Can aphids learn to cope with the presence of endophytic fungi in their food plants?

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Abstract

Plant-endophyte associations can have major impacts on the dynamics of consumer interaction-webs but long-term effects of mycotoxins and the ability of herbivores to adapt to these toxins have not been studied. To understand the potential of aphids to cope with mycotoxins, we compared the life-history parameters for aphids conditioned for several generations on endophyte-infected plants with those of endophyte-naïve aphids on both endophyte-infected and endophyte-free grasses. Aphids conditioned on endophyte-infected plants produced more offspring during the first days of adulthood than endophyte-naïve aphids independent of the endophyte infection of the test environment. However, the endophyte-conditioned aphids tended to have a shorter lifespan, which resulted in similar numbers of total offspring produced for endophyte-conditioned and endophyte-naïve aphids. The difference in life-history parameters caused by the conditioning environment suggests that the effects of endophytes on herbivore life-history traits may represent an adaptive change that should be considered in future studies of endophyte-herbivore interactions.
Keywords: *Rhopalosiphum padi, Neotyphodium lolii, Lolium perenne,* common strain, adaptation, long-term effects, microbes, endosymbionts, reproductive strategy, life-history traits

Introduction

Endophytic fungi live in close association with almost all species of plants (Arnold *et al.* 2000). However, most studies are conducted with agronomic grass systems (Saikkonen *et al.* 2006) where the association between plants and fungus results in the production of invertebrate and vertebrate toxic alkaloids (Clay 1990). Through these toxins the fungus often enhances resistance of plants against herbivores (Breen 1994; Clay & Schardl 2002; Müller & Krauss 2005; Omacini *et al.* 2001). Existing studies focus on short-term effects of endophyte presence using endophyte-naïve herbivores for their tests. Especially short lived and fast reproducing herbivore species may show life-history changes in the presence of the mycotoxins over just a few generations.

Aphids are among the most important pests and populations grow quickly through parthenogenetic reproduction. The lack of genetic recombination in parthenogenetic reproduction does not prevent aphids from adapting to new conditions (Blackman 1981; Loxdale & Lushai 2003). Individuals with an advantageous mutation can pass it to all their descendants and a mutation can spread rapidly through populations (Dixon 1998). The "telescoping" of generations allow aphid embryos to get in contact with, and physiologically react to, any ingested material that passes the haemolymph of their mothers (Via 1991).

The cereal aphid *Rhopalosiphum padi* has shown poor reproduction and survival on the agricultural grass *Lolium perenne* infected with the common endophyte, *Neotyphodium lolii*, in a short-term experiment (Meister *et al.* 2006). However, if aphids experiencing the endophyte for several generations adapt in some way to the endophyte, we would expect aphids conditioned on endophyte-infected grasses to perform differently on endophyte-infected grasses than aphids naïve to endophytes.

Methods

To test how individual aphids react to the presence of endophytes on a longer time scale, aphids reared on endophyte-infected plants for several generations were compared with endophyte-naïve aphids. The stock culture of the aphid *Rhopalosiphum padi* was started with a few individuals collected near the University of Zürich, Switzerland in May 2003. The culture was kept in a controlled temperature room at 20°C and 16:8 h light:dark cycle on *L. perenne* cv. ARION, a commercially available endophyte-free fodder grass (staining of 30 seeds: 0% infection) provided by FAL Reckenholz, Switzerland.

The perennial ryegrass *Lolium perenne* used in the experiment was the New Zealand cultivar Samson, which was either endophyte-free (E -: identity number A 11104) or endophyte-infected by the common strain of *Neotyphodium lolii* (E +: identity number A12038) and was provided by Brian Tapper, AgResearch, NZ. The endophyte status was assessed (1) by staining and microscopic examination of 30 seeds of E- and 30 seeds of E+, and (2) by immunoblot assays ("Phytoscreen field tiller endophyte detection kit" by Agrinostics Ltd. Co. (http://www.agrinostics.com/)) of 120 plants of E- and 120 plants of E+ with a minimum age of 2 weeks grown in the greenhouse. The microscopic staining revealed an infection level of 0% for E- and 93% for E+. The immunoblot assays showed an infection level of 0.008% for E- and 85% for E+.

Before the start of the life-history experiment, the aphids were conditioned in Petri dishes on cuttings of either E- or E+ *L. perenne* for 2 months, which corresponds to approximately 10 aphid generations (further referred to as "conditioning environment"). The Petri dishes were lined with moist filter paper and the cuttings were replaced every second day. The plants providing the cuttings were planted at the beginning of the conditioning phase in a 30 cm x 20 cm seed tray (approximately 1000 seeds). One seed tray was planted with E- seeds, one with E+ seeds. The cuttings used were a random mixture of upper leaf blades and from the lower leaves, the sheath, stem and blade. At the end of the conditioning period, 3rd or 4th instar nymphs were tested in a life-history experiment: aphids conditioned on E- were tested on E- and E+ and aphids conditioned on E+ were tested on E- and E+ (further referred to as "test environment"). Each treatment combination was replicated ten times. Each 3rd or 4th instar nymph was transferred individually into one Petri dish with a wet filter paper and cuttings of either E- or E+ and was followed over the entire lifespan. As life-history parameters, we recorded life-time reproductive success (divided into number of nymphs produced during the first 6 days of adulthood, number of nymphs produced between day 6 and day 11 of adulthood and number of nymphs produced between day 11 and death), adult longevity and developmental time from birth to adulthood for the first one to three nymphs produced by each mother.

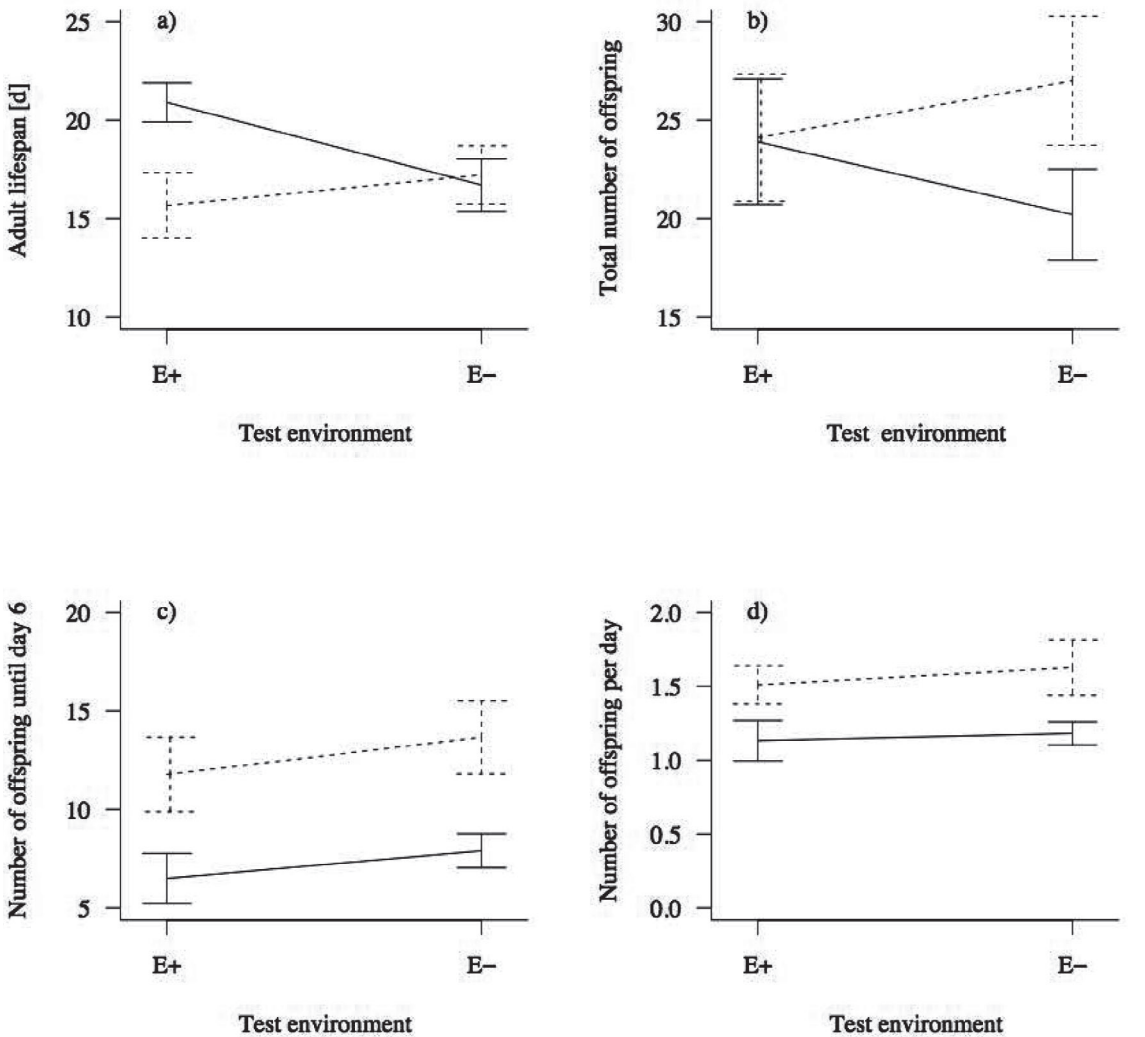
All life-history parameters were analysed separately using two-way ANOVA with the explanatory variables being infection of conditioning environment and infection of test environment and their interaction.

Results

The different life-history parameters measured showed different responses to the conditioning of aphids on endophyte-infected plants. Adult lifespan was affected by an interaction of the conditioning environment and the test environment, with aphids living the longest on E+ when conditioned on E- ($F_{1, 34(interaction)}$=4.40, P=0.043; Fig. 1a) but the test environment and the conditioning environment itself did not affect adult lifespan ($F_{1, 34 (test)}$=1.16, P=0.290; $F_{1, 34 (conditioning)}$=2.95, P=0.095; Fig. 1a). The total number of offspring produced was neither affected by the test nor the conditioning environment, nor the interaction ($F_{1, 34 (test)}$=0.04, P=0.848; $F_{1, 34 (conditioning)}$=1.36, P=0.252; $F_{1, 34 (interaction)}$=1.20, P=0.281; Fig.1b). However, the number of offspring produced during the first 6 days of adulthood was significantly higher for aphids conditioned on E+ independent of the test environment ($F_{1, 34 (test)}$=1.20, P=0.282; $F_{1, 34 (conditioning)}$=13.66, P=0.0008; $F_{1, 34 (interaction)}$=0.03, P=0.871; Fig.1c). In contrast, the number of offspring produced between day 6 and day 11 of adulthood was neither affected by the test nor the conditioning environment or the interaction ($F_{1, 34 (test)}$=0.16, P=0.688; $F_{1, 34 (conditioning)}$=1.39, P=0.246; $F_{1, 34 (interaction)}$=0.47, P=0.495). The same was true for the number of offspring produced between day 11 and death ($F_{1, 34 (test)}$=1.10 P=0.303; $F_{1, 34 (conditioning)}$=0.21 P=0.645; $F_{1, 34 (interaction)}$=1.86, P=0.182). To integrate lifespan and fecundity, we calculated the number of offspring produced per day. Aphids conditioned on E+ produced overall more offspring per day than those on E-, independent of the test environment ($F_{1, 34 (test)}$=0.35, P=0.557; $F_{1, 34 (conditioning)}$=9.11, P=0.005; $F_{1, 34 (interaction)}$=0.06, P=0.808; Fig. 1d).

The time of development to adulthood for the one to three first emerged nymphs was neither affected by the test environment ($F_{1, 33}$=1.78, P=0.191) nor by the interaction of test and conditioning

Figure 1 The life-history parameters measured of *Rhopalosiphum padi* in the test environment on either endophyte-infected (E+) or endophyte-free (E-) *Lolium perenne*. The solid lines represent aphids conditioned on E- plants and the dashed lines represent aphids conditioned on E+ plants. Presented are means ± s.e. for a) adult lifespan, b) total number of offspring produced, c) number of offspring produced during the first 6 days of adulthood and d) number of offspring produced per day. For the results of the statistical analyses refer to the results section.



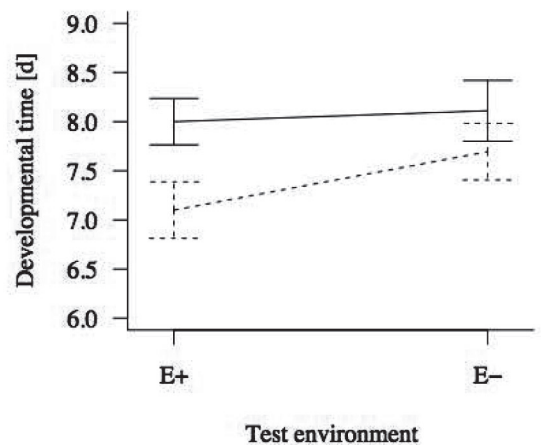
environment ($F_{1, 33}$=0.73, P=0.398), but was by the conditioning environment ($F_{1,33}$=5.55, P=0.024; Figure 2) with aphids conditioned on E+ developing significantly faster than aphids conditioned on E- .

Discussion

For all life-history parameters measured, the endophyte infection of the test environment did not have any influence. This is contrary to the observed changes in life-history traits of *R. padi* in several short-term experiments using endophyte-naïve aphids (e.g. Meister *et al.* 2006). However, the conditioning environment altered the expressed life-history traits: The exposure to endophyte infection over approximately 10 asexual generations appeared to trigger a change in the reproductive strategy. Aphids conditioned on E+ plants produced more offspring during the first few days of adulthood independent of the test environment. However, the total numbers of offspring produced over the entire lifespan remained the same for aphids conditioned either on E+ or on E-.

We speculated that aphids conditioned on endophyte-infected plants over several generations learn to cope with the presence of the mycotoxins and would somehow behave differently from endophyte-naïve aphids. Our results show that the conditioning influences reproduction in the first few days of adulthood, which is similar to 'fecundity compensation' that has been shown as a reaction to the presence of parasites (Minchella 1985; but see Krist & Lively 1998) or as a response in aphids to the presence of secondary parasitoids (van Veen *et al.* 2001) and could be a response to the shorter lifespan caused by the mycotoxin. However, to understand whether this is a general response of herbivores to mycotoxins, further experiments with different aphid clones and different herbivore species are needed. To compare changes in life-history traits for different clones exposed to the same environment would provide insights into the underlying mechanisms and would contrast between adaptation based on genetics or based on experience only (Ferrari *et al.* 2001; Via 1990).

Figure 2 The developmental time (time to adulthood) of the first nymphs produced by *Rhopalosiphum padi* in the test environment on either endophyte-infected (E+) or endophyte-free (E-) *Lolium perenne*. The solid lines represent mothers conditioned on E- plants and the dashed lines represent mothers conditioned on E+ plants. For the results of the statistical analyses refer to the results section.



The influence of the conditioning environment on aphid performance has been observed before for the aphid *Acyrthosiphon pisum*. This species occurs on different host plants and clonal specialisation expressed in different life-history traits on the different host plants has been observed. The specialised host performance does not change after three generations on the alternate host indicating that in this case the host plant experience has a strong influence on aphid life-history traits (Ferrari *et al.* 2006; Via 1991).

The effect of endophytic fungi in *L. perenne* on *R. padi* shows a difference between laboratory experiments and field experiments. In field experiments we found no or little effects of *N. lolii* on *R. padi* (Krauss *et al.* 2007; Krauss *et al.* submitted). One reason for these results may have been the low levels of peramine measured in these field experiments. Another very speculative reason may be that the naturally colonising aphids in the field experiments are not endophyte-naïve and therefore, well adapted to the endophyte presence, whilst the aphids from laboratory cultures had adapted to a non-endophyte environment. In any case, more experiments and surveys of natural endophyte infections and associated herbivores occurring in the grasslands are needed.

We believe our study provides a first indication of the importance of studying effects of endophytes on herbivores over longer time scales to consider the possibility of evolutionary adaptation to the mycotoxins if we want to understand this interaction and its mechanisms in detail. This is especially important for the use of endophyte produced toxins as herbivore controls in applied agriculture as well as for basic ecological research on endophytes and their effects on insect food webs.

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