Pea aphid, *Acyrthosiphon pisum*, cornicle ontogeny as an adaptation to differential predation risk

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Abstract: Aphids possess unique anatomical structures called cornicles through which a defensive secretion containing alarm pheromone is often emitted when a predator attacks an aphid. The levels of alarm pheromone in cornicle droplets from the pea aphid, *Acyrthosiphon pisum* (Harris), vary considerably during development; however, it is not clear how the length of the cornicle changes during ontogeny. The length of the cornicle relative to the lengths of other body structures may have profound effects on aphid defense and alarm signal diffusion. Using previously published morphological measurements of pea aphids and observing interactions between pea aphids and multicolored Asian ladybird beetles, *Harmonia axyridis* Pallas, it was observed that pea aphid cornicles elongate proportionally more than other body parts during the first four instars, when alarm-pheromone levels have peaked, than during the fifth (adult) instar, when pheromone levels decline. pea aphids also are more likely to emit cornicle droplets and daub them onto a predator when the cornicles are undergoing such rapid growth. We suggest that because of a high risk of predation, rapid cornicle growth in juveniles has evolved both for individual defense and for the inclusive fitness benefits of alarm signaling.

Résumé : Les aphis possèdent des structures anatomiques particulières, les cornicules, par lesquels sont émises une sécrétion de défense et une phéromone d’alarme lors de l’attaque d’un prédateur. Les concentrations de phéromone d’alerte dans les gouttelettes issues des cornicules du puceron du pois, *Acyrthosiphon pisum* (Harris), varient considérablement au cours du développement; cependant, les changements dans la longueur des cornicules au cours de l’ontogenèse sont mal connus. La longueur des cornicules, par rapport à la longueur d’autres structures du corps, peut avoir des effets marqués sur la défense de l’aphide et la diffusion du signal d’alarme. À l’aide de mesures morphologiques déjà publiées du puceron du pois et de l’observation des interactions entre des pucerons du pois et des coccinelles asiatiques *Harmonia axyridis* Pallas, nous avons découvert que les cornicules du puceron s’allongent proportionnellement plus que les autres parties du corps pendant les quatre premiers stades, au moment où les concentrations de phéromone d’alarme atteignent leur maximum, par opposition au cinquième stade (adulte) où les concentrations déclinent. Les pucerons sont aussi plus susceptibles d’émettre des gouttelettes par les cornicules et d’en badigeonner un prédateur éventuel pendant cette période de croissance rapide. Nous croyons qu’à cause du fort risque de prédation, la croissance rapide des cornicules chez les jeunes a évolué de façon à assurer la défense individuelle aussi bien que le fitness de tout le groupe par la production d’un signal d’alarme.

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Introduction

To understand a structure or behaviour, the ontogeny and function of the trait should be studied (Tinbergen 1963). By examining ontogeny, it is possible to hypothesize which evolutionary pressures came to shape the development of body structures (Reiss 1989). For example, with respect to predation risk, if a certain age class of an organism is more susceptible to predation than others, there may be strong selection pressure to evolve defenses against the threat during that life stage, but not necessarily during other life stages (Lewis and Magnuson 1999; Perez and Telleria 2001), especially if there is a substantial cost to producing (Wisenden and Smith 1997) or retaining (Hamilton and Barclay 1998) such a defense. These ontogenetic changes may function for direct defense (individual benefits) (Stehouwer 1992; Brodie 1993) and (or) through protection of kin (inclusive fitness benefits), for example through alarm-signaling behaviour (Smith 1976; Miller and Hicinbothom 1991; Mondor et al. 2000).

Aphids have unique anatomical structures called cornicles (paired tubes arising from the 5th or 6th tergite) (Heie 1987) through which a defensive secretion containing alarm pheromone (E-β-farnesene) is often emitted when a predator attacks an aphid (Dixon 1958; Dahl 1971; Kislow and Edwards 1972; Campbell 1977). Not all aphids emit cornicle droplets with equal frequency, however. Juvenile pea aphids, *Acyrthosiphon pisum* (Harris), are much more likely to emit droplets than adults (Nault and Phelan 1984; Mondor et al. 2000), perhaps because they are more susceptible to predation (Frazer and Gilbert 1976). Also, it is known that while the size of the cornicle droplet increases with the size of the pea aphid, pro-
duction of alarm pheromone is initially low, reaches a peak in the second to fourth instars, and then declines significantly in the adult (Mondor et al. 2000). The ontogeny of cornicle growth over the life history of a pea aphid, however, is unknown. Cornicle length may have substantial implications for both protection of the individual, as the droplet may be daubed directly onto a predator (Dixon 1958; Strong 1967; Campbell 1977), and protection of parthenogenetic lineages through raising of the cornicle droplet for alarm-pheromone diffusion (Dahl 1971; Kislow and Edwards 1972; Nault and Phelan 1984).

In this study we hypothesized that the cornicles elongate more than other body parts when aphids are subject to the greatest predation risk, i.e., as juveniles. Cornicles must elongate during development to compensate for increasing body length, as the cornicles are raised and angled forward to reach across the body for defense from predators and (or) to raise the cornicle droplet for alarm signaling. Conversely, the cornicles should grow least in adulthood, when alarm-pheromone levels, as well as the risk of predation, are declining. We tested this hypothesis by comparing the sequential growth of pea aphid cornicles with that of the antennae, cauda, and overall body length. We also observed interactions between pea aphids and adult multicolored Asian ladybird beetles, Harmonia axyridis Pallas, to determine whether aphid instars with cornicles of a particular length were more likely to daub predators with cornicle droplets.

Materials and methods

Morphological measurements

The proportional increase in length of the cornicles, antennae, cauda, and whole body for all juvenile and apterous adult pea aphids were obtained from previously published measurements (Müller et al. 1974) and tested by $\chi^2$ analyses. Thus, firstly, increases in the length of each body structure over successive moults were examined independently to determine whether appendage or body lengths increased proportionally during development. Secondly, we compared the increases in length of all appendages and the body within instars to determine whether body structures grow at different rates. In addition, because aphids experience different temperature regimes during their lifetime, we also compared the proportional increases in cornicle length for aphids raised at different temperatures (10, 15, and 20°C) from published data (Hutchison and Hogg 1983). Development of cornicles was examined across instars for each temperature and within each instar transition at all three temperatures to determine the stability of cornicle development under varying environmental conditions.

These incremental analyses are valid, as body structures do not change appreciably in size during an instar because they are confined by the exoskeleton (Murdie 1969). Body structures increase in length during an instar, but the increase is exhibited only after molting. Use of previously collected measurements also has the advantage that these data were not obtained specifically for our study, thus there is no systematic bias in the measurements (i.e., our study was conducted blind). Furthermore, by conducting this analysis on pea aphids collected from different regions, we are confident that our results are not based on unique adaptations of aphid biotypes (Frazer 1972; Roitberg and Myers 1979), but rather are a general characteristic of pea aphid ontogeny.

Functional analysis of cornicle ontogeny

We observed interactions between pea aphids and adult H. axyridis to determine whether pea aphids emit cornicle droplets to different degrees or have differential success in daubing droplets onto predators during ontogeny. A synchronous colony of first-instar aphids was obtained by placing 100 mature, apterous individuals on a single broad bean (Vicia faba ‘Broad Windsor’) plant for 7 h. Neonates were transferred onto individual leaf pairs excised from 3-week-old bean plants and placed singly in a petri dish (8.5 cm i.d. × 2 cm high). Daily, approximately 1/5 of the adults were removed from the petri dishes, leaving behind a single offspring. After 7 days the petri dishes contained first-instar to adult stages. Aphids were reared at 19–24°C, 40–70% RH, and a 16 h light : 8 h dark photoperiod.

For testing, a single randomly selected aphid was introduced into a petri dish (8.5 cm i.d. × 2 cm high) with a single adult H. axyridis. Aphids were gently transferred with a fine paintbrush to ensure that they did not emit cornicle droplets prior to testing. When an aphid was attacked, emission of a cornicle droplet and contact of this droplet with the predator were recorded by video camera. Only interactions in which aphids were attacked from the anterior (n = 40) were analyzed with regression analyses. We believe this to be a conservative test of our hypothesis, for two reasons. To begin with, when attacked from the anterior, aphids must raise the cornicles and articulate them to a forward position, reaching across the entire length of the body, making it difficult to get a droplet onto a predator. Secondly, we used only adult H. axyridis in the experiments, as this age class consumes aphids faster than the larval instars do, allowing aphids the shortest time to emit droplets and daub them onto a predator. Experiments were conducted under fluorescent lighting at 21–24°C and 30–70% RH.

Results

Morphological measurements

Pea aphids’ body structures do not develop at the same rate: the cornicles ($\chi^2 = 71.46, P < 0.0001$), antennae ($\chi^2 = 19.50, P < 0.001$), and cauda ($\chi^2 = 35.43, P < 0.0001$) grow disproportionately over successive moults. Both the antennae and cauda increase maximally during the transition from first to second instar, and then grow at a constant rate. The cornicles, however, increase maximally during the transition from first to second instar, elongate an intermediate but equal amount during the period encompassing the second and third moults, and elongate the least during the transition from fourth instar to adult (Fig. 1). Overall body length, however, increased at roughly the same rate throughout development ($\chi^2 = 6.21, P = 0.10$) (Fig. 1).

A comparison of all structures after each moult showed a significant difference in the proportional increases in length of the different body structures: first moult, $\chi^2 = 54.70, P < 0.0001$; second moult, $\chi^2 = 21.55, P < 0.0001$; third moult, $\chi^2 = 25.31, P < 0.0001$; fourth moult, $\chi^2 = 9.61, P < 0.05$ (Fig. 1). It was clear that the cornicles increased in length
more than other body structures within all moults except the last one, when length increased an equal amount (but still more than that of the cauda) (Fig. 1).

Temperature does not influence cornicle development in pea aphids to a significant degree, as the cornicles increased in length proportionally more during the transition from first to second instar than during the periods between the second and third instars and between the third and fourth instars at 10°C (proportional increase in length was 118, 64, and 48% after moults 1, 2, and 3, respectively; \( \chi^2 = 35.09, P < 0.0001 \)), 15°C (proportional increase in length was 121, 59, and 48% after moults 1, 2, and 3, respectively; \( \chi^2 = 40.76, P < 0.0001 \)), and 20°C (proportional increase in length was 122, 60, and 51% after moults 1, 2, and 3, respectively; \( \chi^2 = 38.48, P < 0.0001 \)). Cornicle growth was consistent at all three temperatures, as the proportional increases in length did not differ between the three temperatures for the first (proportional increase in length was 118, 121, and 122% at 10, 15, and 20°C, respectively; \( \chi^2 = 0.072, P > 0.9 \)), second (proportional increase in length was 64, 59, and 60% at 10, 15, and 20°C, respectively; \( \chi^2 = 0.230, P > 0.8 \)), or third (proportional increase in length was 48, 48, and 51% at 10, 15, and 20°C, respectively; \( \chi^2 = 0.122, P > 0.9 \)) moulting periods.

Functional analysis of cornicle ontogeny
The number of aphids emitting cornicle droplets during development was best explained by a second-order polynomial regression (\( F_{(2,37)} = 8.74, P < 0.001 \)) (Fig. 2a). First-instar aphids emitted droplets approximately 30% of the time, peaked at 90–100% during the second, third, and fourth instars, and then declined to about 60% in adults. Thus, cornicles elongate preferentially during the transition between first and second instars, when the number of aphids emitting droplets increases dramatically. Cornicle growth only slows to match that of other body parts during the fourth moult (i.e., between fourth instar and adult), at the same time that droplet emission declines (Fig. 2a).

The number of aphids that daubed cornicle droplets onto adult coccinellids is also best explained by a second-order polynomial regression (\( F_{(2,37)} = 2.75, P < 0.10 \)) (Fig. 2b). The number of aphids daubing droplets onto predators increased dramatically between the first and second instars (i.e., after the first moult), stayed high during the second, third, and fourth instars, and then declined when the aphids reached adulthood, when, as mentioned above, droplet emission and cornicle growth also decline (Fig. 2b).

Discussion
Changes in body form during ontogeny are a result of changes in the relative growth rates of body structures (Nijhout and Emlen 1998). Selection on organisms of different ages, as a result of differential mortality, may result in such ontogenetic differences (Lewis and Magnuson 1999; Perez and Telleria 2001). In the present study, we hypothesized that pea aphid ontogeny is influenced by differential mortality during different life stages. As a result, cornicles used for defense and alarm signaling should elongate preferentially over other body parts during early instars, when aphids are subject to the greatest predation risk. Our data confirm this hypothesis, since pea aphid cornicles grew proportionally more than other body structures throughout all the juvenile stadia, corresponding to a peak in alarm pheromone levels (Mondor et al. 2000). Only in the fourth moult (i.e., the tran-
The possibility that the proportionally greater growth of the cornicles and other body parts during the transition from first to second instar reflects developmental constraints on growth inside the maternal aphid cannot be excluded (Dixon 1998). However, we do not believe that developmental constraints can fully explain cornicle-development patterns. While it was previously noted that the relative growth rates of pea aphid appendages show a general decline with age (Holman 1987), the cornicles have a higher relative growth rate than other appendages throughout development. Furthermore, the consistent growth of the cornicles under different rearing temperatures indicates that the development of this structure is not significantly influenced by environmental factors, unlike other aphid traits (e.g., body size (Blackman and Spence 1994; Kairo and Murphy 1999), life-history patterns (Alverson and Allen 1992; Morgan et al. 2001), wing development (Liu and Wu 1994)), which can be strongly affected by temperature.

Differential rates of cornicle growth and of cornicle droplet emission by juvenile aphids both greatly facilitated the transfer of cornicle droplets onto predators. For example, second- and third-instar aphids emitted droplets every time they were attacked, but droplets daubed onto predators increased from 38 to 67%. Only when alarm pheromone levels dropped (i.e., in adults) did droplet emission decline significantly. Cornicle elongation and high alarm pheromone concentrations may have been selected for in juvenile aphids, as they are subject to higher predation rates than adults (Frazer and Gilbert 1976). Immature aphids are also more likely to remain on the natal host plant, where clonemates are located, than are adult aphids (Hodgson 1991; Bailey et al. 1995). While we did not observe aphids escaping from predators, or indeed interrupting or delaying the predation sequence in any way by daubing them with cornicle droplets, it is believed that the droplets do aid in escape when there is less of a size difference between predator and prey (Dixon 1958). Along with any protective benefits, droplets daubed onto a predator can also provide information regarding an approaching predator, as the predator retains the alarm signal when searching for additional prey (Mondor 2001). Daubing droplets onto a predator would be adaptive for species like *A. pisum* that have clonemates dispersed over a host plant, as aphid species that live in denser colonies would be within range of simple molecular diffusion of alarm pheromone (Bossert and Wilson 1963; Bradbury and Vehrencamp 1998).

While we tested aphid responses to adult coccinellid predators only, it is possible that other coccinellid instars, predator species, and (or) parasitoids have selected for ontogenetic changes in aphids. Aphids are attacked by numerous predatory species, of various instars (Pimental and Wheeler 1973; Frazer et al. 1981), but adult coccinellids are thought to be the strongest selective agent on aphids, owing to their abundance and high prey consumption rates (Frazer and Gill 1981; Gutierrez et al. 1990). The extent to which parasitoids select for differential cornicle droplet emission, alarm pheromone levels, and (or) cornicle length is uncertain. Many parasitoids preferentially oviposit in juvenile aphids (Mackauer and Chow 1986; Gerling et al. 1990; Sequiera 1991), and if cornicle droplets do prevent oviposition (Edwards 1966) and (or) warn aphids within a colony of the presence of a parasitoid, then selection would rapidly favour droplet emission in response to oviposition. However, cornicle droplets are seldom emitted in response to parasitoid attack (Goff and Nault 1974). It is possible that some aphid parasitoids inject a substance which causes temporary paralysis at the time of oviposition, thereby preventing cornicle droplet emission (Lagace 1969; Boyle and Barrows 1978). Hence, determining the extent to which different aphid instars and species emit cornicle droplets in response to oviposition by different parasitoid species would clarify the extent to which parasitoids have selected for aphid defensive and alarm signaling traits.

Cornicles vary widely in size and shape among aphid species (Blackman and Eastop 1994, 2000). If, as suggested in this paper, the ontogeny of cornicle development has evolved...
for aphid defense and (or) alarm signaling, it is apparent that not all aphid species derive substantial benefits from having long cornicles. An understanding of how aphid species with negligible cornicles have been successful, and an examination of the factors contributing to differences in cornicle length from a phylogenetic perspective, are required.

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References


