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Duelling aphids: electrical penetration graphs reveal the value of fighting for a feeding site

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SUMMARY

Horned aphids (Cerataphidini) fight each other for access to feeding sites on leaves. An attacker attempts to force another aphid to abandon its feeding site; the victor then appears to insert its stylets into the site relinquished by the loser. This study used electrical penetration graph (EPG) recordings of *Astegopteryx pallida* (Van der Goot) individuals to pinpoint the benefits of fighting. We show that victors take significantly less time to commence feeding in the phloem, measured from the start of probing with their stylets, compared with aphids that initiate a new or discover an abandoned site: 9.0 *versus* 22.9 min, respectively. We also recorded the behaviour of aphids unencumbered with the wire necessary for EPG recordings. Those adult aphids that acquired a feeding site through fighting commenced feeding on average 20 min earlier than those that did not, taking into account the time spent searching and fighting as well as probing. This study clearly establishes that horned aphids use the exact feeding site vacated by another individual and that the benefit they gain is rapid access to the phloem – more rapid, indeed, than has previously been recorded in any aphid.

Key words: aphids, EPG, Cerataphidini, animal fighting, *Astegopteryx pallida*.

INTRODUCTION

Animals often fight members of their own species for access to food or mates. However, intraspecific fighting over food is relatively rare in insects, outside of the highly social species. An exception to this is provided by two aphid subfamilies, the Pemphiginae and the Hormaphidinae, where intraspecific fighting for food resources appears to be routine. There are two distinct contexts for fighting in these aphids. First, aphids fight over scarce gall-initiation sites on developing leaves on the primary tree host (Stern and Foster, 1996). These fighters are first instars and the fights can be lethal (Aoki and Makino, 1982) and may last several days (Whitham, 1979). Second, horned aphids (Hormaphidinae: Cerataphidini) of all instars use their frontal horns to fight conspecifics when feeding in open colonies on the secondary hosts (bamboos and other monocotyledons). In those species where natural colonies have been observed, the aphids use their horns to prize conspecifics (which may or may not be from the same clone) from feeding sites, which subsequently seem to be taken over by the successful attacker (Aoki and Kurosu, 1985; Aoki, 1987; Carlin et al., 1994; Foster, 1996). It is this highly unusual one-on-one combat that is the subject of this paper.

In previous studies, it has been assumed that the victorious horned aphids use the same feeding site as their defeated colony-mate, perhaps utilising the stylet sheath created by the first aphid to guide their stylets to the phloem (Aoki and Kurosu, 1985); this behaviour is described in detail in Foster (Foster, 1996). But gross observations, even down a microscope, cannot give us information about what is taking place within the tissue of the plant. Even if the victor accesses the same feeding site on the leaf's surface, actual penetration of the phloem and commencement of feeding may not take place for several hours after initial stylet insertion (Prado and Tjallingii, 1994; Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1997). Until we have some understanding of the true value of an established feeding site, we cannot understand the advantages of fighting.

Electrical penetration graphs (EPGs) are a proven method allowing objective visualisation of what is happening within plant tissue as a sap-sucking insect penetrates with its stylets (Prado and Tjallingii, 1994; Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1997). The whole animal is incorporated into an electrical circuit, with one electrode being attached to the aphid, while the other is inserted into the soil of its host plant's pot. When the aphid penetrates the leaf with its stylets, it completes the circuit (Tjallingii and Gabrys, 1999). Output EPG waveforms are recorded and vary with the aphid's behaviour and the position of the stylet tip (Lei et al., 1998). These patterns have been painstakingly correlated with aphid feeding behaviour through microscopy (for example, phloem *versus* xylem). By comparison with published material, we can confidently relate EPG patterns to feeding behaviour (Walker, 2000).

In this paper, we used EPG techniques to discover how long it takes individuals of the horned aphid *Astegopteryx pallida* to achieve a feeding site on its bamboo host. We used the time an aphid takes from the start of a successful probe to the commencement of feeding from the phloem as an indicator of the relative ease of access to, and therefore value of, feeding sites. This information was then combined with behavioural studies of unencumbered (i.e. not wired-up) aphids to gain an insight into the total time it takes to commence feeding, taking into account search and fight duration as well as probe times. Our aim was to establish conclusively whether the aphids are fighting over an identical feeding site and to quantify the time benefits of this behaviour.

MATERIALS AND METHODS

Animal maintenance

The experiments were conducted in the Zoology Department, Downing Street, Cambridge, United Kingdom, under DEFRA license [Plant Health Licence No. PHL 55C/3765(3/2001): Licence to Import, Move and Keep Prohibited Invertebrates]. *Astegopteryx pallida* colonies were maintained on two bamboo plants (*Bambusa* sp.) and experiments were carried out on four potted bamboo seedlings in aphid-proof cages and in an aphid-proof room, on a 12 h:12 h light:dark cycle and at 23°C. Owing to a limit on plant numbers, it was inevitable that colonies were repeatedly tested. However, colonies were never tested more often than once every 3 days.

EPG setup

The primary circuit consisted of: a DC voltage source (Tjallingii, 1985; Tjallingii, 2000), feeding substrate (potted bamboo), substrate electrode placed in soil, and the aphid attached to a thin silver wire (the aphid electrode). This preparation was placed in a Faraday cage to minimise electrical interference. Thin, flexible shielded cabling was used to connect the preparation to a ×10 amplifier with 10^9 Ω input resistance. The circuit was then connected to a second (×5) amplifier, then an analogue:digital interface (CED, Cambridge, UK), and finally to the recording computer.

Feeding adult aphids were selected at random. A 3–4 cm silver wire of diameter 25 μm (Johnson Matthey Metals Limited, London, UK) was dextrously attached to the feeding aphid's dorsum by a single drop of benign silver conductive paint (Electrolube Limited, Berkshire, UK; surface resistivity 0.03 Ω m). The aphid was then gently tickled with a paintbrush to induce it to withdraw its stylets, and transferred to a Petri dish. It remained there for 1 to 2 h, to become accustomed to walking with the wire on its back (Prado and Tjallingii, 1997; van Helden and Tjallingii, 2000).

The free end of the wire was then soldered to the aphid electrode. The animal was placed on the experimental leaf, which was secured with its ventral surface uppermost for the duration of the experiment to aid observation (van Helden and Tjallingii, 2000). The substrate electrode was run from the soil of the pot plant to the amplifier. Recordings were made direct to a computer using the program Spike2 (version 3.12, CED).

The characteristic waveforms produced were identified by comparison with published work (Kimmins and Tjallingii, 1985; Prado and Tjallingii, 1994; Reese et al., 2000). Potential drops indicate a living cell has been punctured; sustained drops indicate entry into the phloem (Tjallingii, 1985; Tjallingii and Hogen Esch, 1993; Prado and Tjallingii, 1994). Feeding does not start immediately: initially, aphids salivate into the sieve element, without ingesting. Commencement of feeding is indicated some minutes after the initial sustained potential drop by the appearance of the waveform E2 (Kimmins and Tjallingii, 1985; Prado and Tjallingii, 1994; Reese et al., 2000). This waveform is characterised by a frequency of 0.5 to 4 Hz, and occurs at the 'intracellular voltage level'. It shows rapid small amplitude fluctuations, upon which are superimposed larger dagger-shaped fluctuations. If an aphid sustained feeding (i.e. persisted in the E2 pattern) for at least 30 min, it was taken to have accepted the sieve element (Lei et al., 1998).

In the EPG experiments, the attached wire certainly limited aphid movement. The average 'time to settle' was about four times that for unencumbered aphids. Tether effects of aphids in EPG studies are common (Tjallingii, 1986; van Helden and Tjallingii, 2000). The wire attached to the aphid's back creates torsion that may limit the animal's manoeuvrability. Wired aphids have been shown to have reduced longevity and fecundity, probably due to reduced levels of feeding (Tjallingii, 1986). However, wiring does not affect probing and penetration behaviour, such as reaching the phloem (Tjallingii, 1986; Annan et al., 1997). The tether may make it more awkward for the aphid to search and fight, but once it starts to probe, the electrical patterns are consistent (Tjallingii, 1986).

EPG experiments

For all experiments, it was impossible to distinguish between an aphid taking over an old, abandoned feeding site and initiating a new site (except where the leaves were known to have never previously carried aphids). Aphids that did not achieve a feeding site by fighting are said to have 'found' it. All experimental aphids were adults.

Fighting aphids

Experimental adults (N=26) collected from the colony and wired were placed back in their colony and their behaviour was observed and EPG readings taken. The outcomes of any fights were noted. After the aphid started to feed from the phloem, EPG recordings continued for at least an hour. Aphid behaviour was classified as described in Fig. 1. All probes were recorded. The average 'time to phloem feeding' (Fig. 1) for aphids that 'fought' or found a feeding site was used as an estimate of the true value of the resource.

Virgin leaves

The aphid (N=10) was placed alone on a 'virgin' bamboo leaf, which had never had aphids on it, for at least 3 h. Any attempted probes were recorded.

Cleared leaves

Established colonies were carefully cleared of all aphids, leaving an empty leaf containing many abandoned feeding sites. Not more than 10 min later, the experimental aphid (N=20) was allowed to walk around and search for a feeding site, and any attempts at probing were recorded.

Unencumbered aphids

The 'fighting' aphids in the EPG experiments suffered from some tether effects as a result of the wire on their back, limiting their free range of motion and artificially lengthening the time it took them to settle. To gain an estimate of how long it typically takes an aphid to start feeding under natural circumstances, we observed the behaviour of 'free' individuals without undertaking EPG readings. Each randomly selected aphid (N=42) was marked with a benign dust [daylight fluorescent colours, Swada (London) Limited, UK]. The experimental animal was observed as it walked around the leaf, and whenever a fight ensued, the outcome recorded. Aphids investigated potential feeding sites by touching the surface of the leaf with their antennae, and backing up slowly to insert their stylets into the plant tissue (see Foster, 1996). Their antennae would then be slowly swept back to a horizontal position. Hardie and Powell (Hardie and Powell, 2000) correlated EPG readings with aphid behaviour using video footage, and found that stylet penetration of the leaf occurs at the same time as antennal movements cease. We also observed a clear correlation between the start of stylet penetration and the drawing back of the antennae. Therefore, for this group of aphids, we defined time to settle as being the time from the start of searching to when the antennae were swept back and horizontal after probing for a feeding site (Fig. 1).

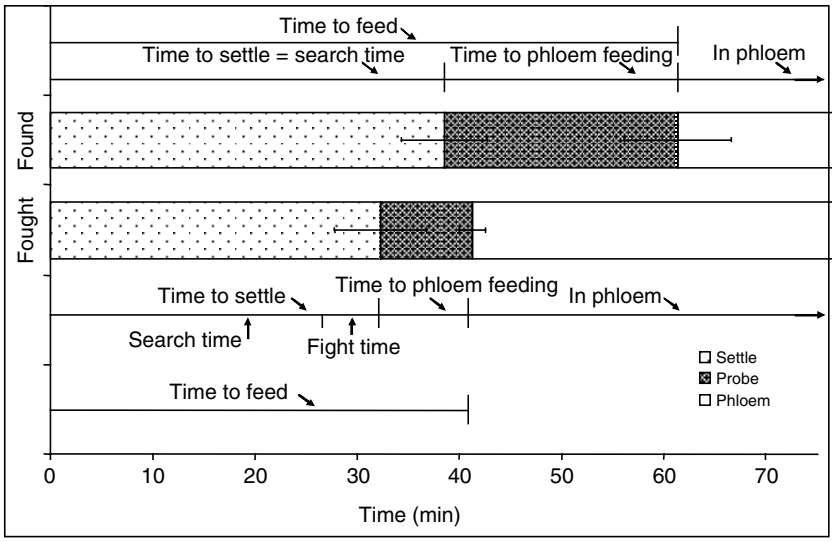

Fig. 1. Definitions of terms used to describe searching and probing periods, for aphids that fight and those that find a feeding site. 'Time to settle' for electrical penetration graph (EPG) aphids is the time taken from being placed on the leaf until the start of the successful probe; for unencumbered aphids, it is the time until the aphid was stationary and had apparently inserted its stylets, with antennae swept back in a horizontal position, as judged entirely from visual observations of behaviour. 'Search time' is the time spent by the aphid searching the surface of the leaf before settling, excluding any final successful fight. Note that for aphids that do not fight, the time to settle is the same as the search time, whereas for fighting aphids it will be search time + 'fight'. Search time also includes any unsuccessful fights. 'Time to feed' includes all searching, fighting and probing time. 'Time to phloem feeding' is the time taken from the start of a successful probe until the phloem is reached and ingestion begins (as indicated by the appearance of waveform E2). This graph also presents the mean (±s.e.m.) time to phloem of EPG aphids, together with the mean (±s.e.m.) time to settle of unencumbered aphids (for aphids that both found and fought for a feeding site).

RESULTS

EPG experiments

The waveforms produced corresponded closely to those previously published. The E2 waveform, indicating phloem ingestion, was well characterised (Figs 2 and 3) (Kimmins and Tjallingii, 1985; Prado and Tjallingii, 1994; Reese et al., 2000).

Fighting aphids

Of those aphids placed within an established colony, 10 achieved a feeding site through fighting, and 16 by finding an abandoned site or initiating a new one (i.e. found). There was no significant influence from either how the aphid probed (fought or found) or the bamboo seedling used (virgin, cleared, or with live colonies) on time to settle [mean ± s.e.m.: 135±23.5 and 117±23.3 min, for fought and found, respectively; generalised linear model (GLM): $F_{1,21}$=0.72, P=0.405].

The duration of time to phloem feeding was significantly influenced by whether the aphid fought for or found a feeding site (GLM: $F_{1,19}$=5.130, P=0.033), but not by the bamboo seedling used or the number of previous probing attempts the experimental aphid had made. In sites that the aphids fought for, the aphids took significantly less time to start phloem feeding than in sites where the aphids found the probing/feeding site (mean ± s.e.m.: 9.0±1.3 and 22.9±5.25 min, for fought and found, respectively; Fig. 1).

Virgin leaves

Ten adult aphids were left to feed individually on virgin leaves, for at least 3 h. None of the 10 started phloem feeding in this time, and indeed two of them had still failed to show any phloem feeding after a total of 10 h on the leaf. Four of the 10 made no attempt whatsoever to probe the leaf. The mean time to first probe was 5 h (range 3.8–5.8 h, N=6).

Cleared leaves

Fifteen of 20 aphids allowed solo access to an empty leaf that had recently (0 to 48 h) been cleared of feeding aphids were able to probe and successfully find the phloem.

Unencumbered aphids

There was no significant difference between the time to settle for aphids that fought (N=27) and those that found (N=15; mean ± s.e.m.: 32.3±4.5 and 38.5±4.1 min, for fought and found, respectively; Student's 2-tailed t-test P<0.37; Fig. 1). It follows, therefore, that the time to feed, made up of the time to settle and

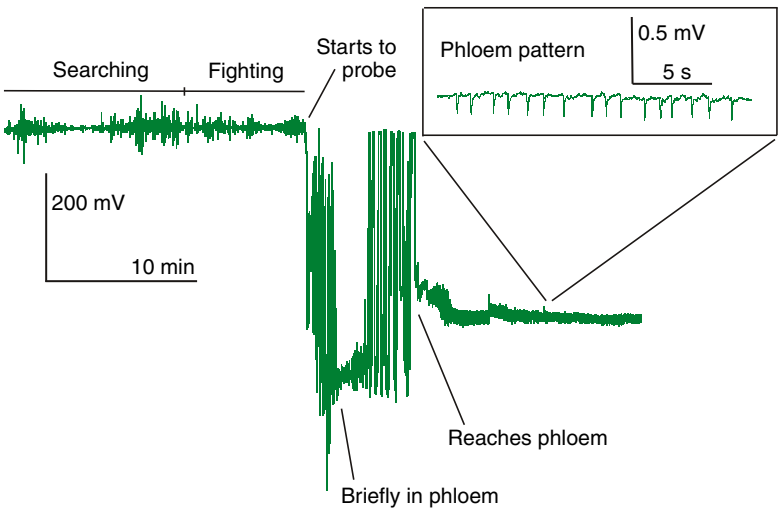

Fig. 2. Typical EPG trace where the aphid fought for a feeding site. Note that it reaches the phloem relatively quickly (less than 10 min after probing starts). Detail shows the waveform produced during sustained feeding from the phloem (i.e. E2 pattern).

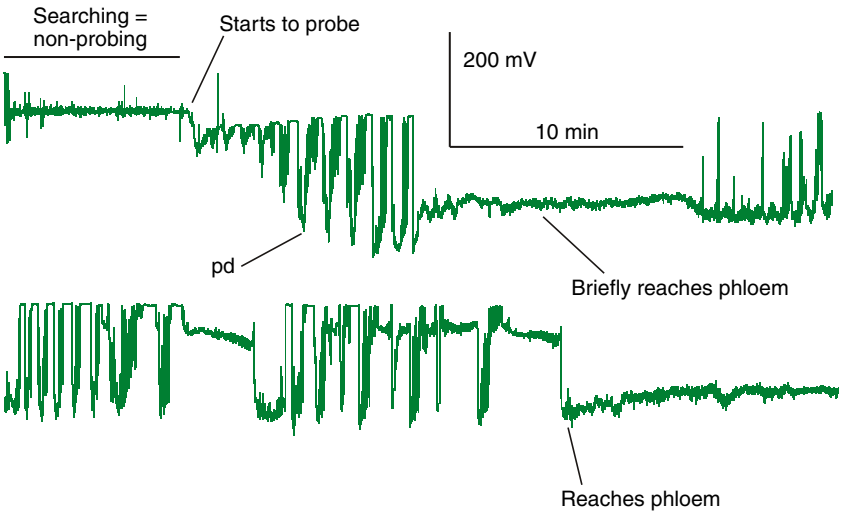

Fig. 3. Typical trace of an aphid that found a feeding site. The trace is continuous in time, but has been cut in two for ease of presentation. Note numerous potential drops (pd) before the phloem is reached; these represent brief punctures of a living cell by the stylets.

the time to phloem feeding, will be significantly longer for the aphids that do not fight, since these individuals take on average 14 min longer to start the phloem feeding (Fig. 1). To provide an estimate of the time taken to feed for unencumbered aphids, we combined the average time to settle with the individual time to phloem feeding data derived from the EPG experiments. This combination of data shows that aphids that fought for a feeding site started feeding from the phloem about 20 min sooner than those that found a feeding site (mean ± s.e.m.: 41.3±0.41 and 61.4±1.3 min, for fought and found aphids, respectively; Student's 2-tailed *t*-test $P<0.002$; Fig. 1).

DISCUSSION

The EPG experiments show us that what the victorious aphid gains from a fight is rapid access to the phloem. This is a prize well worth fighting for. Aphids and plants have been engaged for millions of years in an arms race over access to phloem, and as a result it is always very tricky and time consuming for aphids to find and penetrate the vascular bundle. By parasitising the efforts of another aphid, the victor can bypass this process and thus gain almost immediate access to a source of food. The very shortest times to phloem feeding in this study were less than 5 min; we could find no published times for other aphid species that approached this speed (Table 1). The saving in time will be much greater than our data here suggest (9 *versus* 22.9 min), which must be regarded as a minimal estimate, since unfortunately we cannot be sure whether any of the aphids that found rather than fought for a site had actually initiated their feeding sites *ab initio*. It is likely that many of them had inherited these feeding sites from aphids that abandoned them some time before, since this time (22.9 min) is still low when compared with published results from other homopteran/plant systems (Table 1), as well as with those that we observed on virgin leaves. The speed of access to the phloem establishes that the victor is using the same feeding route as the original aphid, since there is really no other plausible explanation for the short time to find the vascular bundle.

Although the victor gains a decisive time saving from gaining access to a high-quality feeding site, it is conceivable that this is nullified by the extra time taken in searching for and fighting with other aphids. This is unlikely, since Foster (Foster, 1996) showed that third instars of *Astegopteryx minuta* that fought did not take significantly longer to secure a feeding site than those that did not fight. We were able to estimate the total time to feed by combining the time to settle data from unencumbered aphids with the time to phloem data from the tethered EPG aphids. This is legitimate, since although tethering does limit aphid movement it does not affect probing and penetration behaviour (e.g. Tjallingii, 1986) (and see Materials and methods). Using these combined data, aphids that successfully fought had a significant time advantage, even taking into account fight duration: they started feeding on average 20 min sooner (Fig. 1).

Again, this is clearly a minimum estimate of the overall advantage of fighting, since it does not include the time taken to find a feeding site by aphids on virgin leaves. The time cost here is gigantic. In our observations, all of 10 experimental aphids left on virgin leaves from 3 to 12 h failed to find a feeding site, and four of these did not even attempt to probe. Foster (Foster, 1996) found that adult *A. minuta* took about an hour to settle to feed on virgin leaves. However, without the benefit of EPG, he assumed that an aphid was feeding if it had settled for 10 min. We observed several aphids attempting probes that lasted 15–20 min, without successful phloem feeding, after which time they would abandon the site and walk on.

The best tactic to undertake may vary with the aphid's developmental stage. Adults are more aggressive, attacking more frequently and tending to win more fights (Foster, 1996) (W.A.F., personal observations). Fighting is the most time-efficient approach for them. Smaller instars are relatively less successful against defending aphids. Even though they might start feeding more quickly from a fought feeding site, this may not compensate for the extra time

Table 1. 'Time to phloem feeding' and 'time to feed' (Fig. 1) for various homopteran–host plant systems

Homopteran	Host plant	Average time to phloem feeding (min)	Average total time to feed (min)	Reference
Horned aphid, *A. pallida*	Bamboo	9.0 (fought)	–	Present study
Horned aphid, *A. pallida*	Bamboo	22.8 (found)	–	Present study
Cowpea aphid, *Aphis craccivora*	Lupin	23	71.7	(Zehnder et al., 2001)
Rhopalosiphum padi	Barley	73	136	(Ponder et al., 2000)
Nasonovia ribisnigi	Lettuce	24.8	333.3	(van Helden and Tjallingii, 1993)
Grain aphid, *Sitobin avenae*	Wheat	37.3	108.6	(Leszczynski et al., 1995)
Grain aphid, *Sitobian avenae*	Wheat	49	83	(Caillaud et al., 1995)
Sweet-potato whitefly, *Bemisia tabaci*	Tomato	57	188.6	(Jiang et al., 2001)
Greenhouse whitefly, *Trialeurodes vaporariorum*	Cucumber	39	–	(Janssen et al., 1989)

spent trying to defeat a resource holder, or undertaking several initially unsuccessful fights.

It is unlikely that the adult aphids refused to feed on virgin leaves because of a reluctance to feed alone, as they readily fed on leaves that had been cleared of an established colony. This is probably due to the presence of vacated feeding sites on the leaf surface. Exactly how the aphids find abandoned feeding sites would be well worth further detailed study. Foster (Foster, 1996) found that aphids were much faster at finding a feeding site when searching a cleared leaf or one with a crowded colony, compared with starting a new probe on a virgin leaf. Prado and Tjallingii (Prado and Tjallingii, 1997) investigated EPG patterns of aphids allowed to settle on leaves that had just been brushed clean of feeding aphids. They found that the period to first sustained phloem ingestion was shortened. While these results may conceivably be due to searching aphids utilising the stylet sheath of an abandoned site to guide them to the phloem, there are also physiological effects of previous aphid feeding. Telang et al. (Telang et al., 1999) found that *Diuraphis noxia* feeding on susceptible wheat induced a change in phloem content, so that sap contained enhanced levels of essential amino acids. This led to earlier acceptance of the sieve element by the probing aphid. In this case, the effects were systemic through the leaf, rather than highly localised, and not limited to a single feeding site.

It seems unlikely that adults are physically incapable of creating new feeding sites, especially as they have been observed to probe virgin leaves. It may be something they usually do not need to do, and so they have limited motivation in this respect. Under typical circumstances, adult *A. pallida* would not find themselves alone on a virgin leaf. It is the much smaller early instars that tend to migrate from the colony [W.A.F., personal observations, see also observations by Stern et al. (Stern et al., 1997) on a *Pseudoregma* species]. Perhaps adults rely on smaller animals (from either the same or a different clone) to initiate feeding sites, which they then either fight for or take over if they are vacated for some reason (e.g. moulting). Three of five adults placed on virgin leaves with first and second instars were able to establish a feeding site within 10 h; those with other adults and fourth instars together on the leaf did attempt to probe, but could not establish a feeding site (data not presented).

A. pallida individuals have three means of feeding: initiate a new site, find an abandoned site, or fight and take over a site from a feeding aphid. There are obvious time advantages to fighting for a site, at least for larger individuals who are likely to fight successfully. In addition, sites achieved by fighting were of more consistent quality. There was a much wider range for uncontested sites, whether or not these represented abandoned sites or new initiations. The best tactic will vary with the relative size of the individual (relating to its fighting success), and perhaps with colony factors such as size (larger, older colonies may have a higher site turnover, and more sites that were misplaced during moulting). Whether or not individual aphids (both nymphs and adults) use the same feeding sites before and after moulting should be studied further.

Until these aphids have been induced to feed from virgin leaves, it will not be possible to gain an unequivocal measure of how long it takes to initiate a feeding site. However, we now confidently know that the intraspecific fights of the horned aphids are over feeding sites, and that gaining a feeding site by fighting reliably ensures rapid access to the phloem and significantly less time to commence feeding.

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