entire oocyte at later stages. Finally, dorsal/anterior follicle cells migrate to form two dorsal appendages and the operculum as a result of a complex series of patterning events downstream from Grk signalling. These structures are critical in the mature egg for gas exchange and larval hatching, respectively.

Conclusions

In this primer we have introduced the process of *Drosophila* oogenesis and demonstrated the ways in which it is currently being used to investigate key questions in cell biology. It is a system simple enough to be tractable yet complex enough to allow the study of diverse cell behaviours. *Drosophila* oogenesis has provided important insight in the past and should continue to do so in the future.

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Paternal transmission of symbiotic bacteria in malaria vectors

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Bacteria of the genus *Asaia* are associated with different species of malaria vectors and are located in the midgut, salivary glands and reproductive organs of female and male mosquitoes. Based on current evidence, the spreading of these bacteria in mosquito populations occurs through different mechanisms: co-feeding, sexual mating, and maternal transmission [1,2]. Even though paternal transmission of insect symbionts to progeny is not commonplace, the presence of *Asaia* in the male reproductive organs makes this additional transmission route worth being investigated. Here, we show that male-borne *Asaia* are transferred to females during the mating of *Anopheles stephensi* mosquitoes. Subsequently, the bacteria acquired by the female are vertically transmitted to the progeny. It would thus be possible to use male mosquitoes, which do not bite, to spread *Asaia* strains interfering with malaria transmission.

Although many insect symbionts are known to be vertically transmitted from mother to offspring [3], paternal transmission has rarely been reported [4]. We recently identified acetic acid bacteria belonging to the genus *Asaia* that are stably associated with different species of *Anopheles* vectors of malaria. *Asaia* is located in various tissues and organs, particularly the midgut, salivary glands and reproductive organs. *Asaia* cells are transmitted vertically from mother to offspring, as well as via horizontal routes, such as co-feeding and venereal contact [1,2]. The abundant presence of *Asaia* cells in the gonads of *Anopheles* males prompted us to investigate whether there is also paternal transmission to progeny.

All the experiments were performed using a colony of *Anopheles stephensi* reared since 1985 at University of Camerino. Mosquitoes were maintained at standard aseptic conditions of 30°C and 95% humidity. Larvae were grown in tanks filled with distilled water containing sterile minced commercial mouse food. Adult male mosquitoes were fed with sugar solutions containing one of the two different recombinant strains of *Asaia*, labelled respectively with Gfp or DsRed fluorescent proteins, constitutively expressed from genes carried on a plasmid or on the chromosome. The generation of GFP-tagged strain SF2.1(GFP) has already been described [1] and was based on the use of a plasmid carrying a Kan^R gene cassette that confers resistance to kanamycin. Strain SF2.1(DsRed) was generated with the purpose of having stably labelled bacteria after introducing the marker gene into the bacterial chromosome by insertion of a mini-Tn5 gene cassette containing the *dsRed* gene by conjugation and transposition as described in [5]. Strain SF2.1(DsRed) indefinitely retained the DsRed cassette without any antibiotic selective pressure.

Two sets of experiments were performed in duplicates for each of the two recombinant strains, SF2.1(GFP) or SF2.1(DsRed). Prior to mating, male mosquitoes were fed with sugar solution containing a concentration of 10^8 recombinant bacterial cells per millilitre. Forty-eight hours after sugar feeding, they were transferred to a cage containing virgin females, separated from males at the pupal stage. To avoid symbiont transfer by co-feeding, no food was provided. Males and females were left free to mate for 48 hours. Females were then transferred into a different cage for blood feeding and oviposition. Eggs were permitted to develop into adults, and the resulting males and females were carefully analysed by both fluorescent microscopy (IX71, Olympus, Melville, NY) and MRC600 laser scanning confocal microscopy (BIO-RAD).

The percentage of F_1 individuals detected to carry fluorescent *Asaia* cells varied depending on the recombinant strain provided to the parental males. In the progeny of males infected with *Asaia* strain SF2.1(DsRed) 63% of the offspring (19 out of 30) harboured red-fluorescent bacteria, while the percentage of F_1 individuals carrying green-fluorescent

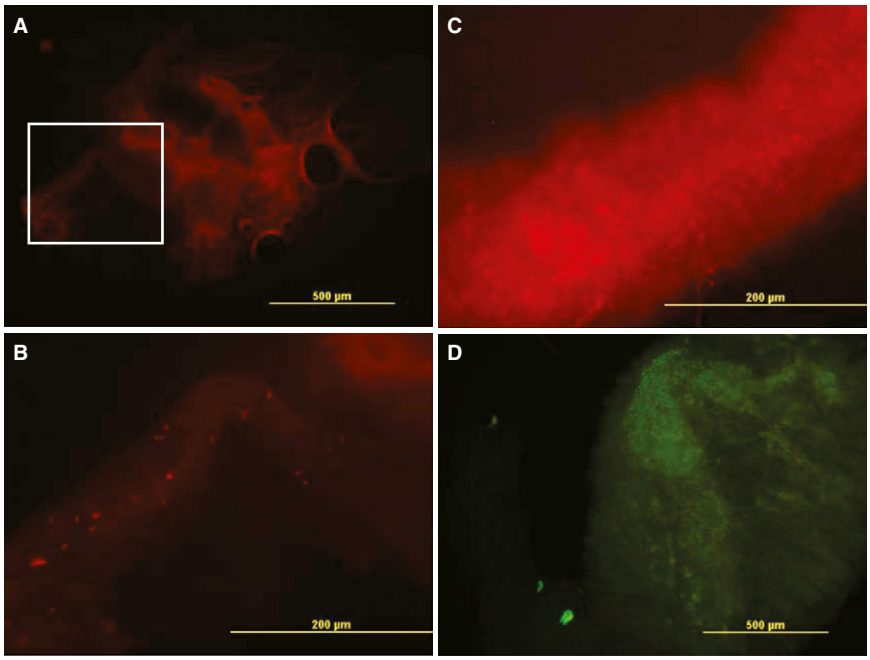

Figure 1. Paternal transmission of fluorescent *Asaia* strains.

(A) Ventral diverticulum colonization of F1 mosquitoes by *Asaia* sp. strain SF2.1(DsRed). (B) Magnification of the white square of (A) showing single red cells of the reporter strain. (C) Midgut colonization by *Asaia* sp. of strains SF2.1(DsRed) and (D) SF2.1(GFP), showing microcolonies of the fluorescent bacteria.

bacteria was 27% (8 out of 30) in the progeny of males infected with *Asaia* sp. strain SF2.1(GFP). Neither red- or green-fluorescent spots were observed in the mosquitoes from control cages. Similarly, only red- or green-fluorescence was observed in mosquitoes exposed to strains SF2.1(DsRed) or SF2.1(GFP), respectively. In the case of GFP-tagged *Asaia*, the experiments were performed in presence of antibiotic added to sugar solution and to larval breeding water (Kanamycin, 100 μg/ml) to permit plasmid selection. Partial degradation of the antibiotic and limited diffusion within the mosquito body are expected anyway and explain the reduced transmission rate of strain SF2.1(GFP) (27%) compared to the DsRed-tagged strain (63%), where the marker gene is carried on the chromosome.

Asaia fluorescent cells massively colonized the midgut and ventral diverticulum and were also found in the salivary glands (Figure 1). Our results confirm venereal transmission of *Asaia* in *A. stephensi* [1,2] and, more importantly, demonstrate effective paternal transmission of these symbionts. This mode of transmission is uncommon in arthropods, and the results here reported represent the first unambiguous demonstration of paternal transmission of bacterial symbionts in mosquitoes. Previously, this mode of transmission was demonstrated for beneficial symbionts in aphids [4].

We have also provided evidence for the trans-stadial transfer of a symbiotic bacterium, i.e. *Asaia*, from larvae to pupae and from pupae to adults, a controversial issue in mosquitoes [6–8]. Previous studies suggested an environmental route of symbiont infection of adults, for instance through the breeding water [8]. In our experiments, both culturing and PCR analysis revealed no evidence for the presence of *Asaia* in the larval breeding water, thus weakening the former hypothesis.

In conclusion, the paternal route can be added to the previously reported horizontal and maternal routes of transmission of *Asaia* in *A. stephensi* mosquitoes. Our findings have implications for *Asaia*-based paratransgenic protocols to control malaria. In addition to the direct release of modified bacteria in mosquito breeding sites to 'infect' larvae and, via trans-stadial transmission, adults, the release of paratransgenic non-biting mosquito males is now a potential strategy. The release of males would overcome several ethical objections to releasing female mosquitoes in the wild. Finally, the paternal route of transmission has broad evolutionary implications, as already discussed by Moran and Dunbar [4].

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