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Individual animals are a transient habitat for populations of biotrophic microorganisms, and only those microbial populations with propagules which disperse to other animals persist beyond the life span of each animal. Consequently, the patterns of transmission are crucial to the ecology of microorganisms associated with animals. Transmission of microorganisms between animal hosts may be vertical (i.e., from a parent animal to its offspring) or horizontal (where the donor and recipient host are not necessarily related) (8, 11). The mode of transmission can be deduced from the following two complementary approaches: ecological, in which the abundance and distribution of microorganisms in different animal tissues, especially the reproductive organs, and the free-living environment are determined (2, 16, 22); and evolutionary, in which the historical incidence of vertical and horizontal transmission is deduced from the level of congruence of the phylogenies of the animal and microorganisms (14, 21, 23). The ecological approach offers accurate information on the prevalence of horizontal and vertical transmission and underlying processes, while the evolutionary approach can identify historical events too rare to be identified by the ecological methods.

Many of the microorganisms associated with animals are commensals that have less than universal prevalence; i.e., they have no detectable significance to the animal host and are borne by some, but not all, members of the host population. The prevalence of these microorganisms is expected to be determined by their transmission dynamics, but despite increasing research interest in commensal microorganisms (1, 12, 17), their transmission patterns are poorly known.

In this study we used an insect-borne bacterium, a γ-proteobacterium known informally as pea aphid *Bemisia*-like symbiont (PABS), also known as T-type, which is widely but not universally distributed in natural populations of the pea aphid, *Acyrthosiphon pisum*. The vertical transmission of PABS to asexual and sexual morphs and sexually produced eggs was demonstrated by a diagnostic PCR-based assay, and the maximum estimated failure rate was 2%. Aphids naturally lacking PABS acquired PABS bacteria administered via the diet, and the infection persisted by vertical transmission for at least three aphid generations. PABS was also detected in two of five aphid honeydew samples tested and in all five siphuncular fluid samples tested but in none of 15 samples of salivary secretions from PABS-positive aphids. However, PABS-negative aphids did not acquire PABS when they were cocultured with PABS-positive aphids; the maximal estimated level of horizontal transmission was 18%. A deterministic model indicated that the force of infection by a horizontal transmission rate of 3% is sufficient to maintain a previously described estimate of the prevalence of PABS-positive aphids (37%), if the vertical transmission rate is 98%. We concluded that PABS infections in *A. pisum* can be maintained by high vertical transmission rates and occasional horizontal transmission, possibly via the oral route, in the absence of selection either for or against aphids bearing this bacterium.
diets at a density of approximately 10
by the method described by Harrison et al. (15) and were aseptically added to the
5
light and 6 h of darkness with a daily 1°C reduction in temperature to 13°C and
were obtained by culturing the plant-reared aphids first for 3 days with 18 h of
each aphid, collected as it was born onto a sterile DNA-free pipette tip; 20 sexual
to contain PABS.

of distilled water and a positive control consisting of aphid DNA template known
bromide, and visualization under UV illumination. All gels included as molecular
at ca. 1,330 bp after electrophoresis on 2% agarose gels, staining with ethidium
hairs of a paintbrush and collected in a microcapillary tube. Finally, groups of five
covered with tinfoil positioned below each leaf was collected in 25
undersides of plant leaves, and the honeydew deposited over 2 days onto a strip
sachets incubated without aphids were used as a negative control. The second type of samples comprised the fluid exudates
as a negative control. The first type, five replicate groups of five aphids were allowed to feed on the
In the first type, the general bacterial 16S ribosomal DNA primers used were 5'-
and 5'-GCT TAA CAC ATG CAA G-3'
round, the general bacterial 16S ribosomal DNA primers used were 5'-
generations in the summer months, followed by a single sexual generation that
Kingdom), comprising 100- to 1,000-bp markers in 100- bp increments and one
JF98/24
Trifolium pratense, July 2001,
University of York
None
ACD01/04
Trifolium pratense, July 1995,
University of York
PAUS
Aphis fabae HR 91/3
Vicia faba, June 1991, Abingdon,
Oxon
PAUS

Materials and Methods

The aphids listed in Table 1 are cyclical parthenogens with multiple asexual
generations in the summer months, followed by a single sexual generation that
produces the overwintering eggs. They were maintained as parthenogenetic lines
on Vicia faba cv. The Sutton at 18°C with a daily cycle consisting of 18 h of light
and 6 h of darkness and were also raised on an aseptic chemically defined diet,
formulation A (24) with 0.15 M amino acids and 0.5 M sucrose.

The incidence of PABS in the samples was determined by a specific nested
PCR assay (5). DNA was extracted with a DNeasy tissue kit (Qiagen, Crawley,
United Kingdom) by following manufacturer’s instructions. For the PCR assays we used the cycling conditions and primers described previously (5). For the first
round, the general bacterial 16S ribosomal DNA primers used were 5'-GCT
TAA CAC ATG CAA G-3' and 5'-AGC GCC AGT GTG TAC AAG ACC-3',
corresponding to nucleotide positions 41 to 61 forward and 1405 to 1385 reverse
in Escherichia coli; and for the second round, the specific forward primer was
5'-AGC GCA GTT TAC TGA GTT CA-3', corresponding to nucleotide positions 806 to 826 reverse in Vicia faba.

Vertical transmission of PABS. Among the parthenogenetic aphids, every mother and offspring of A. pisum lines UY2 and
LMB95/28 bore PABS, and every individual of line JF98/24 was PABS-free. All the oviparous (sexual females) and males of lines UY2 and LMB95/28 and all the fertile sexual eggs of line
LMB95/28 tested were also positive for PABS. When the binomial
distribution was applied, the minimum transmission rates were calculated to be 74% for each sample of male
aphids (n = 10) and 86% for each sample of parthenogenetic aphids, oviparae, and eggs (n = 20). Combining the data for all
samples across the data (for UY2, n = 50; for LMB95/28, n = 70), a minimum overall transmission rate of 98% was obtained.

Over the course of this study, the routine parthenogenetic cultures of A. pisum lines UY2, LMB95/28, and JF98/24 were
monitored for the presence of PABS over an estimated 114, 297, and 111 parthenogenetic generations. Lines UY2 and
LMB95/28 were stably PABS positive, and JF98/24 was PABS negative throughout this period.

Horizontal transmission of PABS. PABS was detected in the
siphuncular fluid samples collected from all five aphids tested and in two (40%) of the five honeydew samples but in none of the
diet sachets from which PABS-positive aphids had fed (Fig. 1a). The diet sachets probed by the aphids (but not the aphid-
free sachets) did, however, become contaminated by bacteria other than PABS, as indicated by a product in the first round of the
nested PCR assay, but the source and identity of the bacteria were not investigated further in this study. As determined
by the binomial distribution, the minimal occurrence of PABS in siphuncular fluid was 55% (n = 5), and the maximum occurrence of release of PABS into the food substrate during feeding was 18% (n = 15).

When aphids of the PABS-negative lines A. pisum JF98/24 and A. fabae HR91/3 were fed on diets bearing bacteria from A. pisum clone LMB95/28, they were positive for PABS, and the third-generation descendants of these aphids maintained on plants were also PABS positive. The cultures of clones
JF98/24 and HR91/3 derived from aphids fed bacterium-free diets were PABS negative (Fig. 1b).

In the final experiment, groups of the PABS-negative lines *A. pisum* ACD01/04 and *A. fabae* HR91/3 were cultured with the PABS-positive *A. pisum* line LMB95/28 on *V. faba* plants for three parthenogenetic generations. At the start and at the end of the experiment, all of the aphids of line LMB95/28 tested were PABS positive, and none of the aphids of lines ACD01/04 and HR91/3 bore PABS. Representative data for the ACD01/04 line are shown in Fig. 1c.

**Modeled impact of transmission patterns on frequency of PABS in aphid populations.** The deterministic model was used to calculate the per capita force of infection required to maintain a stable frequency of PABS-positive aphids (19). For a calculated maximum rate of failure of vertical transmission of 2% (see above) and a frequency of PABS-positive aphids in natural populations of 37% (5), the force of infection was 3% (Fig. 2). Figure 2 also shows that when the rate of failure of vertical transmission was elevated, the force of infection required to maintain a stable frequency of PABS in the aphid

**FIG. 1.** Incidence of horizontal transmission of PABS. (a) Release of PABS from *A. pisum* line LMB95/28 via siphuncular fluid (lanes 1 to 5), honeydew (lanes 6 to 10), and feeding on diet sachets (lanes 11 to 15). Lane 16 contained a PABS-positive control, and lane 17 contained a negative control. (b) Acquisition of PABS by *A. pisum* line JF98/24 (lanes 1 and 2) and *A. fabae* line HR91/3 (lanes 3 and 4) from diet sachets that either bore PABS (lanes 1 and 3) or were microbiologically sterile (lanes 2 and 4). After feeding on the test diets, aphids were reared on plants for three generations prior to analysis. Lane 5 contained a PABS-positive control, and lane 6 contained a negative control. (c) PABS status of *A. pisum* lines ACD01/04 and LMB95/28 in coculture. ACD01/04 (lane 1) and LMB95/28 (lane 2) were examined at the start of the experiment; one of five replicate aphids of line ACD01/04 from each cage (lanes 3 to 5) and of LMB95/28 from one cage (lane 6) were examined at the end of the experiment. Lane 7 contained a PABS-positive control, and lane 8 contained a negative control.

**FIG. 2.** Force of infection in a frequency-dependent model required to maintain the proportion of PABS-positive aphids in a population with 2 to 50% loss through failure of vertical transmission.
population increased dramatically at low PABS frequencies but that high PABS frequencies could be maintained by a low force of infection even with 50% loss via vertical transmission.

DISCUSSION

The central question addressed in this study is whether the observed prevalence of PABS in natural aphid populations can be accounted for by the transmission patterns in the absence of selection either for or against aphids bearing PABS. Under these conditions, PABS can be described as a commensal that can exploit the aphid habitat without having an impact on aphid fitness.

For the observed high fidelity of vertical transmission (>98%) in A. pisum, for the model output shown in Fig. 2, low levels of horizontal transmission (e.g., 3%) were required to maintain the 37% prevalence of PABS reported previously (5), and even lower transmission levels were required for the higher prevalence of PABS in some A. pisum populations (7). The question, therefore, is whether a 3% force of infection is realistic for the aphid-PABS system. The estimated incidence of horizontal transmission of <18%, as obtained from coculturing aphid lines containing and lacking PABS, is compatible with the expectation of the model output (if the estimated values for horizontal transmission had been appreciably higher than 3%, the data would have been indicative of selection against PABS-positive aphids); and the experiments in which we examined the release and acquisition of PABS by the oral route suggested that PABS can be transmitted horizontally. Siphuncular fluid, which consistently bore PABS in our experiments, comprises modified hemolymph, and access of hemolymph-borne PABS to this material is not restricted by any anatomical barriers. The presence of PABS in honeydew and the stable vertical transmission via the insect ovaries of PABS acquired from ingested food together indicate that PABS can readily breach the aphid gut wall in both directions (i.e., to and from the gut lumen). PABS has previously been detected associated with dissected guts of A. pisum (6), and the site of transfer across the gut wall is an important issue for future research. Many other microbial taxa are known to be acquired by insects via the gut (18).

The low predicted rate of horizontal transmission between cocultured aphids (<18%), when combined with the estimated incidence of release (>55%) and assured acquisition of PABS, suggests that unidentified factors may restrict the incidence of horizontal transmission. Perhaps the populations of PABS released from aphids may have low infectivity or low viability, either at the time of release or because of inhospitable conditions on the plant surface (27). Also, aphids which have specialized mouth parts for feeding on plant sap may rarely ingest material from the plant surface. These issues may not be specific to PABS because an accessory bacterium known as PASS (R-type) also is not transmitted at a high frequency by coculture of PASS-positive and PASS-negative aphids (4).

In summary, our findings suggest that PABS can be maintained at the frequency observed in aphid populations by a combination of vertical transmission with high fidelity and occasional horizontal transmission. A priority for future research is to obtain more precise quantitative data for vertical and horizontal transmission rates by using increased sample sizes.

Environmental factors, especially temperature and rearing plant, may affect horizontal transmission by influencing the density or characteristics of released PABS cells, the proximity between aphids, and aphid probing behavior, as well as the persistence and vertical transmission of the bacterial cells in the insect tissues. In addition, horizontal transmission of these bacterial through aborted attack by a parasitoid whose ovipositor is contaminated with bacteria from a previous aphid victim has been suggested (5, 25), but this has not been investigated experimentally.

The conclusions of this study are fully compatible with the finding that aphids from natural populations containing and lacking PABS did not differ significantly in terms of fitness under field conditions on the host plant V. faba (7). However, these conclusions apply strictly to aphids on V. faba in the absence of natural enemies under summer conditions in the United Kingdom. The possibility that the prevalence of PABS-positive aphids under different conditions may be influenced by selective factors has been raised by several studies in which possession of accessory aphids other than PABS has been correlated with plant affiliation and temperature (4, 20, 26). A priority for future research is to elucidate the contribution of transmission and selective factors to the observed prevalence of accessory bacteria in their animal hosts across different biotic and abiotic regimes.

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