Sexual acquisition of beneficial symbionts in aphids

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A noted cost of mating is the risk of acquiring sexually transmitted infections that are detrimental to the recipient. But many microbial associates of eukaryotes are mutualistic, raising the possibility that sexual contact provides the opportunity to acquire symbionts that are beneficial. In aphids, facultative bacterial symbionts, which benefit hosts by conferring resistance to natural enemies or to heat, are transmitted maternally with high fidelity and are maintained stably throughout hundreds of parthenogenetic generations in the laboratory. Data from field populations indicate that horizontal transfer of these facultative symbionts is frequent, and transfections are readily achieved by microinjection or ingestion in artificial diet. However, no natural mechanism for the horizontal transfer of these symbionts has been identified. Here we demonstrate that during sexual reproduction, male-borne symbionts can be acquired by females and subsequently transferred to sexually and parthenogenetically produced progeny, establishing stable, maternally transmitted associations. In our experiments, sexually transmitted symbionts resulted in (i) infection of previously uninfected matrilines, (ii) a double infection in a matriline already bearing a different symbiont, and (iii) replacement of the maternal symbiont. We also observed some cases in which maternal symbionts failed to become established in sexually produced progeny. Microscopy indicated that symbionts were abundant in the male reproductive system, which demonstrates a natural route of non-maternal transfer of insect symbionts. Because such transfer can generate coinfections, thereby creating opportunities for symbiont competition and recombination, paternal inheritance has major consequences for expectations regarding symbiont evolution.

Acyrtosiphon pisum | mutualism | symbiosis

Aphids harbor a variety of mutualistic bacterial symbionts. Their primary symbiont, *Buchnera aphidicola*, is required for normal development and reproduction; it is transmitted maternally by colonization of developing eggs and embryos, and it provides aphid hosts with required nutrients (1, 2). In addition to *Buchnera*, aphids sometimes harbor several other vertically transmitted symbionts. In the pea aphid, *Acyrtosiphon pisum*, three species of symbionts, all members of the Enterobacteriaceae (“*Candidatus* Hamiltonella defensa,” “*Candidatus* Serratia symbiotica,” and “*Candidatus* Regiella insecticola”), occur at high frequencies in field populations. Individual aphids can be infected with zero, one, or two symbiont types (3–7). Strains of each of these facultative symbionts have been shown to confer major fitness benefits on their aphid hosts, such as the ability to overcome infection by wasp parasitoids (8, 9), resistance to fungal pathogens (10), expansion of host-plant range (11), and tolerance to heat stress (12–15).

The usual aphid life cycle consists of a series of fast-developing, all-female parthenogenetic generations from spring until autumn, at which time a single generation of sexual females and males is produced in response to a shortened photoperiod. Sexual individuals mature and mate, and females deposit overwintering eggs, which hatch in spring to produce an all-female parthenogenetic generation, completing the cycle. During parthenogenesis, embryos develop prenatally, and they are inoculated at an early stage by symbionts from the mother; maternal transmission of single infections of these three facultative symbionts is effectively 100%, at least under our laboratory condi-

tions (for details, see *Materials and Methods*). The only losses observed have been in lines that were initially doubly infected; these lines typically eliminate one of two facultative symbionts, giving rise to a stable infection by a single facultative symbiont (3). Thus, coinfections are often unstable, although some may persist for numerous generations (16).

Prior Evidence for Horizontal Transfer of Aphid Facultative Symbionts.

The regular occurrence of coinfections in field populations (3, 6, 7, 17) is one of several indicators that the horizontal transmission of facultative symbionts among maternal lineages must occur regularly in pea aphid populations. Horizontal transmission within and among species is also demonstrated by molecular phylogenetic analyses (3, 16, 17). Furthermore, transfections by microinjection of hemolymph from an infected aphid into one with no secondary symbiont or with a different symbiont species or strain yield high rates of establishment as stable heritable symbioses (8, 13, 18), indicating that these secondary symbionts have the capacity to invade cells, hemocoels, and embryos of naïve hosts. Aphids can also acquire infections from feeding on an artificial diet into which symbionts from infected aphids have been introduced (19). However, transmission by host plants is unlikely; symbionts have never been detected in or isolated from plant tissues used by infected aphids, and no transfers have been observed between infected and uninfected aphids feeding on the same plant, despite experiments designed to detect them (19).

Male Transfer of Symbionts to Progeny. We hypothesize that a regular opportunity for transfer of symbionts among aphid matrilines is male–female contact during the autumn sexual generation. During copulation, male insects transfer sperm as well as a variety of other substances, such as specialized proteins produced in accessory glands (e.g., ref. 20). Ejaculate components have been studied only in a small proportion of insect species. Sexually transmitted viruses and eukaryotic pathogens from insects are known (21). Venereal transmission of bacteria apparently has not been reported to date (21), which likely reflects a general lack of knowledge of bacteria infecting insects. In aphids, such transfer would be biologically significant only if the symbionts were transmitted maternally to progeny as stable infections during the subsequent parthenogenetic generations.

Results

By mimicking seasonal conditions in plant growth chambers, we carried out crosses of aphid clones differing in symbiont infection type and then established progeny clones for at least seven subsequent parthenogenetic generations, with each cross requiring ≈ 8 months (for details, see *Materials and Methods*).

Initially, we performed a single cross of uninfected mothers to fathers infected with *R. insecticola*; 13 progeny clones were obtained, of which all 13 acquired the paternal infection (Table 1). All of these clones maintained the paternal infection for as long as the clones were monitored, at least eight generations. In later crosses, mothers were usually themselves infected with a symbiont different from that of the father. When we screened

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Table 1. Symbiont transmission to progeny from sexual crosses of pea aphid lineages of different secondary symbiont infection status

Cross no.	Symbiont of maternal clone	Symbiont of paternal clone	No. of progeny lines	Losses of maternal symbiont	Acquisition of paternal symbiont by progeny clones	
					Generations 2 and 3	Generations 5–7
1	Uninfected	<i>R. insecticola</i>	13	NA	13	13
2	Uninfected	<i>H. defensa</i> -1	1	NA	—	0
3	<i>R. insecticola</i>	<i>H. defensa</i> -1	33	3	18	3
4	<i>R. insecticola</i>	<i>S. symbiotica</i>	3	0	—	0
5	<i>H. defensa</i> -1	<i>R. insecticola</i>	8	0	—	0
6	<i>H. defensa</i> -1	<i>S. symbiotica</i>	8	0	—	0
7	<i>H. defensa</i> -1	<i>H. defensa</i> -2	10	0	—	0
8	<i>H. defensa</i> -2	<i>S. symbiotica</i>	2	0	—	0
9	Uninfected	<i>S. symbiotica</i>	4	NA	—	0
10	<i>S. symbiotica</i>	Uninfected	4	0	NA	NA
			86 (total)	3 (of 68)* (4.4%)	31 (of 46)* (67.4%)	16 (of 82)* (19.5%)

NA, not applicable; —, generations 2 and 3 were not screened for these crosses.

*No. of possible observations, based on nos. of relevant crosses screened.

individuals from the second or third generation after the cross with a threshold of detection at 100 chromosome copies per aphid, a majority (67%) were confirmed to have the paternal symbiont, often at low titer. Of these infected lines, only a few retained the infection until the subsequent screening, at the seventh generation. Three paternal transfers of *H. defensa* became established in progeny lines (Table 1). In two of these cases, a dual infection was established and inherited for at least seven generations. In both, the paternal (*H. defensa*) infection was subsequently lost by generation 10, so that the line reverted to the maternal (*R. insecticola*) infection. In the third case, the maternal *R. insecticola* was replaced by the paternal *H. defensa*, which was stably maintained until at least generation 10. Only for *S. symbiotica* was paternal transfer not observed, possibly because of low sample sizes ($n = 17$). Therefore, at least two of the three symbiont species tested can be transferred from father to progeny and established in the new matriline. Furthermore, our experiments demonstrate that paternal transfer can be the source of single infection in previously uninfected matrilines, dual infection in singly infected matrilines, and replacement of maternal with paternal symbionts.

Localization of Symbionts Within Male Reproductive Organs. To determine the location of the symbionts within male reproductive systems, we used microscopy with FISH, employing oligonucleotide probes designed to complement regions of the symbiont 16S rRNA. These studies were conducted with males from the same *R. insecticola*-infected line (line 2a) used in crosses (Table 1). We detected *R. insecticola* both in testes and in accessory glands, with highest densities apparent in the latter (Fig. 1). Symbionts were not apparent within the sperm heads themselves. Controls performed with uninfected males showed limited green fluorescence in the junction of the testes, presumably because of autofluorescence of the insect tissues, but none in the accessory glands.

Imperfect Maternal Transfer During the Sexual and Egg Stages. Crosses with infected mothers allowed us to assess the efficiency of maternal transfer through the sexual and egg stages. Although most progeny acquire maternal infections, we confirmed three losses of the maternal symbiont, all in cross 3 (Table 1). As noted above, transmission through the parthenogenetic generations approaches 100% for single infections, at least under our standard laboratory conditions. Thus, the sexual egg stage may be a major source of uninfected maternal lines within populations. The frequency of loss is likely to be even higher in the field,

where winter temperatures are typically far lower than those experienced in our laboratory crosses. In one case of maternal loss, the paternal symbiont (*H. defensa*) replaced the maternal symbiont (*R. insecticola*). In the other two cases, progeny were uninfected even though both parents had symbionts.

Fig. 1. Localization of symbionts within the male reproductive system by using FISH with probe matching the 16S rRNA sequence of *R. insecticola* (green and bright yellow) and with propidium iodide DNA counterstain (red). (A) Male of clone 2a (infected with *R. insecticola*). The testes are on the right; the greatest concentration of symbionts is within the accessory glands (center and left). (B) Control with male of clone 5A (uninfected) showing lack of hybridization or autofluorescence in accessory gland and green autofluorescence in part of external aedeagus, on the right. (C) Same as B, showing low level of green autofluorescence near the testes. (Scale bars, 0.1 mm.)

Discussion

Our experiments demonstrate venereal transfer of bacteria in insects as well as venereal transmission of microorganisms that are commonly beneficial to hosts. For aphid secondary symbionts, transfer at mating allows movement among aphid matriline, providing a likely explanation for several previous observations indicating that such movement occurs regularly in pea aphid populations (3, 5–7, 16, 17).

Mechanisms of Symbiont Transfer. Symbionts appear to be transferred to females with seminal materials but not within sperm cells, based on the absence of hybridization of our probe within the sperm heads (Fig. 1). The observed efficiency of transfer varied from 0% to 100% among our crosses; further work will be needed to determine the role of factors such as timing of mating relative to oviposition, host genotype, prior infection, and the possible effects of symbionts on the mortality of eggs. Regarding the last point, egg mortality was high (as is common in aphid-breeding experiments), so it was not possible to determine whether symbiont status affected egg mortality directly, with consequences for observed transfer frequency. Some juveniles died before hatching or during the process of hatching. In PCR-based screens, some showed evidence of transfer of *S. symbiotica* from the paternal line (data not presented). Thus, although we did not obtain surviving lines receiving paternal *S. symbiotica*, we suspect that this symbiont also undergoes sexual transmission, as observed for *H. defensa* and *R. insecticola*.

Information regarding the genomic content of *H. defensa* indicates that host invasion may depend on mechanisms encoded in the symbiont genome, enabling it to colonize novel hosts. The *H. defensa* genome appears to encode two intact type III secretion systems, which are used by many pathogens for invading and manipulating host cells (22). All strains of *H. defensa* studied also contain variable phage genomes that sometimes encode toxins targeted to eukaryotic cells (22).

The Significance of Paternal Transfer of Symbionts. Facultative symbionts are known to confer major benefits on pea aphids, enabling them to resist heat or to overcome parasitoids or fungal pathogens (4, 8–15). Although experimental transfections have shown that these symbionts can be deleterious when introduced to novel host species (13, 17), the mutualistic effects of *H. defensa* in protecting pea aphids against parasitoids appear to be largely independent of host genotype (9). Some symbiont strains affect host life-history traits, such as the tendency to produce winged forms, which are not linked to fitness in a clear manner but are considered to have a major genetic component in aphids (23). Our results show that such symbiont-dependent effects can depend on the contributions of males to progeny.

Paternal transmission has broad implications for symbiont dynamics in populations and for evolution of symbiont effects on hosts. Matings between individuals infected with different symbiont strains may be the primary source of coinfecting lines, which are observed regularly in pea aphids and other aphid species even though they appear to be impermanent in parthenogenetic lineages (3, 7, 16). Coinfections may enable symbiont strains to recombine or to exchange phage or phage genes that affect interactions with hosts (22). Second, the frequent establishment of double infections creates the potential for antagonistic coevolution among symbiont species or strains, possibly explaining the rapid changes in titer induced by certain combinations (16). Third, although many maternally transmitted symbionts increase their transmission by causing hosts to increase production of daughters at the expense of sons (24), selection to favor daughters will be decreased or eliminated if males can transmit symbionts to offspring. Even if the success of transfer per mating is not 100%, an infected male can potentially infect more

progeny than are produced by one female because a male can mate with many females. Similarly, transmission by males may facilitate faster sweeps of new symbiont strains through host populations. A recent study showed that infection with another strain of *R. insecticola* increases male production in some pea aphid lines (23), raising the possibility that this symbiont has evolved to manipulate host investment in males as a route to infecting more matriline.

Aphids are unusual in possessing a life cycle expected to be particularly resistant to the evolution of virulent venereal diseases. Male production in autumn depends on the survival and reproduction of a succession of numerous (typically 5–15) summer parthenogenetic generations, and this dependence is expected to promote mutualistic effects of symbionts on female reproduction. Deleterious, sexually transmitted pathogens would generally be eliminated unless they also conferred a benefit during parthenogenetic generations.

The facultative symbionts in our study are a major source of phenotypic variation among pea aphid lineages. For example, in a set of pea aphid clones showing wide variation in resistance to parasitoid attack, the resident *H. defensa* strain accounted for most of the variation, and none was attributable to aphid genotype (9). Under the common assumption that heritable symbionts are maternally transmitted, symbiont effects on host phenotype would be potentially detectable as maternal effects in genetic studies. If not recognized, occasional transfer of symbionts from males will cause ambiguous and misleading results. Because female aphids often mate with more than one male, males other than the genetic father might be a source of symbionts. Indeed, attempted matings between species sometimes occur in aphids (N.A.M., unpublished observations) and might serve as a route of interspecies symbiont transfer even when fertilization is not possible.

If male-mediated transfer is widespread, it may offer a means to manipulate the symbiotic status of pest insect populations in useful ways. For example, tsetse, the vector of African trypanosome, harbors a symbiont, *Sodalis glossinidius*, with similarities to the symbionts in this study: it is within the Enterobacteriaceae, possesses type III secretion systems (25), establishes heritable infections after artificial transfer by microinjection (26), and undergoes horizontal transmission among matriline through an unidentified mechanism (27). *Sodalis glossinidia* affects vector competency (28), and introduction of trypanosome-incompatible strains has been proposed as a means of reducing vector efficiency. Paternal transfer might provide a route for introducing symbionts into populations because a single infected male potentially can transfer symbionts to the progeny of several females.

Materials and Methods

Transmission of Facultative Symbionts During Parthenogenetic Generations. Our laboratory conditions for long-term rearing of clonal lineages of aphids consist of constant 20°C temperature in plant-growth chambers, with fava bean seedlings as food plants. Since June 1999, we have reared continuously a set of 9–15 infected pea aphid lines. During this period, some for up to 7 years, no instance of loss of all facultative symbionts has occurred in laboratory cultures, which were last screened in April 2006. Summing across our cultured, infected lines, we have observed transfer across 2,500 generations, based on a conservative estimate of 30 generations per year. (Parthenogenetic generations overlap. The time from birth to first reproduction is 9 days at 20°C; most progeny are born within the first 7 days after a female's first reproduction.)

Criteria for confirming the presence of a symbiont include PCR with diagnostic primers for a large portion of the 16S rRNA-encoding DNA plus sequencing of 500–700 nucleotides from the 5' end; primers for each symbiont species are given in ref. 17. Criteria for establishing the absence of symbionts include negative results obtained by using quantitative PCR with diagnostic primers, with a sensitivity of <100 chromosome copies,

based on calibration of absolute copy numbers with a dilution series from cloned fragments.

Sexual Crosses. We used standard methods to induce sexual females and males by subjecting colonies to long nights, to set up crosses on caged host plants, to overwinter the resulting eggs, and to establish progeny as new clonal lines on separate host plants (29), except that our eggs were hatched after 60–80 days rather than 100 days. For each cross, 10–15 females in the final juvenile (fourth) instar were placed on an aphid-free plant and caged, and 3–6 males from the chosen line were introduced to the cage. Cages were made from polystyrene cups (18). Females cannot mate before reaching adulthood, so this procedure ensures that females were virgins (i.e., had not mated within their natal colony). Crosses were performed in three sets, consisting of cross 1, crosses 2–8, and crosses 9 and 10, with different aphid clones involved in each set. Both mothers and fathers were derived from one uninfected line (line 5A) and four infected lines, one with *S. symbiotica* (line 9-2-1), one with *R. insecticola* (line 2a), and two with different strains of *H. defensa* (lines 82B and A2C). Collection information for these lines is given in Russell and Moran (13) and Oliver *et al.* (9). All facultative symbionts were distinguishable through DNA markers. Aphid matrilines were distinguishable through a combination of two *Buchnera* polymorphisms (consisting of one indel and one nucleotide difference) in the intergenic region between *hslU* and *ibpA*.

Screening for Facultative Symbionts in Progeny. Each progeny line was maintained for at least seven generations (10 weeks) after hatching from the sexually produced eggs. (The steps for each cross and screening, from induction of sexual females and males, through egg production and diapause, to hatching and culturing of progeny lines, require ≈ 8 months.) To detect symbiont infections in progeny lines, diagnostic PCR was performed by quantitative PCR on a LightCycler (Roche, Indianapolis, IN). Calibration based on dilutions of cloned template indicated that these assays are able to detect <100 gene copies. PCR primers for diagnosing the three symbionts were based on distinctive sites in *dnalK* (*H. defensa*, as in ref. 4; *R. insecticola*, U70F1 5'-GATTTTCGCTTTCTCTGCTG-3' and U70R1 5'-ATACCCATCTCGGTGGTG-3'; and *S. symbiotica*, R70F1 5'-TGGCGGGTGATGTGAAG-3' and R70R1 5'-CGGGATAGTG-GTGTTTTTGG-3'). To determine which symbionts established long-term infections, diagnostic PCR was performed on generation 7 and generation 10. In some cases, it was performed on generation 2 or 3. In cases in which quantitative PCR indicated that a paternal symbiont had been acquired, larger-scale PCR was performed with a symbiont-specific 16S rRNA primer (R1279F 5'-CGAGAGCAAGCGGACCTCAC-3',

T1279F 5'-CGAGGGAAAGCGGAACCTCAG-3', or U1279F 5'-CGAACGTAAGCGAACCTCAT-3') combined in each case with a more universal 23S rRNA primer, 35R (18). Amplified 16S rRNA products were sequenced at the Genomic Analysis and Technology Core (GATC) facility (University of Arizona, Tucson, AZ). Transfers of *H. defensa* were also verified by PCR and sequencing of genes from APSE-2 phage by using primers listed for P2 and P28 (22). Quantitative PCR conditions were described in ref. 22.

Verifying Matriline by Using Markers from Primary Symbionts. To rule out contamination of the cultures with individuals from a different line, we verified maternal identity by using known polymorphisms in the genome of *B. aphidicola*, which is known to be maternally inherited by inoculation of overwintering eggs within the mother (1). All cases of transfer were verified with diagnostic PCR for at least three individuals, and they were sequenced to confirm that the PCR product did correspond to the facultative symbiont of the father. As above, sequencing was performed at the University of Arizona GATC facility. Additionally, our crosses included lines differing in aphid nuclear genotype for a single-locus color polymorphism, and color of the progeny lines corresponded to the expected ratios. For example, in cross 1, mothers were heterozygous with pink-dominant, fathers were homozygous green, and the *B. aphidicola* genotype differed between maternal and paternal lines. Progeny consisted of seven pink and six green lines, and all contained the maternal and not the paternal *B. aphidicola* genotype. Therefore, infection of progeny with *R. insecticola* could have occurred only through paternal transfer. Similar controls were applied in each case of paternal transfer.

Microscopy. To localize the symbionts within male reproductive systems, FISH was performed on reproductive systems dissected from males of line 2a, by using probes that matched with the 16S rRNA of *R. insecticola*. Males from line 5A (uninfected with secondary symbionts) were used as controls, as were males of 2a, for which no probe was added. Entire reproductive systems, including testes, vas deferens, and accessory glands, were removed from males in phosphate buffer and hybridized by using methods and a fluorescent-tagged oligonucleotide probe (Pro-319) described in ref. 30. Propidium iodide was used as a general DNA counterstain.

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- Buchner, P. (1965) *Endosymbiosis of Animals with Plant Microorganisms* (Wiley Interscience, New York), pp. 297–332.
- Baumann, P. (2005) *Annu. Rev. Microbiol.* **59**, 155–189.
- Sandström, J. P., Russell, J. A., White, J. P. & Moran, N. A. (2001) *Mol. Ecol.* **10**, 217–228.
- Moran, N. A., Russell, J. A., Fukatsu, T. & Koga, R. (2005) *Appl. Environ. Microbiol.* **71**, 3302–3310.
- Ferrari, J., Darby, A. C., Daniell, T. J., Godfray, H. C. J. & Douglas, A. E. (2004) *Ecol. Entomol.* **29**, 60–65.
- Tsuchida, T., Koga, R., Shibao, H., Matsumoto, T. & Fukatsu, T. (2002) *Mol. Ecol.* **11**, 2123–2135.
- Haynes, S., Darby, A. C., Daniell, T. J., Webster, G., van Veen, F. J. F., Godfray, H. C. J., Prosser, J. I. & Douglas, A. E. (2003) *Appl. Environ. Microbiol.* **69**, 7216–7223.
- Oliver, K. M., Russell, J. A., Moran, N. A. & Hunter, M. S. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 1803–1807.
- Oliver, K. M., Moran, N. A. & Hunter, M. S. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 12795–12800.
- Scarborough, C. L., Ferrari, J. & Godfray, H. C. J. (2005) *Science* **310**, 1781.
- Tsuchida, T., Koga, R. & Fukatsu, T. (2004) *Science* **303**, 1989.
- Montllor, C. B., Maxmen, A. & Purcell, A. H. (2002) *Ecol. Entomol.* **27**, 189–195.
- Russell, J. A. & Moran, N. A. (2006) *Proc. R. Soc. London Ser. B* **273**, 603–610.
- Chen, D. Q. & Purcell, A. H. (1997) *Curr. Microbiol.* **34**, 220–225.
- Chen, D. Q., Montllor, C. B. & Purcell, A. H. (2000) *Entomol. Exp. Appl.* **95**, 315–323.
- Oliver, K. M., Moran, N. A. & Hunter, M. S. (2006) *Proc. R. Soc. London Ser. B* **273**, 1273–1280.
- Russell, J. A., LaTorre, A. L., Sabater-Munoz, B., Moya, A. & Moran, N. A. (2003) *Mol. Ecol.* **12**, 1061–1075.
- Russell, J. A. & Moran, N. A. (2005) *Appl. Environ. Microbiol.* **71**, 7987–7994.
- Darby, A. C. & Douglas, A. E. (2003) *Appl. Environ. Microbiol.* **69**, 4403–4407.
- Wolfner, M. F. (2002) *Heredity* **88**, 85–93.
- Knell, R. J. & Webberley, K. M. (2004) *Biol. Rev. Camb. Philos. Soc.* **79**, 557–581.
- Moran, N. A., Degnan, P. H., Santos, S. R., Dunbar, H. E. & Ochman, H. (2005) *Proc. Natl. Acad. Sci. USA* **102**, 16919–16926.
- Leonardo, T. E. & Mondor, E. B. (2006) *Proc. R. Soc. London Ser. B* **273**, 1079–1084.
- Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. (1999) *Annu. Rev. Microbiol.* **53**, 71–102.
- Toh, H., Weiss, B. L., Perkin, S. A., Yamashita, A., Oshima, K., Hattori, M. & Aksoy, S. (2006) *Genome Res.* **16**, 149–156.
- Cheng, Q. & Aksoy, S. (1999) *Insect Mol. Biol.* **8**, 125–132.
- Aksoy, S., Chen, X. & Hyspa, V. (1997) *Insect Mol. Biol.* **6**, 183–190.
- Aksoy, S. & Rio, R. V. (2005) *Insect Biochem. Mol. Biol.* **35**, 691–698.
- Via, S. (1992) *Entomol. Exp. Appl.* **65**, 119–127.
- Moran, N. A., Tran, P. & Gerardo, N. M. (2005) *Appl. Environ. Microbiol.* **71**, 8802–8810.