Direct vs. inclusive fitness in the evolution of aphid cornicle length

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Abstract

By comparing the relative sizes of anatomical structures among phenotypes, selective pressures that shape species’ morphologies can be evaluated. Aphids emit droplets containing an alarm pheromone/defensive secretion from unique anatomical structures called cornicles, upon being attacked. As aphids live in colonies of high relatedness, it is uncertain whether direct or inclusive fitness benefits have chiefly promoted cornicle evolution. Morphological measurements for apterous parthenogen, alate parthenogen, female sexual and male sexual morphs of 43 species (21 genera, one subfamily) were assessed to distinguish between the hypotheses that: (1) cornicles evolved for mechanical defence against natural enemies (direct fitness); (2) cornicles evolved for alarm signalling (inclusive fitness); or (3) cornicle length has been largely constrained by flight aerodynamics. Our results generally support the inclusive fitness hypothesis; cornicle length decreases as the relative number and relatedness of offspring decreases. As cornicle length is greatest in apterous parthenogenetic morphs, inclusive fitness benefits of protecting highly related kin may have been a key factor selecting for cornicles, and increased cornicle length, in aphids.

Introduction

By examining the relative sizes of morphological structures among species, it is possible to contemplate the evolutionary pressures that have come to shape an organism’s structure and function (Reis, 1989; Price, 1996). As natural selection acts on phenotypes (Lewontin, 1970), a comparable analysis of trait development among distinct morphs, within a species, can also be performed (Schlichting & Pigliucci, 1998; Emlen & Allen, 2004). For example, if phenotypes experience differential predation risks, defensive traits should be more pronounced in high-risk phenotypes than in those with lower predation risk (Tollrian, 1993; Wiaczkowski & Staronska, 1999; Teplitsky et al., 2005). As traits evolve not just through direct defence (direct fitness benefits) (Dobzhansky, 1962; Endler, 1986) but also through protection of kin (inclusive fitness benefits) (Hamilton, 1964; Dawkins, 1989; Griffin & West, 2003), colony structure (i.e. number and relatedness) must also be correlated with trait development to determine the relative strengths of direct and indirect evolutionary forces on diverse polyphenisms.

Aphids have unique anatomical structures on the fifth or sixth tergite called cornicles (Hottes, 1928; Wynn & Boudreaux, 1972). When a predator or parasitoid attacks an aphid, a droplet of fluid is frequently emitted through these structures and daubed onto a natural enemy (Dixon, 1958; Dahl, 1971; Kislow & Edwards, 1972). Intraspecifically, longer cornicles enable aphids to daub cornicle droplets directly onto predators with greater efficacy (Mondor & Roitberg, 2004). The ultimate function of these cornicle droplets, however, has long been debated. It has been hypothesized that cornicles evolved for individual protection, as droplets daubed on a natural enemy sometimes result in prey escape (Dixon, 1958; Edwards, 1966; Butler & O’Neil, 2006). An equally plausible hypothesis is that this trait evolved by increasing the survivorship of clone-mates, through enhanced alarm signal transmission, as the droplets contain an alarm pheromone that promotes aphid dispersal from areas of high predation risk (Nault & Phelan, 1984; Mondor et al., 2002; Mondor & Roitberg, 2004). It is also conceivable, however, that cornicle length has simply been constrained by flight...
Apterous parthenogen. An unwinged, parthenogenetic phenotype that is prevalent during the growing season of the host plant (i.e. the summer months). This morph produces the largest number of live, genetically identical offspring of any morph. As the phenotype is incapable of long-distance dispersal, colony sizes increase rapidly.

2 Alate parthenogen. A winged, parthenogenetic phenotype that is also readily abundant during the growing season. This phenotype is commonly produced by apterous parthenogens in response to crowding or declining host plant quality. As wing production and use are believed to be metabolically costly, this morph produces fewer offspring than do aperous morphs. Unlike aperous parthenogens, this phenotype is capable of dispersing to new host plants. Thus, this morph is generally associated with smaller colony sizes than are aperous parthenogens.

3 Female sexual. An unwinged, sexual phenotype that is produced in response to declining light levels and/or temperature. This morph produces eggs, which are a robust life stage necessary for overwintering in harsh climates. As eggs are sexually produced, they are of lesser relatedness than are asexually produced offspring. Female sexuals can be very different in size, shape, and colour from parthenogenetic morphs; however, asexual and sexual morphs are the most alike in the Aphidinae (Blackman & Eastop, 2000).

4 Male sexual. Like the female sexual, this phenotype is only produced in regions where egg production for overwintering is required. Multiple matings are believed to occur, thus the paternity of eggs may be uncertain, resulting in a relatedness of less than 0.5. Males are similar in appearance to alate parthenogenetic females, although usually of smaller size (Blackman & Eastop, 2000).

We used these four morphs for our analysis as, indicated above, each phenotype differs qualitatively or quantitatively with respect to offspring number, offspring relatedness and wing condition (Table 1). To determine whether there are differences in cornicle length among phenotypes, relative cornicle lengths (cornicle length divided by body length) rather than absolute cornicle lengths were analysed, to eliminate any bias because of differences in overall body size among taxa (i.e. species may have longer cornicles because they are of larger overall size) (Mondor et al., 2002). This may also be particularly important when examining different life stages, as females tend to be much larger than males (Palmer, 1952; Blackman & Eastop, 2000).

Table 1 Relative offspring production, offspring relatedness and wing condition of the aphid phenotypes used in our comparative analysis.

<table>
<thead>
<tr>
<th>Aphid phenotype</th>
<th>Direct relative offspring production</th>
<th>Relatedness of offspring to parent</th>
<th>Wing condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apterous parthenogen</td>
<td>Many live young</td>
<td>1.0</td>
<td>Unwinged</td>
</tr>
<tr>
<td>Alate parthenogen</td>
<td>Fewer live young</td>
<td>1.0</td>
<td>Winged</td>
</tr>
<tr>
<td>Female sexual</td>
<td>Eggs</td>
<td>0.5</td>
<td>Unwinged</td>
</tr>
<tr>
<td>Male sexual</td>
<td>None</td>
<td>0.5*</td>
<td>Winged</td>
</tr>
</tbody>
</table>

*If paternity of offspring (i.e. sexually produced eggs) is certain.
As species are not truly independent, comparative analyses must be approached from a phylogenetic perspective. Unfortunately, an accurate phylogeny for Macrosiphini aphids is not currently available (von Dohlen et al., 2006). In the absence of an accurate phylogeny, we attempted to control for phylogenetic, and hence statistical, dependence between species using the phylogenetic subtraction method (Stearns, 1983). By utilizing the most accurate taxonomic information available (Remaudière & Remaudière, 1997), variation in trait values attributed to lower level taxonomic associations were removed, leaving residual values which are independent of taxonomic, and presumably phylogenetic, associations (Morrow & Gage, 2000). In our study, generic means for relative cornicle lengths were calculated and subsequently subtracted from the trait values for each morph of each species.

As phenotypes are also not independent, a comparative analysis of cornicle length between phenotypes was conducted, using the previously obtained residual values, by repeated measures ANOVA (Zar, 1984). Post hoc tests were conducted using Tukey’s honestly significant difference (HSD) test (Zar, 1984). All statistical analyses were conducted using JMP IN 5.1 (SAS Institute Inc., 2003).

**Results**

Cornicle lengths vary widely among aphid taxa, with nearly an eightfold difference in length among genera in the subfamily Macrosiphini (relative cornicle length range, 0.086–0.66) (Fig. 1). Although the majority of aphid taxa follow the same general pattern of cornicle length among phenotypes, species in some genera, such as Amphorophora, Sitobion and Wahlgreniella, deviate widely. In these taxa, male sexuals have cornicles as long as, and sometimes longer than, parthenogenetic morphs (Fig. 1).

When factoring out species relatedness, using phylogenetic subtraction methodology, relative cornicle length was indeed found to differ among aphid phenotypes ($F_{3,40} = 17.72, P < 0.0001$). Cornicles, longest in apterous parthenogens, steadily decrease in length as offspring number and relatedness decline (Fig. 2). Cornicles decrease in length in the transition from apterous parthenogen to alate parthenogen to female sexual to male sexual respectively.

**Discussion**

Because of the differential costs and benefits associated with the development of these appendages, aphids exhibit vast differences in cornicle length between species (Hottes, 1928; Blackman & Eastop, 2000). Intraspecifically, cost–benefit trade-offs of producing morphological structures also results in diverse anatomical growth trajectories and body forms among phenotypes (Lees, 1966; Holman, 1987; Moran, 1988; West-Eberhard, 2003). By analysing morphological measurements of four distinct aphid phenotypes with vastly different life history

![Fig. 1 Comparison of relative cornicle lengths (cornicle length divided by body length) for apterous parthenogen, alate parthenogen, female (apterous) sexual and male (alate) sexual morphs in different genera of the subfamily Macrosiphini.](image-url)
traits, we attempted to distinguish between selection on cornicle length because of direct and indirect effects. As cornicle lengths within the Macrosiphini are much longer in parthenogenetic as opposed to sexual morphs, the results most clearly match the inclusive fitness hypothesis (Nault & Phelan, 1984; Mondor et al., 2002; Mondor & Roitberg, 2004). This does not, however, completely preclude factors other than kin selection from having contributed to differences in cornicle length. For example, it is not currently possible to directly assess the importance of direct defence, by correlating cornicle length with the degree of predation risk encountered by different species. Nevertheless, our analysis indicates that indirect effects likely play a key role in cornicle evolution.

The ancestral condition for aphids is believed to be winged and without cornicles (Heie, 1987). Thus, the unwinged phenotype and cornicle presence/development are both derived traits. The key question then becomes: what conditions have lead to the evolution of these traits? It is easy to postulate the advantages of an apterous lifestyle; wings, wing muscles and metabolic energy for flight are all costly (Groeters & Dingle, 1989; Dixon & Kindlmann, 1999). As asexual morphs evolve, colony structure would immediately change. Colonies would be larger, more sedentary, and of high relatedness because of reduced dispersal. Consequently, conditions would promote strong selection for an alarm pheromone to protect offspring of high relatedness (Nault & Phelan, 1984; Bradbury & Vehrencamp, 1998). As alarm pheromones are emitted through the cornicles, it is also highly likely that cornicle evolution is related to alarm pheromone transmission (Mondor et al., 2002; Mondor & Roitberg, 2004).

Functionally, longer cornicles enable aphids to daub cornicle droplets directly onto predators with greater efficacy (Mondor & Roitberg, 2002). Cornicle droplets on predators provide directionality as to the threat of predation, so that aphids disperse only when absolutely necessary (i.e. it is costly to give up feeding sites) (Roitberg & Myers, 1979; Dill et al., 1990). If alarm pheromone transmission is the chief function of cornicles, then these structures should be longer in parthenogenetic morphs, as only these individuals have clonal offspring. Conversely, cornicles would be less adaptive in egg-laying sexual morphs because of a lesser degree of relatedness between, and the lack of any dispersal ability by, the clutch. The pattern of cornicle length among phenotypes supports this hypothesis.

Most enigmatic is that this overall pattern of cornicle development differs widely in some species, and even some genera. This may be true for at least two nonmutually exclusive reasons. First, it is possible that cornicles are not adaptive in these taxa (e.g. these species might not have an alarm pheromone). Although alarm pheromones have been identified in approximately 30 genera of aphids (El-Sayed, 2006), their presence in many taxa remains to be examined. This hypothesis would suggest that cornicles may be present in these species because of other factors, e.g. a direct defensive function. Secondly, cornicles may not be necessary for alarm signal diffusion in every taxon. A difference in colony structure has previously been proposed to explain interspecific difference in cornicle length (Mondor et al., 2002). Species that live in a compact colony structure, i.e. within range of simple diffusion of alarm pheromone, tend to have shorter cornicles than species that live in a more diffuse colony. When clone-mates are more dispersed over a host plant, the selective advantage of having longer cornicles to daub alarm pheromone droplets directly onto natural enemies, and therefore turn the pheromone droplet into a dynamic signal, is believed to outweigh the costs of producing these structures (Mondor et al., 2002).

It should not be overlooked that asexual and sexual aphids may be very different both ecologically and physiologically (Dixon, 1977; Dixon et al., 1993). As the majority of aphid research has been conducted on viviparous morphs, our knowledge of female and male aphid ecology is extremely limited (Dixon, 1977). It is possible that different species or genera are subjected to different selective forces, resulting in the deviating pattern of cornicle development in some taxa. Further analyses are required to determine which factors apart from colony structure, mutualistic interactions and other morphological adaptations (Mondor et al., 2002) have, or have not, selected for increasing cornicle length both within and among taxa.

As model organisms, aphids have enabled researchers to address a number of key ecological and evolutionary questions (e.g. the effects of climate change on gene frequencies, Mondor et al., 2005; the evolution of sex,
Simon et al., 2002; symbiont-mediated gene flow, Leonardo & Mondor, 2006). By examining trait differences among distinct phenotypes of a species, we can evaluate the selective pressures that have come to shape not only interspecific, but also intraspecific, traits (Schlichting & Pigliucci, 1998; Emlen & Allen, 2004). Factors leading to the evolution and maintenance of aphid cornicles have long remained untested, both empirically and theoretically (Hottes, 1928; Wynn & Boudreaux, 1972). As an initial step towards addressing this question, we have demonstrated that cornicles are of greater magnitude in parthenogenetic, as opposed to sexual, morphs irrespective of wing presence/absence. As parthenogens live in colonies of extremely high relatedness (i.e. r ≈ 1 in small colonies) (Hebert et al., 1991), we suggest that cornicle development may have been shaped not just by direct, but also through inclusive, fitness benefits.

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