixius nervosus* are infected by bacteria before the differentiation of cystocytes into oocytes and trophocytes.

In several species of heteropteran bugs belonging to the families Artheneidae (*Chilacis typhae*), Blissidae (*Ischnodemus sabuleti*), and Lygaeidae (*Arocatus longiceps*, *Kleidocerys resedae*, *Nysius ericae*, and *Nithecus jacobaeae*), which possess the ring-shaped "infection zone" within the tropharium (Figs. 3.2a, b, c and 3.4f), the symbiotic bacteria infect the previtellogenic oocytes occupying this part of the tropharium (Kuechler et al. 2010, 2011, 2012; Matsuura et al. 2012; Schneider 1940; Swiatoniowska et al. 2013). The mode of transmission of symbionts to the next generation has been thoroughly studied in two species belonging to Lygaeidae: Orsillinae—*Nysius ericae* and *Nithecus jacobaeae* only (Swiatoniowska et al. 2013). In these species, the cytoplasm of the bacteriocytes contains numerous, large, elongated gammaproteobacteria (Fig. 3.2b, c). In addition, *Nysius ericae* contains small, rod-shaped bacteria of an unknown identity and function (Fig. 3.2c). In adult females, the symbionts leave the bacteriocytes and enter the previtellogenic oocytes (Fig. 3.4f, g). Initially, i.e., in previtellogenic oocytes within the infection zone, the bacteria are dispersed throughout the entire cytoplasm (Fig. 3.4f); however, in developing oocytes in the vitellarium, they gather in the anterior pole of the oocyte, forming a "symbiont ball" (Fig. 3.4h).

In the carpenter ant, *Camponotus floridanus*, the symbiotic gammaproteobacteria *Blochmannia floridanus* infect the follicular cells surrounding the lower part of the germarium of the polytrophic ovarioles (Kupper et al.

Fig. 3.4 (continued) tropharium containing rod-shaped bacteria (*black arrows*) in the vicinity of the trophocyte nuclei. TEM, scale bar = 2 μm. (**f**) *Nysius ericae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the ovariole containing the infection zone (longitudinal section). *White arrow* indicates the symbiotic bacteria that leave the bacteriocyte cytoplasm and enter the oocyte. Fluorescence microscope, DAPI + propidium iodide, scale bar = 20 μm. (**g**) *Nysius ericae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the infection zone. The large elongated bacteria (*white asterisks*) and small rod-shaped bacteria (encircled by a *black dotted line*) leave the bacteriocyte and enter the oocyte. *Black arrowheads* indicate the oolemma. TEM, scale bar = 2 μm. (**h**) *Nithecus jacobaeae* (Hemiptera, Heteroptera, Lygaeidae: Orsilinae). Fragment of the early vitellogenic oocyte (longitudinal section). Note the "symbiont ball" (encircled by a *white dotted line*) at the anterior pole of the oocyte. Methylene blue, scale bar = 20 μm. *bc* bacteriocyte, *cc* cystocyte, *cn* cystocyte nucleus, *fc* follicular cells, *nc* nutritive cord, *oc* oocyte, *on* oocyte nucleus, *tn* trophocyte nucleus

2016). At the time the cystocytes differentiate into oocytes and trophocytes, the symbionts leave the follicular cells and begin to invade early previtellogenic oocytes via an endocytic pathway. Thus, in these insects, bacteria never infect the trophocytes. In contrast to *Camponotus floridanus*, in which germaria are infected, in some mallophagans (e.g., in a slender pigeon louse *Columbicola columbae*), the symbiotic bacteria after leaving the ovarial ampullae begin to invade the posterior ends of polytrophic ovarioles containing previtellogenic oocytes (Buchner 1965; Fukatsu et al. 2007). The infestation of young, previtellogenic oocytes has also been observed in the panoistic ovarioles of termites and cockroaches (Buchner 1965; Sacchi et al. 1988, 2000).

3.3.3 Modes of Infestation of the Older Oocytes

In most insects, symbionts infect the posterior pole of the terminal oocytes; however, there are significant differences in the course of this process (Buchner 1965; Cheung and Purcell 1999; Kobiałka et al. 2015, 2016; Kot et al. 2012, 2014; Michalik et al. 2009, 2013, 2014a; Niżnik and Szklarzewicz 2007; Pyka-Fosciak and Szklarzewicz 2008; Sacchi et al. 2008; Szklarzewicz and Moskal 2001; Szklarzewicz et al. 2006, 2013, 2016).

In the oviparous females of aphids (i.e., in all generations of the Adelgoidea and oviparous generations of Aphidoidea), the bacteriocytes begin to adhere closely to the choriogenic oocytes located terminally within the ovariole (Fig. 3.5a). Subsequently, symbionts leave the bacteriocytes and start to invade the ovarioles. As a rule, symbionts (both P-symbionts and S-symbionts) migrate to the perivitelline space (i.e., space between the follicular epithelium and oocyte) through the wide spaces between neighboring follicular cells (Fig. 3.5b). In some species ((*Adelges viridis* (Adelgoidea: Adelgidae), *Clethrobius comes* (Aphidoidea: Aphididae: Drepanosiphinae), *Schizolachnus pineti* (Aphidoidea: Aphididae: Lachninae)), however, symbionts also pass through the cytoplasm of the follicular cells (Fig. 3.5c) via an endocytic/exocytic pathway (Michalik et al. 2013, 2014a). After passing through the follicular epithelium and perivitelline space (Fig. 3.5b), symbionts enter the ooplasm eventually forming the characteristic "symbiont ball" at the posterior pole of the oocyte (Fig. 3.5d, e). It is worth mentioning that the time of the infestation of the oocyte by bacteria correlates with the process of choriogenesis (Michalik et al. 2013, 2014a; Pyka-Fosciak and Szklarzewicz 2008). During the migration of the bacteria from the body cavity to the ovarioles, the egg envelopes are incomplete: apical and lateral regions of the oocyte are surrounded by egg envelopes, whereas the oocyte posterior pole remains free of envelopes, enabling symbionts to enter the oocyte. The follicular cells begin to synthesize precursors of missing egg envelopes only after the symbionts have entered the oocyte (Michalik et al. 2013, 2014a; Pyka-Fosciak and Szklarzewicz 2008). It should be stressed that symbiont transmission that relies on internalization of symbionts in the ooplasm of the posterior pole of older (i.e., choriogenic) oocytes is very rare among insects and,
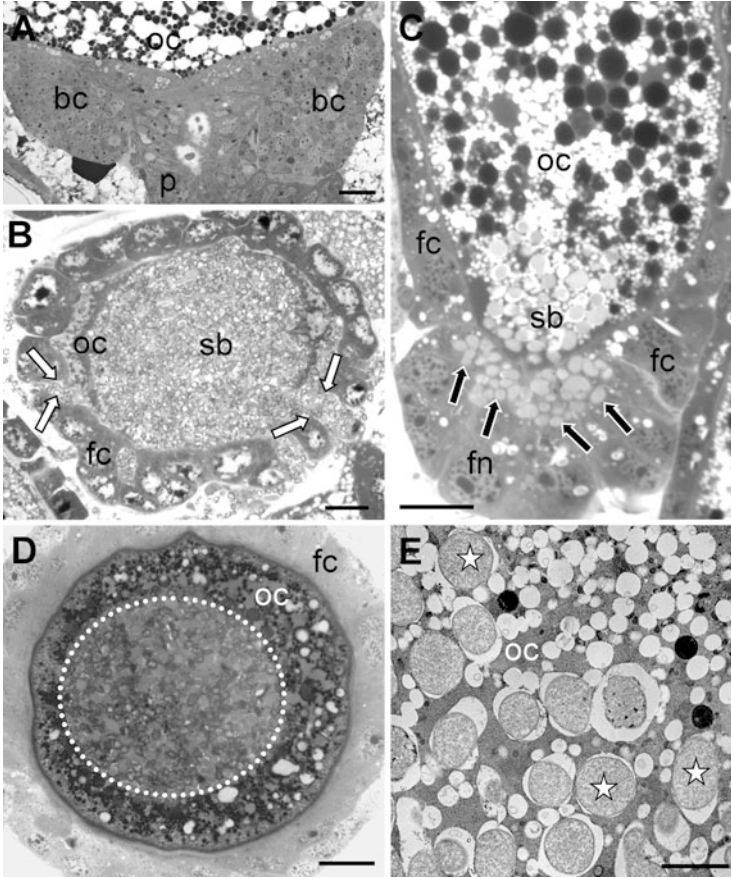
Fig. 3.5 Transovarial transmission of symbionts in oviparous aphids (Hemiptera, Aphidomorpha). (**a**) *Prociphilus fraxini* (Aphidoidea, Aphididae: Eriosomatinae). Bacteriocytes with *Buchnera aphidicola* bacterium gathered around the posterior pole of the terminal oocyte (longitudinal section). Methylene blue, scale bar = 10 μm. (**b**) *Sipha* (*Rungsia*) *maydis* (Aphidoidea, Aphididae: Drepanosiphinae). Posterior pole of oocyte (cross section). *White arrows* indicate symbiotic microorganisms, which migrate to the oocyte through the spaces between neighboring follicular cells. Methylene blue, scale bar = 10 μm. (**c**) *Adelges* (*Sacchiphantes*) *viridis* (Adelgoidea, Adelgidae). Posterior pole of the oocyte (longitudinal section). *Black arrows* indicate symbiotic microorganisms, which migrate to the oocyte through the cytoplasm of follicular cells and through the spaces between neighboring follicular cells. Methylene blue, scale bar = 10 μm. (**d**) *Eulachnus rileyi* (Aphidoidea, Aphididae: Lachninae). Symbiotic microorganisms at the posterior pole of the terminal oocyte in the form of the "symbiont ball" (encircled by a *white dotted line*) (cross section). Methylene blue, scale bar = 10 μm. (**e**) *Adelges (Sacchiphantes) viridis* (Adelgoidea, Adelgidae). Fragment of the "symbiont ball." Note symbiotic bacteria (*white asterisks*) in the oocyte cytoplasm. TEM, scale bar = 10 μm. *bc* bacteriocyte, *fc* follicular cells, *fn* follicular cell nucleus, *oc* oocyte, *p* ovariolar stalk (pedicel), *sb* "symbiont ball"

to our knowledge, apart from oviparous aphids, this mode of transmission has not been reported for other insects.

In psyllids, some scale insects (e.g., *Icerya purchasi* and *Palaeococcus fuscipennis* (both from Monophlebidae family) and auchenorrhynchans, the initial stages of symbiont transmission are similar to that in oviparous aphids (i.e., symbiotic microorganisms leave the bacteriocytes, begin to migrate toward ovaries, and gather around the posterior pole of the terminal oocyte) (Buchner 1965; Kobiałka et al. 2015, 2016; Kot et al. 2012, 2014; Niżnik and Szklarzewicz 2007; Sacchi et al. 2008; Szklarzewicz et al. 2006). There are, however, significant differences in the manner of the infestation of the ovariole in aphids and other insects: (1) in aphids, symbionts migrate mainly through the wide spaces between follicular cells (Fig. 3.5b), whereas in most insects, this migration takes place through the follicular cells (via endocytic/exocytic pathway) (Fig. 3.6b, c, d, e), and (2) in aphids, after passing through the follicular epithelium and perivitelline space, the microorganisms immediately enter the ooplasm (Fig. 3.5b, c, d, e), whereas in most insects, they gather in the deep invagination of the oolemma, forming a "symbiont ball" outside of the oocyte (Fig. 3.6f, g). The microorganisms residing in the perivitelline space closely adhere to one another (Fig. 3.6g, h). The symbionts stay in the perivitelline space until the end of oocyte growth.

It should be noted that in psyllids, scale insects, and auchenorrhynchans, prior to leaving the bacteriocytes, the pleomorphic bacteria change their shape into an almost spherical (Fig. 3.6a). Simultaneously, they begin to stain very intensely with methylene blue (Fig. 3.6a). It seems that the above changes are connected with the reorganization of bacterial cytoskeleton during the migration into the ovaries.

In a few families of scale insects (Pseudococcidae, Eriococcidae, Putoidae), the symbiotic bacteria infest the anterior region of the telotrophic ovariole (Buchner 1955; von Dohlen et al. 2001; Michalik et al. 2016; Szklarzewicz et al. 2017). At the time the ovarioles contain oocytes in the stage of late vitellogenesis, the bacteria (or whole bacteriocytes in *Puto superbus*; see below) begin to surround the neck region of the ovariole (i.e., the region between the tropharium and vitellarium). During symbiont/bacteriocyte migration, the posterior and lateral regions of the oocyte are surrounded by egg coverings. The only region of the oocyte surface that is devoid of eggshells is its anterior pole, which is attached to the nutritive cord (Fig. 3.7a). The bacteria may migrate either through the follicular cells (via endocytic/exocytic pathway) or through the spaces between follicular cells (Fig. 3.7b), whereas the bacteriocytes crawl between neighboring follicular cells (not shown). After reaching the perivitelline space (Fig. 3.7c), the bacteria/bacteriocytes accumulate in the depression of the oolemma at the anterior pole of the oocyte (Fig. 3.7d). At that time, in Eriococcidae family, the nutritive cord degenerates, and the bacteria start to enter the oocyte (Fig. 3.7f), whereas in Pseudococcidae and Putoidae, the bacteria/bacteriocytes remain in the invagination of oolemma until the end of oocyte growth (Figs. 3.7e and 3.8e). It should be stressed that although the symbiotic association between *Gossyparia spuria* and *Acanthococcus aceris* represents an evolutionary young condition (Michalik et al.
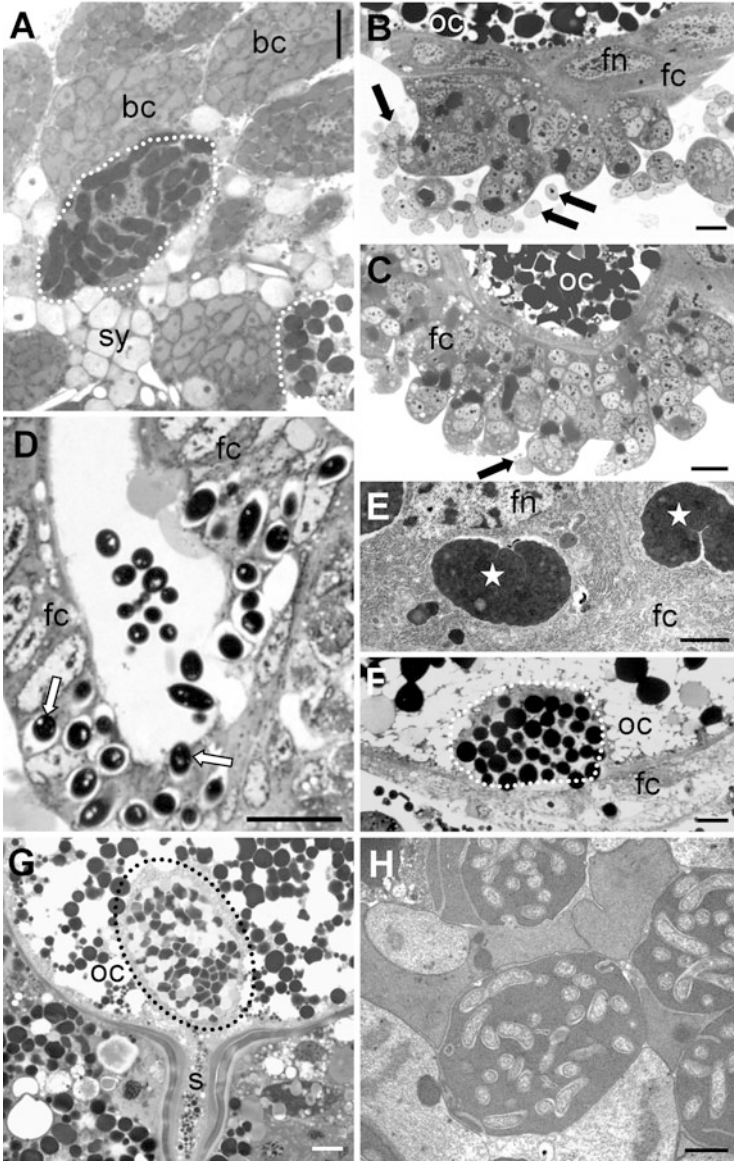
Fig. 3.6 Infestation of the terminal pole of the choriogenic oocyte by symbiotic bacteria. (**a**) *Cacopsylla mali* (Hemiptera, Psyllomorpha, Psyllidae). Fragment of the bacteriome. The symbiotic bacteria (encircled by a *white dotted line*) before migration to the ovaries stain intensively with methylene blue and change shape to almost spherical. Methylene blue, scale bar = 10 μm. (**b, c**) *Evacanthus interruptus* (Hemiptera, Cicadomorpha, Cicadellidae: Evacanthinae). Posterior pole of the terminal oocyte (*b*, longitudinal section; *c*, cross section). Consecutive stages of migration of symbionts into the perivitelline space through the cytoplasm of follicular cells. *Black arrows* indicate the bacteria that invade the projections of follicular cells. Methylene blue, scale bar = 20 μm. (**d**) *Conomelus anceps* (Hemiptera, Fulgoromorpha, Delphacidae). Posterior end of the ovariole (longitudinal section). *White arrows* indicate the yeast-like symbionts which invade the ovariole through the follicular epithelium. Methylene blue, scale bar = 20 μm. (**e**) *Cacopsylla ulmi* (Hemiptera,

2016), these scale insects have already developed and established a stable mode of symbiont transmission.

In insects, as a rule, before the migration to the ovaries, the bacteria are released from the bacteriocyte cytoplasm; however, in some insects such as cockroaches (Sacchi et al. 1988), mallophagans (Buchner 1965), whiteflies (Buchner 1965; Costa et al. 1993; Szklarzewicz and Moskal 2001), and scale insect *Puto superbus* (Putoidae) (Buchner 1965; Szklarzewicz et al. 2017), the whole and intact bacteriocytes penetrate the ovaries. In the cockroach *Blattella germanica*, bacteriocytes enter the ovaries; however, shortly before entry into the ovarioles, the symbionts leave bacteriocyte and individually migrate through the ovariole sheath and follicular epithelium to the perivitelline space (Sacchi et al. 1988). In the mallophagan genus *Goniodes*, the bacteriocytes traverse the epithelium of the ovariolar stalk (pedicel); however, during migration, they degenerate and release the symbionts (Buchner 1965). In consequence, just as in *Blattella germanica*, the oocytes of *Goniodes* are infected by individual bacteria. In whiteflies, in the last larval instar (puparium), bacteriocytes containing both primary and secondary symbionts separate from the bacteriomes and gather around the posterior ends of ovarioles (Fig. 3.8a). In adult females, which possess early vitellogenic oocytes, whole and intact bacteriocytes migrate through the spaces between follicular cells surrounding the posterior pole of early vitellogenic oocytes into the perivitelline space (Fig. 3.8b, c). During this migration, bacteria become elongated and stain more intensely with methylene blue (Fig. 3.8c). In the perivitelline space, the bacteriocytes accumulate in the deep invagination of the oolemma over the hydropylar stalk (Fig. 3.8d). In the whitefly *Aleurochiton aceris* and in the scale insect *Puto superbus* (Fig. 3.8e) (see above), the bacteria do not leave the bacteriocyte cytoplasm until the end of oocyte growth.

Fig. 3.6 (continued) Psyllomorpha, Psyllidae). Fragment of the follicular cell with migrating bacteria (*white asterisks*). TEM, scale bar = 2 μm. (**f**) *Palaeococcus fuscipennis* (Hemiptera, Coccomorpha, Monophlebidae). The posterior pole of the oocyte (longitudinal section). Pleomorphic bacteria (encircled by a *white dotted line*) accumulate in the deep depression of the oolemma. Methylene blue, scale bar = 10 μm. (**g**) *Aphalara polygoni* (Hemiptera, Psyllomorpha, Aphalaridae). Concentration of symbionts ("symbiont ball") (encircled by a *black dotted line*) in the deep invagination of oolemma over the stalk which will serve to anchor the egg to the host plant (longitudinal section). Methylene blue, scale bar = 1 μm (**a**, **e**, and **g**—courtesy of Marta Kot, Jagiellonian University, Kraków, Poland). (**h**) *Macrosteles laevis* (Hemiptera, Cicadomorpha, Cicadellidae: Deltocephalinae). The bacteria forming the "symbiont ball" closely adhere to each other. TEM, scale bar = 2 μm. *bc* bacteriocyte, *fc* follicular cells, *fn* follicular cell nucleus, *oc* oocyte, *s* stalk of the oocyte, *sy* syncytial region of the bacteriome containing the secondary symbionts
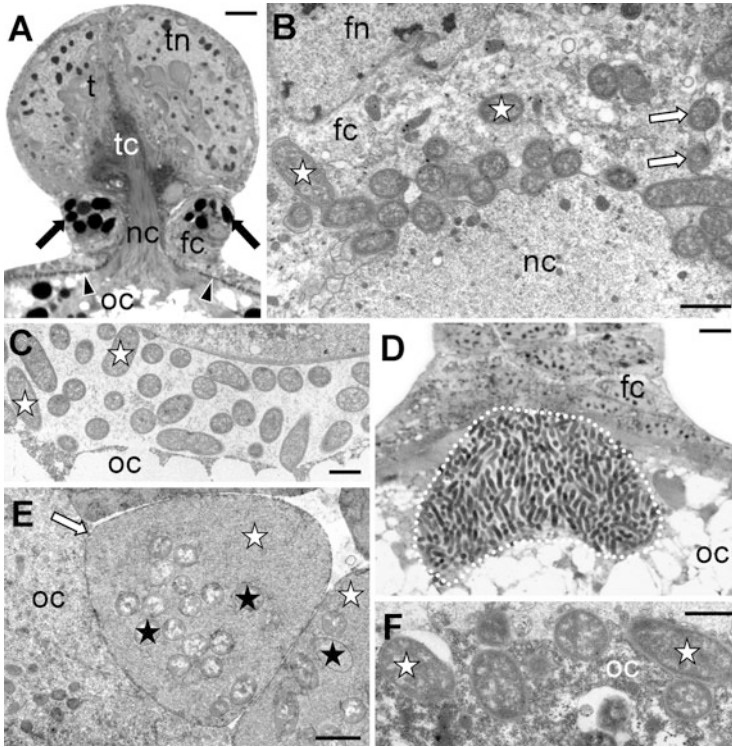
Fig. 3.7 Infestation of the anterior pole of the oocyte by symbiotic microorganisms in scale insects (Hemiptera, Coccomorpha). (**a**) *Phenacoccus aceris* (Pseudococcidae: Phenacoccinae). Anterior end of the ovariole (longitudinal section). Symbiotic bacteria (*black arrows*) inside follicular cells surrounding the neck region of the ovariole. *Black arrowheads* indicate egg envelopes. Methylene blue, scale bar = 10 μm. (**b**) *Gossyparia spuria* (Eriococcidae). Symbiotic bacteria migrate through the cytoplasm of follicular cells (*white asterisks*) and between neighboring cells (*white arrows*). TEM, scale bar = 1 μm. (**c**) *Acanthococcus aceris* (Eriococcidae). Bacteria (*white asterisks*) begin to accumulate in the perivitelline space. TEM, scale bar = 1 μm. (**d**) *Acanthococcus aceris* (Eriococcidae). The neck region of the ovariole (longitudinal section). The accumulation of bacteria (encircled by a *white dotted line*) in the perivitelline space. Methylene blue, scale bar = 10 μm. (**e**) *Pseudococcus longispinus* (Pseudococcidae: Pseudococcinae). Pleomorphic bacteria (*white asterisks*) containing rod-shaped bacteria (*black asterisks*) localized in the deep depression of the oolemma. *White arrow* indicates the oolemma. TEM, scale bar = 1 μm. (**f**) *Gossyparia spuria* (Eriococcidae). Symbiotic bacteria (*white asterisks*) enter the oocyte. TEM, scale bar = 1 μm. *fc* follicular cells, *fn* follicular cell nucleus, *nc* nutritive cord, *oc* oocyte, *t* trophocyte, *tn* trophocyte nucleus, *tc* trophic core

3.4　Future Perspectives

Earlier histological studies and more recent ultrastructural observations have revealed that different insect taxa developed typical (i.e., uniform among all the representatives of the taxon) mode of symbiont transmission. The one exception to
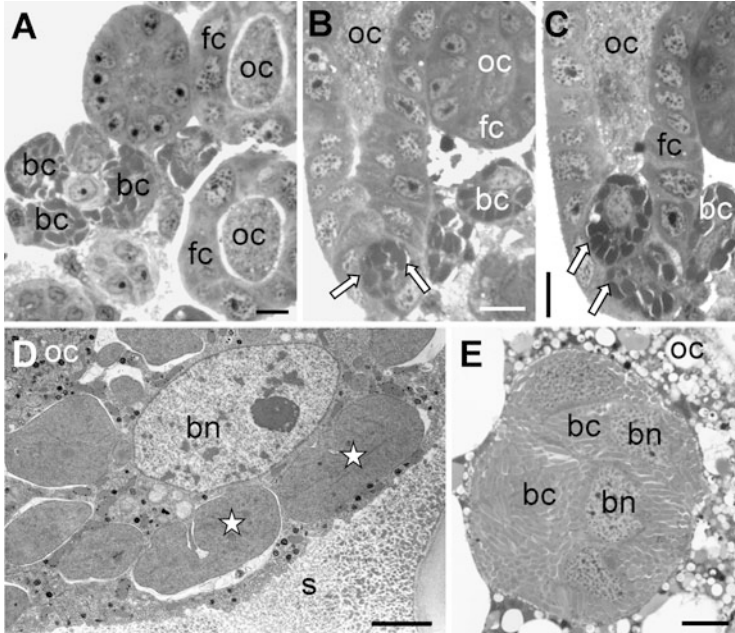
Fig. 3.8 Infestation of the ovariole by bacteriocytes which are whole and intact. (**a**) *Aleurochiton aceris* (Hemiptera, Aleyrodomorpha, Aleyrodidae). Bacteriocytes gather among the posterior ends of ovarioles (cross section) of the last instar before imago (termed puparium). Methylene blue, scale bar = 10 μm. (**b, c**) *Aleurochiton aceris* (Hemiptera, Aleyrodomorpha, Aleyrodidae). Consecutive stages of migration of the whole intact bacteriocytes (*white arrows*) into the perivitelline space in the adult female (longitudinal section). Methylene blue, scale bar = 10 μm. (**d**) *Aleurochiton aceris* (Hemiptera, Aleyrodomorpha, Aleyrodidae). Bacteriocyte with pleomorphic bacteria (*white asterisks*) in the deep invagination of the oolemma over the hydropylar stalk (longitudinal section). TEM, scale bar = 2 μm. (**e**) *Puto superbus* (Hemiptera, Coccomorpha, Putoidae). Bacteriocytes localized at the anterior pole of the full-grown oocyte ("symbiont ball") (cross section). Methylene blue, scale bar = 10 μm. *bc* bacteriocyte, *bn* bacteriocyte nucleus, *fc* follicular cells, *oc* oocyte, *s* hydropylar stalk

this rule is observed in scale insects, which are characterized by very diverse symbiotic systems in terms of the type of symbionts, their localization in the host insect body, and the mode of transmission to the next generation. Moreover, it has been shown that even close relatives—members of the same family (e.g., Eriococcidae) or related families (e.g., Monophlebidae and Marchalinidae), may have different symbionts, which are inherited in a different manner (Buchner 1965; Matsuura et al. 2009; Michalik et al. 2016; Niżnik and Szklarzewicz 2007; Szklarzewicz et al. 2006, 2013). According to the Polish coccidologist Jan Koteja (1985), this large diversity of symbionts, associated with scale insects, may result from the permanent contact of these insects with microorganisms present in the primary habitat (forest leaf litter). Koteja (1985) hypothesized that scale insects living in the litter were saprophagic but during further evolution, the different groups of scale insects changed their feeding behavior at different times and

became plant sap feeders. Changing the feeding habit, scale insects acquired microorganisms, which then became their symbionts. In consequence, the symbionts of scale insects are polyphyletic. It is worth noting that the members of some scale insect families (e.g., Steingeliidae, Matsucoccidae, Dactylopiidae) have been regarded as asymbiotic (Tremblay 1977); however, recent, more detailed ultrastructural and molecular studies have revealed that they also contain symbiotic microorganisms (Koteja et al. 2003; Ramirez-Puebla et al. 2010; Szklarzewicz et al. 2014). Thus, the symbiotic systems of scale insects should be more extensively studied.

3.5 Conclusions

Insects, as hosts to obligate symbiotic microorganisms, developed different modes of transmission from one generation to the next: (1) the symbionts may invade dividing germ cells (cystocytes) (e.g., in some scale insects, bedbug *Cimex lectularius*, some planthoppers), early previtellogenic oocytes (in some heteropterans, carpenter ant *Camponotus floridanus*, mallophagans, cockroaches, termites), or full-grown oocytes (in most insects); (2) the microorganisms may be released from the bacteriocytes (in most insects), or whole and intact bacteriocytes may enter the ovarioles (in whiteflies, scale insect *Puto superbus*); (3) the symbionts may invade the anterior (in Pseudococcidae and Eriococcidae scale insects) or posterior pole of the oocyte (in most insects); and (4) the symbionts may enter the ooplasm (in aphids and some Eriococcidae scale insects) or may gather in the perivitelline space in the deep invagination of the oolemma (in most insects). The above observations strongly indicate that the course of transmission of symbionts between generations is related to the course of oogenesis of host insect and correlates with the stage of oocyte development.

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