Interspecific symbiont transfection confers a novel ecological trait to the recipient insect

Tsutomu Tsuchida1,2, Ryuichi Koga2, Shogo Matsumoto1 and Takema Fukatsu2,*

1 Molecular Entomology Laboratory, RIKEN Advanced Science Institute, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
2 Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba 305-8566, Japan
*Author for correspondence (t-fukatsu@aist.go.jp).

In Japan, pea aphids Acyrthosiphon pism mainly feed on vetch and clover, and many aphid clones produce more progeny on vetch than on clover. In this context, particular genotypes of the facultative symbiont Regiella insecticola enhance reproduction of infected pea aphids specifically on clover, thereby broadening the suitable food plant range of the insect. A species that is sympatric to A. pism, vetch aphids Megoura crassicauda, are commonly found on vetch but not on clover. Laboratory rearing of M. crassicauda strains revealed active reproduction on vetch but substantially no reproduction on clover. Experimental transfection of Regiella from A. pism to M. crassicauda by haemolymph injection established stable and heritable infection in the recipients, although no Regiella infection has been detected in natural populations of M. crassicauda. Different strains of Regiella-transfected M. crassicauda grew and reproduced on vetch, but exhibited lower fitness in comparison with corresponding uninfected aphid strains. Strikingly, the Regiella-transfected M. crassicauda exhibited improved survival and some reproduction on clover. These results suggest that Regiella has the potential to confer an ecological trait, adaptation to clover, on novel insect hosts, and also account for why Regiella is able to infect M. crassicauda but is scarcely found in these aphid populations.

Keywords: Acyrthosiphon pism; Megoura crassicauda; Regiella insecticola; plant adaptation; fitness effects

1. INTRODUCTION

Many insects are obligatorily associated with bacterial symbionts of mutualistic nature. The best-studied case is the aphid endosymbiont Buchnera aphidicola, which provides essential amino acids and other nutrients deficient in the plant sap diet of the host aphids (reviewed in [1]). In addition to the obligate symbiont, a number of aphids also harbour one or a few facultative bacterial symbionts. Recent studies have revealed that these facultative symbionts confer condition-dependent fitness advantages to the host aphids, such as heat tolerance, parasite resistance, plant adaptation, etc. (reviewed in [2]).

‘Candidatus Regiella insecticola’ (hereafter simply called Regiella) is a gammaproteobacterial symbiont associated with diverse aphids [3–5]. In Japan, pea aphids Acyrthosiphon pism mainly feed on vetch and clover, and many aphid clones produce more progeny on vetch than on clover. Notably, Regiella infection was detected from aphids on clover at significantly higher frequencies than on vetch [6]. Similar associations between Regiella infection and clover infestation have been identified in pea aphid populations worldwide [7–9]. Experimental works showed that some Regiella strains are capable of improving the host fitness specifically on clover but other strains are not [10–12]. Hence, at least some Regiella strains are involved in plant adaptation in natural populations of A. pism.

In Japan, sympatric to A. pism, vetch aphids Megoura crassicauda are commonly found on vetch but not on clover. An extensive field survey detected no Regiella infection in natural populations of M. crassicauda [13]. Notwithstanding this, experimental transfection of Regiella from A. pism to M. crassicauda by haemolymph injection established stable and heritable infection for over 50 generations [13]. Biological effects of Regiella transfection on M. crassicauda are of interest but not yet investigated.

Here we report a series of experiments to address the following questions: (i) why is Regiella able to infect M. crassicauda but not maintained in natural aphid populations? (ii) Does Regiella transfection transfer the ecological trait, adaptation to clover, to the recipient M. crassicauda?

2. MATERIAL AND METHODS

(a) Insects

Three isofemale strains of vetch aphid M. crassicauda, namely HGm, TSKm and KKn, were collected from vetch Vicia sativa at three localities in Japan: Hongo, Tokyo, in 1999; Tsukuba, Ibaraki, in 1999; and Kyoto, Kyoto, in 2004. Diagnostic PCR and 16S rRNA gene cloning and sequencing confirmed that these strains harboured the obligate endosymbiotic B. aphidicola but no facultative symbionts. A Regiella-infected strain, TUt, of pea aphid A. pism was used as a donor of Regiella, wherein Regiella infection caused enhanced host fecundity specifically on white clover Trifolium repens [12]. A Regiella-cured strain of the same genotype, TUTrem, was also used. These aphid strains were maintained on seedlings of the broad bean Vicia faba at 20°C in a long-day regimen (16 L : 8 D).

(b) Symbiont transfection

Regiella-infected strains of M. crassicauda were established by haemolymph transfer as described [13]. In short, haemolymph from adult TUT insects was injected into CO2 anaesthetized third-instar HGm, TSKm and KKn insects using a glass capillary under a dissection microscope. Haemolymph of the Regiella-cured strain TUTrem was used for control treatments. From 11–13 days after injection, nymphs of the injected insects were collected and individually reared to adulthood on broad bean plants. After collecting a sufficient number of nymphs, the adult insects were subjected to diagnostic PCR for detection of Regiella and Buchnera infections as described [13]. The Regiella-infected and uninfected aphid lines were maintained on seedlings of broad bean, and were regularly checked for Regiella infection by diagnostic PCR.

(c) Plants

Broad bean plants were used two to three weeks after sowing. Vetch and clover plants were grown from field-collected seeds and used as pre-flowering plants three to four weeks after sowing. The plants were grown on medium nutrient compost at 20°C in a long-day regimen (16 L : 8 D).
Table 1. Fitness effects of Regiella infection on M. crassicauda reared on broad bean and vetch. Asterisks indicate statistically significant differences between Regiella-infected and uninfected insects of the same genotype (p < 0.05, Mann–Whitney’s U-test).

<table>
<thead>
<tr>
<th>plant</th>
<th>clone</th>
<th>Regiella infection</th>
<th>n</th>
<th>fresh body weight of 8-day-old insect (mg)</th>
<th>pre-reproductive period (days)</th>
<th>fecundity (number of offspring)</th>
<th>longevity (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>broad bean</td>
<td>HGm</td>
<td>uninfected</td>
<td>27</td>
<td>3.46 ± 0.55</td>
<td>8.26 ± 0.53</td>
<td>96.87 ± 16.55</td>
<td>30.37 ± 5.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>23</td>
<td>3.39 ± 0.58</td>
<td>8.52 ± 0.59</td>
<td>89.77 ± 14.03*</td>
<td>27.78 ± 4.70</td>
</tr>
<tr>
<td></td>
<td>KKm</td>
<td>uninfected</td>
<td>19</td>
<td>3.09 ± 0.61</td>
<td>8.42 ± 0.51</td>
<td>99.37 ± 18.44</td>
<td>31.95 ± 4.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>20</td>
<td>3.18 ± 0.39</td>
<td>8.30 ± 0.47</td>
<td>81.95 ± 18.61*</td>
<td>25.65 ± 5.24*</td>
</tr>
<tr>
<td></td>
<td>TSKm</td>
<td>uninfected</td>
<td>19</td>
<td>3.88 ± 0.56</td>
<td>8.40 ± 0.94</td>
<td>90.47 ± 12.79</td>
<td>30.84 ± 5.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>21</td>
<td>3.59 ± 0.38</td>
<td>9.00 ± 1.07</td>
<td>91.62 ± 6.88</td>
<td>30.38 ± 3.02</td>
</tr>
<tr>
<td>vetch</td>
<td>HGm</td>
<td>uninfected</td>
<td>14</td>
<td>2.45 ± 0.76</td>
<td>8.71 ± 0.47</td>
<td>78.86 ± 14.71</td>
<td>43.50 ± 5.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>13</td>
<td>1.98 ± 0.65</td>
<td>9.31 ± 0.85*</td>
<td>63.46 ± 14.86*</td>
<td>35.46 ± 4.33*</td>
</tr>
<tr>
<td></td>
<td>KKm</td>
<td>uninfected</td>
<td>9</td>
<td>2.02 ± 0.66</td>
<td>9.22 ± 0.83</td>
<td>75.00 ± 14.71</td>
<td>40.56 ± 5.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>11</td>
<td>1.94 ± 0.77</td>
<td>9.64 ± 1.36</td>
<td>71.00 ± 25.07</td>
<td>32.36 ± 5.07*</td>
</tr>
<tr>
<td></td>
<td>TSKm</td>
<td>uninfected</td>
<td>16</td>
<td>1.79 ± 0.83</td>
<td>10.75 ± 2.49</td>
<td>79.25 ± 24.02</td>
<td>40.63 ± 9.57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>infected</td>
<td>16</td>
<td>1.96 ± 0.54</td>
<td>9.63 ± 0.96</td>
<td>70.19 ± 19.75</td>
<td>34.06 ± 6.93*</td>
</tr>
</tbody>
</table>

*aTotal number of insects examined.
*bMean ± s.d.

d) Fitness measurement
Fitness measurements were conducted 31 months for HGm (equivalent to about 94 generations), 23 months for KKm (about 70 generations) and 24 months for TSKm (about 73 generations) after the injection treatments. In all the transfected aphid strains, Regiella infection was stably maintained during the period. Unwinged aphids were subjected to the experiments. In order to minimize winged morph production, a limited number of aphids (ca 10) were maintained on a seedling of broad bean in each rearing cage. Ten-day-old adult aphids were allowed to deposit nymphs for 6 h, and the newborn nymphs were defined as 0 day old and individually transferred to a new plant of broad bean or vetch. Fresh body weight, pre-reproductive period, number of offspring and longevity of the insects were monitored every day. To examine the aphid performance on clover, the nymphs produced on broad bean plants within 6 h were kept on the same plants for 3 days. In 3-day-old aphids, presumptive unwinged nymphs and presumptive winged nymphs are distinguishable by the absence and presence of wing-pads on their thorax using a dissection microscope. After removing insects with wing-pads, the remaining unwinged-to-be nymphs were transferred to rearing cages with clover plants in 10 groups of 10 individuals for each of the Regiella-infected aphid strains. For each of the rearing cages, longevity of the insects was monitored every day, and, if larviposition occurred, the nymphs were counted and then removed. All the experiments were conducted at 20°C in a long-day regimen (16 L: 8 D). The plants were replaced by fresh ones every week.

3. RESULTS AND DISCUSSION
(a) Fitness cost of Regiella infection in M. crassicauda on laboratory host plant V. faba
On broad bean V. faba, all the three genotypes of M. crassicauda, namely HGm, KKm and TSKm, grew well and reproduced actively. Fitness parameters for the Regiella-infected strains were generally equivalent or inferior to those with the uninfected strains of the same genotype. Significant reductions were identified in fecundity in HGm and longevity in HGm, KKm and TSKm (table 1).
(b) Fitness cost of Regiella infection in M. crassicauda on native host plant V. sativa
On vetch V. sativa, all the three genotypes of M. crassicauda grew well and actively reproduced, although their fitness values were generally lower than those attained on broad bean. Fitness parameters with the Regiella-infected strains were generally equivalent or inferior to those with the uninfected strains of the same genotype. Significant reductions were identified in fecundity in HGm, fecundity in HGm and longevity in HGm, KKm and TSKm (table 1).

d) Regiella transfection to M. crassicauda conferred potential ability to survive and reproduce on non-host plant
These results illuminate an intriguing biological consequence of the Regiella transfection to vetch aphid M. crassicauda. Originally, M. crassicauda is almost unable to survive and reproduce on clover. However, upon Regiella transfection, their survival and reproduction on the non-host plant are slightly but significantly improved. Considering that the Regiella strain used in

this study improves fecundity of A. pisum specifically on clover [12], these results can be interpreted as the transfer of an ecological trait, viability on clover, from A. pisum to M. crassicauda by symbiont transfection. Recently, a number of studies have demonstrated environment-dependent fitness advantages conferred by facultative symbionts, and the possibility has been argued that such ecological traits might be moving around between different species in association with horizontal symbiont transfers (reviewed in [2]). Our study suggests that symbiont-mediated acquisition of a novel host plant is not only theoretically but also practically feasible.

4. CONCLUSION AND PERSPECTIVE
The fitness data of Regiella-infected insects in this study, namely slightly negative effects on the native host plant V. sativa and only marginal positive effects on the non-host plant T. repens, account for the reason why Regiella can potentially be a symbiont of M. crassicauda but is scarcely found in natural populations [13]. Furthermore, it should be noted that Regiella infection has been detected from a diverse array of aphid species [4,5]. We expect that future experimental work on different donor–recipient combinations might identify more drastic effects of Regiella transfection, leading to the establishment of a tractable model system to investigate the process of symbiont-mediated acquisition of novel host plant. Previous work on A. pisum has demonstrated that Regiella is involved not only in plant adaptation but also in pathogen immunity and parasitoid resistance [14,15]. Intra- and interspecific horizontal transfers of Regiella infection may play some roles in the

Figure 1. Survival of M. crassicauda on clover. (a) Strain HGm. (b) Strain KKm. (c) Strain TSKm. Survival rates of Regiella-infected strains and uninfected strains are shown as thick lines and dotted lines, respectively (n = 100 each). Statistical significance was evaluated by the generalized linear model with gamma error.

Figure 2. Reproduction of M. crassicauda on clover. (a) Strain HGm. (b) Strain KKm. (c) Strain TSKm. Each dot represents the total number of offspring in a rearing cage (§2). Statistical significance was evaluated by the generalized linear model with gamma error.
evolutionary dynamics of these ecologically important traits in natural aphid populations.

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