Induced niche shift as an anti-predator response for an endoparasitoid

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When two developmental stages do not share the same ecological niche, the control of the niche shift through a change in developmental timing, referred to as 'heterokairy', can provide an adaptive advantage for the individual (e.g. if mortality risk is higher in the first niche). For endoparasitic species that develop inside another (host) species, mortality of the host may directly induce mortality risk for the parasite. Thus, endoparasitoid larvae should be selected for response to host predation. In this study, aphids previously parasitized by the endoparasitoid *Endaphis fugitiva*, Gagné and Muratori (Diptera: Cecidomyiidae), were experimentally exposed to increased mortality risks. Both simulated attack and actual predator attacks against aphid hosts induced early emergence of the parasitoid larvae. Parasitoid emergence from the aphids occurred several minutes before the predator finished feeding on the aphid, allowing enough time for the parasitoid larvae to avoid direct predation. Predator-induced emergence produced significantly smaller parasitoid larvae than controls, but, interestingly, no effect on *Endaphis* adult size was found. To our knowledge, this is the first evidence of induced emergence in an insect parasitoid, but we suggest that this mechanism might be at work in many other species where plasticity in development time allows the individual to perform an adaptive niche shift.

Keywords: niche shift; anti-predator strategy; plasticity; aphid parasitoids; Cecidomyiidae; Aphididae

1. INTRODUCTION

Life histories of many animals are characterized by niche shifts (Warkentin 1995). When two developmental stages do not share the same ecological niche, referred to as 'ontogenetic niches' (Werner & Gilliam 1984), the control of the niche shift through a change in developmental timing can be advantageous for the individual (e.g. when mortality risk is different in the two niches). Recently, Spicer & Burggren (2003) proposed the term 'heterokairy' to refer to plasticity in the timing of onset of developmental events at the level of an individual. Heterokairy is the individual equivalent of evolutionary heterochrony (Warkentin 2007), and can arise either from plasticity in developmental rate (ontogenetic shift, Rowe & Ludwig 1991; Spicer & Burggren 2003) or by a purely behavioural decision. Selective pressure can occur when mortality risk varies between the niches of the different developmental stages; it thus becomes adaptive for the individual to modulate the timing of an ontogenetic trait depending upon external factors (Sih & Moore 1993).

Induced heterokairy in response to predation risk is especially well documented for amphibians that show plasticity in both timing of egg hatch and larval development (Warkentin 2007). Early hatching of eggs has been reported for tree frogs (*Hyla regilla*) and cascade frogs (*Rana cascadae*) exposed to predatory leeches (Chivers

et al. 2001); for wood frogs (*Rana sylvatica*) and American toads (*Bufo americanus*) when infected by water mold (Gomez-Mestre *et al.* 2006, 2008); and for the red-eyed tree frog (*Agalychnis callidryas*) in the presence of wasp or snake predation (Warkentin 1995, 2000). Water-borne cues emitted by *Pseudomonas*-infected eggs induced early hatching in the whitefish (*Coregonus* sp.) (Wedekind 2002). On the other hand, Ponton *et al.* (2009) reported a counter-intuitive case where juveniles of the crustacean gammarid *Gammarus insensibilis* exited the marsupial brood pouch of infected females significantly later than those from uninfected mothers, despite the fact that infected mothers are reportedly more susceptible to predation.

In invertebrates, the only known example of an adaptive shift in the timing of egg hatch occurs in *Scytodes pallida*, the egg-carrying spitting spider, in which eggs hatch sooner in the presence of the predaceous jumping spider, *Portia labiata* (Li 2002). Flexibility of anti-predator strategy may also be achieved by integrating gross morphological adaptations with behavioural decision-making processes (Lima & Dill 1990), as shown in larval newts of two species in the genus *Triturus* that respond with both behavioural and developmental niche shifts when predators are present (Van Buskirk & Schmidt 2000).

Very few endoparasite species have direct predators of the parasitic stage, but most are vulnerable to the predators of their hosts (Thomas *et al.* 2002). For parasites that develop inside another host species, the mortality risk to the host species is usually directly translated to mortality risk for the parasitic species. Insects that contain endoparasitoids are often as vulnerable to predation as

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unparasitized ones (Lawton & Hassell 1981). To minimize predation risk, a parasitic species should be selected either for host-manipulation strategies (Fritz 1982; Brodeur & McNeil 1989; Brodeur & Boivin 2004; Harvey *et al.* 2008) or host-leaving decision strategies (Ponton *et al.* 2006a).

Life-history theory predicts that developmental switch points should be adjusted to minimize the ratio of costs to benefits across stages (Werner & Gilliam 1984). In parasitoid species, leaving the host early generally decreases the final size of the imago, since it is generally not possible for the parasitoid to enter a second host (Godfray 1994).

In this study, we show that an endoparasitoid of aphids, *Endaphis fugitiva* Gagné and Muratori (Diptera: Cecidomyiidae), is able to modify the timing of emergence from its host, the banana aphid, *Pentalonia nigronervosa* Coquerel (Hemiptera: Aphididae), in response to predator attack on the aphid. Adult *E. fugitiva* females lay their eggs near aphid colonies. The newly hatched larvae actively search for hosts, climb upon the aphids' abdomen, and enter the aphids between the coxa and the thorax to develop as solitary endoparasitoids feeding on aphid body tissues. Once mature, the larvae emerge from the host through the anus to pupate in the soil (Muratori *et al.* 2009). During immature development, mortality risk for the aphid translates directly to mortality risk for *Endaphis* larvae. For this species, leaving the host has a fitness cost for an individual parasitoid, since the parasitoid larva will have less time to use the host resource and will be unable to enter a new host. In this case, it is assumed that the parasitoid larva will cease growth and enter metamorphosis with fewer resources than a fully grown larva.

We investigated plasticity in the timing of emergence of *Endaphis* to test the prediction that this life-history trait is modulated by the risk of mortality to the host. Under laboratory conditions, we studied the behavioural response of parasitoid larvae inside aphids that are exposed to increasing mortality risks.

2. MATERIAL AND METHODS

(a) Biological material

Eggs of the parasitic fly *E. fugitiva* were obtained by exposing red ginger plants (*Alpinia purpurata*) infested with cohorts of the banana aphid, *P. nigronervosa*, in a banana field at the Kauai Agricultural Research Centre (University of Hawaii at Manoa). The aphid colonies with parasitoid eggs were incubated in wooden-framed cages in a greenhouse (approx. 26°C; relative humidity (r.h.) 72%). Four days after field exposure, the parasitized aphids were easily recognizable by the appearance of dark spots on the dorsal abdomen. Eggs of the aphid predator *Micromus timidus*, the brown lacewing (Neuroptera: Hemerobiidae) were sampled on eggplant adjacent to the banana field at the Kauai Agricultural Research Center. Newly emerged predator larvae were maintained in the laboratory (approx. 24.1°C; r.h. 62%) and fed with mixed-age *P. nigronervosa* until the fifth instar. When the behavioural study was conducted, all aphids were 9 days old and had been parasitized 4 days earlier.

(b) Behavioural experiment 1: effect of artificial injuries

Between 5 and 6 days after field exposure, parasitized aphids were sorted into two groups according to the maturity of the

Endaphis larvae (which can be easily determined visually through the host cuticle): yellow immature larvae (which are as yet unable to pupate), and orange mature larvae (which can pupate successfully). Thirty aphids of each group were artificially injured in a standardized manner by removing the two hind legs and crushing both eyes. We manipulated the aphids as gently as possible for less than 1 min, taking care to avoid any physical contact between our instruments and the parasitoid larvae. The aphids were then placed in groups of 10 on a red ginger leaf in a Petri dish (diameter 5.5 cm). Thirty control aphids were given the same treatment except that they did not receive injuries. The number of emerged parasitoid larvae was recorded once per hour for 24 h. Treatment effect was statistically tested using survival analysis (log-rank test).

(c) Behavioural experiment 2: effect of predator attack

We isolated a single fourth instar parasitized aphid with one-fifth instar predator in a Petri dish (diameter 5.5 cm). Once the predator attacked the aphid, we observed the reaction of the parasitoid larva through the cuticle of the aphid using a stereomicroscope. For each attack, we recorded the time required for the parasitoid to emerge from its host (beginning at the instant of predator contact), and the total time the predator spent feeding on the aphid. A total of 74 parasitized aphids under attack were observed: 50 of these contained a mature larva, and 24 contained an immature larva. Only last instar predator larvae ($n = 12$) were used, each one tested against five to six individual aphids. Thirty parasitized aphids isolated in small vials without predators were used as controls. The time for emergence of the control larvae was calculated from the beginning of the first observation in the attack treatment.

(d) Cost of induced early emergence

In order to assess the cost of the induced emergence, we collected the mature parasitoid larvae that emerged during the previous experiment, both after escape from predator attack ($n = 41$), and from unattacked (control) aphids ($n = 30$). At the same time, we collected the aphid cadavers after parasitoid emergence from both predator-attacked and control treatments. All insects were maintained in ethyl alcohol (host and parasitoid associated in one vial) for further measurement. Subsequently, another batch of early emerged larvae were produced by applying artificial injuries to hosts, as explained for experiment 1. These larvae ($n = 40$ for each treatment) were individually reared in plastic cups (diameter 3 cm) filled with moist sand to allow pupation and emergence of the adults (Muratori *et al.* 2009). Adult emergence was checked daily in order to evaluate the pupation duration. All insects were fixed on microscopic slides using Canadian balsam. Images of the insects were captured using a Moticam 2300 (Motic, China) mounted on a Leica stereomicroscope. Aphid hind tibia, *Endaphis* hind tibia and *Endaphis* larval length were measured using IMAGEJ (Rasband 2004). In the two groups, aphid tibia lengths were not significantly different between the two treatments ($F = 1.19$, $p = 0.564$ and $F = 1.405$, $p = 0.317$).

Statistical analyses were performed using PRISM 5 for MacOSX (Graph Pad Inc.) and R 2.9.0.

3. RESULTS

Parasitoid larvae developing in aphids that were subject to injury emerged significantly earlier than those

Figure 1. Effect of host injury on the timing of emergence by endoparasitic *Endaphis fugitiva* larvae. Open circles denote injured hosts; closed circles denote controls (uninjured hosts). Both (a) immature and (b) mature larvae responded to injuries by emerging earlier than from uninjured hosts.

Figure 2. Emergence of the parasitoid *Endaphis fugitiva* from its aphid host: (a,b) without predator; and during host attack by a (c) brown lacewing or (d) by a syrphid larva.

developing in control aphids; this held true for both immature ($\chi^2 = 4.495$, $p = 0.034$; figure 1a) and mature larvae ($\chi^2 = 61.42$, $p < 0.001$; figure 1b).

When parasitized aphids were attacked by the brown lacewing, *M. timidus*, the parasitoid larvae emerged from the aphid and escaped from the predator successfully in most cases (83%, $n = 60$); (figure 2); (see video in the electronic supplementary material). The control parasitoid larvae in hosts unexposed to predation emerged several hours later ($m = 11.1 \pm 0.9$ h, $n = 30$); (figure 3). After initial predator contact, it took an average of 3.5 min (212 ± 18 s, $n = 50$) for *Endaphis* larvae to emerge, after which the predator continued feeding on the aphid remains for an additional 9.5 min (574 ± 25 s, $n = 50$); (figure 4); allowing the *Endaphis* larvae to perform jumps and escape from further predator attack.

We noted that there was a delay between the first reaction of the larva (obvious rapid movements observed through the cuticle of the aphid) and the actual beginning of larval emergence (mean delay time = 61.4 ± 7.5 s, $n = 50$). In a few rare cases direct predation by Hemerobiids on the parasitoid larvae were observed.

The mature parasitoid larvae that emerged from aphids exposed to predator attack were significantly smaller than the larvae that emerged from control aphids (1467 ± 16 μ m and 1503 ± 32 μ m, respectively; $F = 2.28$, $p = 0.0031$). When we control for aphid size, the relative size of the parasitoid larvae (larva total length/aphid tibia length) was significantly lower for attacked than for non-attacked larvae (figure 5a; log-transformed data, $F = 1.945$, $p = 0.019$). By contrast, *Endaphis* adult size was not significantly affected by early emergence

Figure 3. Effect of maturity on the escape ability of *Endaphis* larvae when the hosts are attacked by predators. 'In' (black bar) denotes proportion of larvae inside the host; 'out' (grey bar) denotes proportion of larvae outside the host. Unattacked hosts were used as a control.

Figure 4. Time needed for the parasitoid to escape from host (white bar) and aphid handling time (HT) by predator on a host from which the parasitoid escaped (grey bar) or on an aphid with *Endaphis* larva (black bar). Error bars are standard errors.

(absolute size: $F = 1.25$, $p = 0.537$), even when controlling for aphid size (figure 5b; relative size: $F = 1.41$, $p = 0.347$). Parasitoid pupation success (number of emerged adults/incubated larvae) was not significantly different between attacked (84.6%) and control aphids (95%) (Fisher's exact test: $p = 0.154$). Finally, the pupation duration was longer for attacked larvae than for control ones (13.63 ± 0.15 days and 13.36 ± 0.08 days, respectively; log-rank test: $\chi^2 = 3.93$, $p = 0.047$).

4. DISCUSSION

It has been estimated that 80 per cent of all animal species have life histories that include metamorphosis, and many of the remaining species undergo ontogenetic niche shifts without metamorphosis (Rowe & Ludwig 1991). For many systems, predation risks affect the ontogenetic timing (Gomez-Mestre *et al.* 2006). This study shows that the timing of emergence from a host by an endoparasitoid can be under behavioural control in response to predation of the host.

To be efficient, anti-predator adaptations should be sensitive to the current level of predation risk (Lima & Dill 1990). Generally, there is a large amount of behavioural evidence for the use of chemical cues by aquatic

Figure 5. Relative size distribution between emerged *Endaphis* from an attacked host and from non-attacked host (control). Data shown are the ratio between size trait of the parasitoid and size trait of the aphid. (a) Relative size of the parasitoid larva. (b) Relative size of the adult parasitoid. Error bars indicate standard errors.

animals for the assessment of predation risk (Sih & Moore 1993; Kiesecker *et al.* 1999; Wisenden 2000; Chivers *et al.* 2001; Wedekind 2002). Bullfrog tadpoles (*Rana catesbeiana*), for example, avoid contact with infected conspecifics using water-borne chemical cues (Kiesecker *et al.* 1999). In arthropods, it has been shown that kairomones released from the draglines of the spider-eating jumping spiders *Portia labiata* are sufficient to elicit changes in the egg-hatching traits of the egg-carrying spitting spider *Scytodes pallida* (Li 2002). In the endoparasitoid system presented here, it seems unlikely that the larvae could detect the presence of a predator from volatile chemical cues, since these would have to permeate through to the inside of the host body. Three alternative mechanisms can be speculated. First, predator detection can be mediated by cues in the host haemolymph, such as the release of a stress factor by the attacked host. For *E. fugitiva*, the head of the final instar larva possess two large antennae and has several sensory papillae (Muratori *et al.* 2009). Second, direct physical contact between the larval integument and the predator mouthparts might trigger the parasitoid response. Finally, since the predator feeds on the aphid by sucking the haemolymph, the larva might detect a change in the host internal body pressure. Which mechanism is actually at work remains to be investigated.

Our experiments showed that the parasitoid larva takes time to respond to the predator attack on its host. This is in line with the trade-off analysis between costs of remaining and costs of fleeing predation (Ydenberg & Dill 1986). The parasitoid will leave only when cost of mortality risk increases above the cost of leaving the host,

and therefore stops its development. The rapidity of response will depend on the efficacy of the larval escape behaviour and also on the aphid's ability to flee from predation. If a predator aborts its attack on the aphid, the larva will do better to go on feeding within the aphid. Moreover, it might depend on the mortality risk assessed by the larva. Relyea (2001) showed that the responses of different anuran prey were related to the level of predation risk posed by each of the predators. Here, we observed that artificial injuries induced early emergence of the larva within hours of the aphid manipulation, while real predator attack-induced emergence occurred within minutes.

Few failures are as unforgiving as failure to avoid a predator: being killed greatly decreases future fitness (Ydenberg & Dill 1986; Lima & Dill 1990). Nevertheless, the benefit of an efficient anti-predator behaviour may be balanced by reduced fitness costs and persistence of plasticity itself has costs and benefits (Relyea 2002). Here, we showed that a niche shift could be controlled by a behavioural trait of the larva. First, the maintenance of sensory and regulatory machinery needed for plasticity may require energy and material expenses to process signals coming from a predator attack (DeWitt *et al.* 1998). A less-responsive individual could save resources on sensory machinery for other functions that might have a positive effect on individual fitness. In the absence of predation risk, non-plastic emergence would then be favoured. Second, other costs of early ontogenetic shifts are incomplete development or reduced size of stressed individuals. Incomplete development of some morphological traits that persist after hatching might negatively affect the survival of prematurely hatched larvae (Warkentin 1995; Gomez-Mestre *et al.* 2006). The energy cost of escape in itself is likely to be negligible, as shown for predator-induced early emergence in hairworms (*Paragordius tricuspidatus*), which produce similar offspring as controls (Ponton *et al.* 2006b).

Depending upon the cues available from predators and other sources of mortality, selection against early hatching could limit the expression of the escape hatching response (Warkentin 2000). In the present study, even though body size of prematurely emerged larvae was smaller than in controls, adult size was not affected. To explain this trend, two resources re-allocation hypotheses can be put forward. First, there might be a trade-off between adult size and pupation duration (Gotthard *et al.* 1994; Ellers & Van Alphen 2002; Fisher *et al.* 2004; Yadav & Singh 2007). Control larvae that are larger than attacked ones develop into adults of the same size, but they do so at a faster rate than control individuals. The second hypothesis is that there might be a trade-off between adult size and other traits that have not been measured in this study (such as fat body, egg load, wing muscles, etc.) (Yadav & Singh 2007). Based on our data, we cannot state which hypothesis is the most probable.

None of the aphid species, nor their parasitoids, are native to the Hawaiian Islands. For *P. nigronervosa* and *E. fugitiva*, the areas of origin are unknown. Thus, we have little information on the selective forces that acted upon the behavioural responses of the parasitoid larvae to predation. As shown for hatching plasticity in amphibians, early parasitoid emergence from hosts clearly increases fitness in the context of host predation, but it

is not possible with these data to argue that host predation was the original selective force in the evolution of the observed emergence plasticity (Warkentin 1995). It is even possible that this early emergence of the larvae could have evolved from ancestral phytophagous Cecidomyiidae for which plastic larval emergence from gall on the host plant has been observed (Yukawa 2000).

In the present system, anti-predator behaviour was induced by attacks from several different predator species (figure 2; F. Muratori & S. Borlee 2007, unpublished data). As mentioned above, ontogenetic niche shifts in response to mortality risk have been shown in amphibians (Warkentin 1995; Chivers *et al.* 2001), fishes (Wedekind 2002), spiders (Li 2002) and hairworms (Sanchez *et al.* 2008). To our knowledge, this is the first evidence of an induced niche shift in a parasitoid, and we suggest that this mechanism might be at work in many other parasitoid species where plasticity in development time allows the individual to perform an adaptive niche shift.

Some endoparasitoid species (Hymenoptera: Braconidae: Microgastrinae) feed only on host haemolymph, and emerge from the still living host to pupate externally (Harvey *et al.* 2000; Harvey & Strand 2002). One might speculate that host predation can be a selective force that maintains host-leaving ability in the parasitoid larvae, since this frees the parasitoid from the constraint of consuming the entire host tissue to pupate. The plasticity in behavioural decisions that modulates life-cycle timing of the insect should be considered as one of the fitness-enhancing strategies of organisms under threat (Agrawal *et al.* 1999).

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