

Seasonal extension of the soldier instar as a route to increased defence investment in the social aphid *Pemphigus spyrothecae*

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Abstract. 1. The possibility that the duration of the soldier instar could be extended over the course of the galling season was examined in *Pemphigus spyrothecae* Passerini (Hemiptera: Aphididae). In this species, the individuals of one morph of the dimorphic first instar are non-sterile specialised defenders known as soldiers.

2. In the face of a declining birth rate, the proportion of first-instar soldiers to first-instar non-soldiers was found to increase. This provides strong evidence of extension of the soldier instar over time.

3. Instar extension provides a mechanism for post-natal investment in soldiers and allows individuals with insufficient time to mature to remain in the defensive stage so that they may continue to make an indirect contribution to their clone's reproductive success.

Key words. Altruism, aphid soldiers, caste ratio, clone.

Introduction

The defensive *soldier* instars of social aphids tend to be the earliest and it is not unusual for these instars to be considerably longer than later non-soldier instars (Aoki & Kurosu, 1986; Akimoto, 1992; Rhoden, 1997; Withgott *et al.*, 1997). Indeed, this dichotomy is epitomised in those dimorphic species in which the soldiers are sterile (e.g. Aoki, 1977; Aoki & Miyazaki, 1978; Aoki, 1980; Aoki & Kurosu, 1992): the soldier instar has an indefinite duration that continues until death. Some secondary-host soldiers of *Pseudoregma bambucicola* (Takahashi) have been known to live for up to 116 days (Sakata & Itô, 1991). Within a species, variability among non-soldier instars may also exist. The first instars of the non-soldier morph of secondary-host *P. bambucicola* have a development time of approximately double that of other non-soldier instars (Sakata & Itô, 1991). This may be because, in *Pseudoregma*, aphids of this instar and caste are specialised for the potentially time-consuming task of dispersal (including wind dispersal: Aoki *et al.*, 1981; Akimoto, 1992).

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The duration of an instar can change if required. Death of the fundatrix in *Pemphigus obesinymphae* Aoki results in acceleration of the developmental rate of the monomorphic first-instar soldiers within her gall (Withgott *et al.*, 1997). This, of course, means that the duration of the instar is shortened. Similarly, those soldiers of *P. obesinymphae* which successfully migrate to the gall of another clone tend to exhibit an increased developmental rate (as well as a reduction in defensive behaviour) (Abbot *et al.*, 2001). First-instar soldiers of the monomorphic galling species *Hemipodaphis persimilis* Akimoto are subject to a temporal change in instar duration. The duration of their instar increases gradually over the galling phase (Akimoto, 1992). It is thus clear that, in some cases, the developmental rate of the first instar is proximately influenced.

Instar extension is an increased investment in defence. Because the soldiers of many aphid species are behaviourally or morphologically specialised yet can nevertheless moult to become non-soldiers, a delay in the moulting of soldiers translates to an increase in defence investment for the clone. Instar extension is significant to the evolution of defence because it provides a post-production method by which the number of soldiers in a colony can be modulated.

The proportion of soldiers in an aphid clone before predation is a reliable indicator of the instantaneous defence investment of the clone. By optimising the reduction in

predation or predation risk, this investment should maximise the number of successful reproductives produced.

The proportion of soldiers in secondary-host colonies of *P. bambucicola* may be influenced by the immediate environment. Larger colonies tend to have higher proportions of soldiers (Schütze & Maschwitz, 1991; Shibao, 1999), perhaps because larger colonies are more attractive to predators (Shibao, 1998). A higher proportion of soldiers is also found in colonies which contain alate adults, in colonies on thicker bamboo shoots, and in colonies living on shoots branching from older stems (Sakata *et al.*, 1991). The proportion of soldiers is also related to the season and drops to almost zero in summer (Sunose *et al.*, 1982). Ant-tending has also been found to have a definite effect on colonies of *Pseudoregma sundanica* (Van der Goot), with tended colonies having a lower proportion of soldiers (Shingleton & Foster, 2000). This effect may be due to the effect that ants have in reducing predation.

In an aphid species with obligately sterile soldiers (and hence dimorphic first instars), modifying the number of soldiers produced at birth is the only way for a clone to adjust its defence investment. In a species with facultatively sterile soldiers, however, the trade-off between investing in defence and investing in reproduction can be adjusted in two ways: (1) a change can be made in the proportion of soldiers produced at birth and/or (2) the duration of the soldier instar can be modified. In a species in which the facultatively sterile soldiers occur in a monomorphic first instar, a reduction in birth of soldiers is equivalent to a reduction in the overall birth rate of the colony; however, the soldier proportion of a colony will decrease only if a compensating decrease in soldiers' moulting rate does not occur concomitantly. Such a compensating mechanism is known to occur in monomorphic *H. persimilis* (Akimoto, 1992). In a species in which facultatively sterile soldiers are part of a dimorphic first instar, any resource surplus resulting from a reduction in soldiers at birth can be reallocated to direct production of reproductives. Even in such cases where flexibility in defence investment is enhanced by instar dimorphism, reduction in birth of soldiers will cause a corresponding reduction in the proportion of soldiers only if the duration of the soldier instar does not increase. The possibility of temporal instar extension in an aphid species with instar dimorphism has not been examined.

Soldiers of the current study species, *P. spyrothecae*, occur in a dimorphic first instar and the duration of their instar is certainly greater than that of first-instar non-soldiers as well as those of all other instars. Aoki and Kurosu (1986) reported that only two (0.7%) of the 304 soldiers that they examined were pharate, whereas 18 (19%) of the 94 first-instar non-soldiers were in this pre-moult phase. By rearing a small number of aphids in the laboratory, Rhoden (1997) estimated that, in the third galling generation, soldiers have an instar duration of approximately 20 days, a period that is 2.3 times as long as that of second-instar virginoparae. In contrast, the instar durations of the first and second sexuparous instars were approximately equal.

There is currently no evidence that aspects of the immediate environment such as colony size or gall size affect the proportion of soldiers found in *P. spyrothecae* clones, but it is apparent that a large proportion of soldiers persists throughout the galling phase (Foster, 1990). The proportion of the population represented by *P. spyrothecae* soldiers at the galling phase's population peak and beyond is stable between 0.4 and 0.6 (Foster, 1990; Rhoden, 1997; Braendle, 1999). This is not true for the mature galls of those other species of *Pemphigus* which lack nymphal dimorphism.

Given the potential challenges to detecting soldier instar extension in aphid species with facultatively sterile soldiers and instar dimorphism (namely, low moulting rate and resource reallocation to non-soldier production), its successful detection by purely demographic methods is likely to indicate that its role as a defence investment strategy is more crucial than may be readily apparent. By examining the relationship between temporal changes in ratios of soldiers to other instars and temporal changes in birth rate, the current study aimed to establish if instar extension is a significant component of the defence investment strategy of *P. spyrothecae*.

Methods

Study organism

Pemphigus spyrothecae is a species with a dimorphic first instar which is comprised of facultatively sterile soldiers and quick-moulting non-soldiers. Soldiers are always and exclusively confined to the first-instar virginoparous morph. Two to three generations occur in the galling phase of *P. spyrothecae* (which is described schematically in Fig. 1). The first generation (which is made up entirely by the offspring of the foundress) is monomorphic and virginoparous. The first-instar soldiers pass through a further three non-soldier instars to become adult virginoparae. These first adult virginoparae give birth to sexuparae as well as virginoparae and are thus responsible for the dimorphic second gall generation. Mature sexuparae represent the reproductive success of the gall as it is these which emigrate to give rise to the sexuals that produce the overwintering eggs (Lampel, 1960). Some proportion of second-generation virginoparae may manage to avoid predation and pass through the first (soldier) instar and later (non-soldier) instars to successfully reach adulthood (Rhoden, 1997). These second-generation adult virginoparae also produce both sexuparae and virginoparae. The opportunity for development of these third-generation individuals, although dependent on predation, is largely determined by the amount of time remaining before the host plant sheds the leaves on which the aphids make their galls.

Analysis of soldier proportion

The ratio of first-instar soldiers to all other aphid individuals was calculated for *P. spyrothecae* and, to facilitate

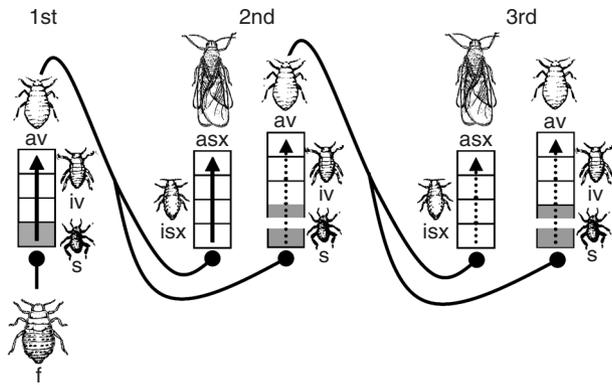


Fig. 1. The three gall generations of *Pemphigus spyrothecae*. The first generation is produced by the foundress (f) and consists of a single virginoparous morph that matures from first-generation soldiers (s) to immature virginoparae (iv), and finally to adult virginoparae (av). These adult virginoparae produce the second gall generation and may give birth to both virginoparous and sexuparous morphs. The immature sexuparae (isx) have no defensive function and mature to adults (asx) which leave the gall to continue the life cycle. The immature virginoparae soldiers (s) in the first instar and non-soldiers (iv) in the three later instars) have the potential to mature to adult virginoparae. These second-generation adult virginoparae produce the third galling generation, which is identical to the second generation, but is likely to be cut short by leaf fall of the host. Grey shading indicates the soldier instar. Round arrows indicate birth, triangular arrows indicate development. Dashed lines indicate development that is facultative or circumstance-dependent. The broken box in the soldier instars of the second and third gall generations denotes the possibility of instar extension, which is the subject of the current research.

comparison, for *Pemphigus bursarius* Linnaeus. (Unlike the morphologically distinct soldiers of *P. spyrothecae*, the soldiers of *P. bursarius* belong to the monomorphic first instar and are discernible only because of their defensive behaviour. Because colony fitness in *P. bursarius* is consequent upon the maturation of the single morph present, marked instar extension is unlikely.) In addition, for *P. spyrothecae*, the ratio of first-instar soldiers to first-instar non-soldiers was calculated as a demographic indicator of the relative difference in the early developmental rate of the two morphs. Ratios were calculated over the entire 1999 galling phase (11 May to 22 October), for destructive sampling events that were approximately evenly interspersed and carried out across four sites in Cambridge, U.K.: (a) *Cement Works* (52°11'49"N, 0°9'52"E), (b) *Coton Footpath* (52°12'31"N, 0°4'8"E), (c) *Leys Playing Field* (52°11'22"N, 0°6'58"E), and (d) *Pembroke Sports-ground* (52°11'25"N, 0°6'5"E). Both *P. spyrothecae* and *P. bursarius* form galls on the petioles of black poplar, *Populus nigra* Linnaeus. A destructive sampling event involved the collection of 25 unpredated galls from mature Lombardy poplar trees (*P. nigra* var. *italica* Du Roi) at each of the four field sites.

Pilot study of moulting rate

Akimoto (1992) successfully demonstrated instar extension in *Hemipodaphis persimilis*, a species with monomorphic first instars, by slide mounting nymphs to identify pharate individuals. A pilot study of slide-mounted soldiers of *P. spyrothecae* demonstrated that estimation of moulting rate by this method was impractical because of the low numbers of first-instar soldiers in the pharate phase. Whereas the moulting rates (instantaneous proportion of individuals ready to moult) for *H. persimilis* were approximately 0.5 for non-soldier instars and between 0.54 and 0.18 for soldiers (Akimoto, 1992), the moulting rates of *P. spyrothecae* were considerably lower. None of the 200 July 1999 soldiers examined in a pilot study was found to be pharate. Estimates from the study of Aoki and Kurosu (1986) indicate that the September moulting rate of *P. spyrothecae* is approximately 0.2 for first-instar sexuparae, but as low as 0.007 for soldiers.

Birth rate

The birth rate in colonies of *P. spyrothecae* was calculated from the time of maturation of the first galling generation to the end of the galling phase. From destructive samples of each of the dates 23 July, 31 July, 15 August, 30 August, and 30 September 1999, 20 adult virginoparae were selected for dissection. (Four virginoparae were selected randomly from each of five galls.) Each virginopara was dissected and the number of mature embryos (>0.2 mm in length) contained in her ovarioles was counted. The change in number of adult virginoparae per gall was then examined in the context of the number of embryos within each virginopara. In this manner, it was possible to ascertain whether the birth rate of the colony as a whole was increasing or decreasing.

Results

'Soldier' ratio in *Pemphigus bursarius*

The ratio of first instars (behavioural soldiers) to all other instars decreased consistently over the galling phase in *P. bursarius* (linear regression, $F_{1,97} = 104.9$, $P < 0.001$, $r^2 = 0.614$). This gradual decrease in the proportion of first instars is plotted in Fig. 2.

Soldier ratios in *Pemphigus spyrothecae*

The percentage of the colony made up by soldiers was extremely high at the beginning of the galling phase, reflecting the fact that only the foundress is present at this early period and all her first-instar offspring are soldiers. By the time that the first generation of adult virginoparae had matured (day 60 onwards), the proportion of soldiers had fallen to approximately 40%. It remained at this relatively high level for the remainder of the galling phase.

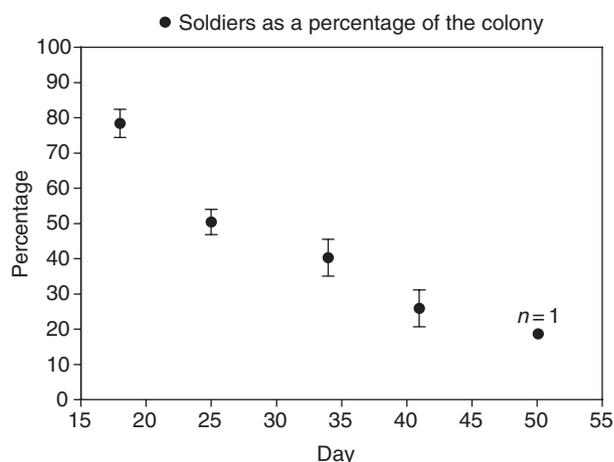


Fig. 2. *Pemphigus bursarius*: the change over the galling season in the number of first instars (behavioural soldiers) as a percentage of the total colony population. $n=25$ for each sampling date, except for days 41 and 50 when only six and one of the 25 galls collected remained inhabited. Error bars indicate ± 1 SE.

The ratio of soldiers to first-instar sexuparae did not exhibit the same constancy. Mid-phase (day 80), there were one to three times more soldiers. As the season progressed, the ratio increased markedly as the number of first-instar sexuparae decreased. For example at *Coton Footpath*, the mean number of first-instar sexuparae decreased from 14 ± 2 on day 100 to 4 ± 1 on day 120 whereas the corresponding decrease for soldiers was comparatively slight, from 54 ± 8 to 40 ± 9 . This divergence represents the fact that there were 10 times as many soldiers as first-instar sexuparae by the end of the season. At all sites, linear regression analysis for the period of day 80 to season's end highlighted a significant difference between the slopes calculated for soldiers as a percentage of the first instar and the slopes calculated for soldiers as a proportion of the entire colony. For example, at *Cement Works*, the regression coefficient of 1.02 for soldiers as a percentage of first instars was significantly different from the regression coefficient of 0.36 for soldiers as a percentage of the entire colony ($F_{1,536} = 51.67$, $P < 0.001$). Quantitatively similar results were found for all other sites. These changes in soldier proportion, including the increase of soldiers relative to first-instar non-soldiers, are plotted in Fig. 3.

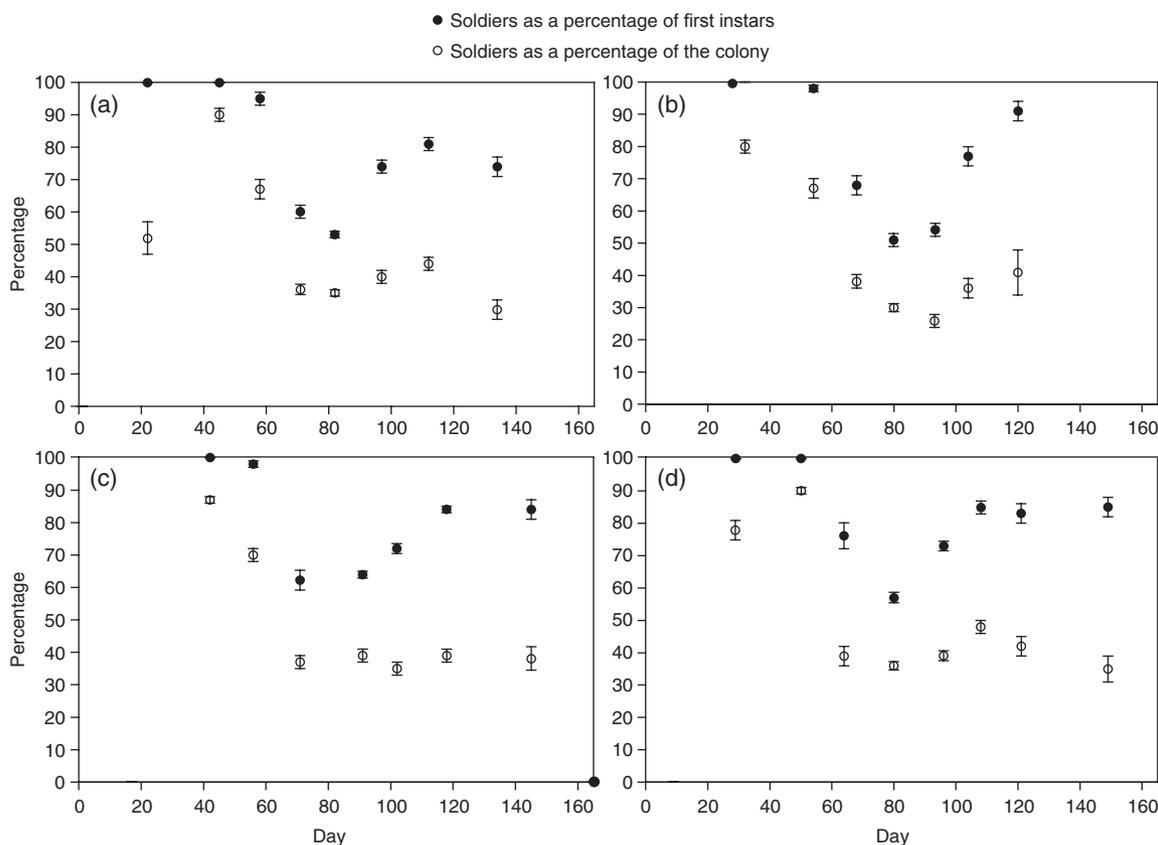


Fig. 3. *Pemphigus spyrothecae*: the changes over the 1999 galling season in the percentage of soldiers in the colony as a whole and in the percentage of soldiers in the first instar only (first-instar virginoparae and sexuparae). Results are plotted for each field site: (a) Cement Works, (b) Coton Footpath, (c) Leys Playing Field, and (d) Pembroke Sportsground. $n=25$ for each sampling date. Error bars indicate ± 1 SE.

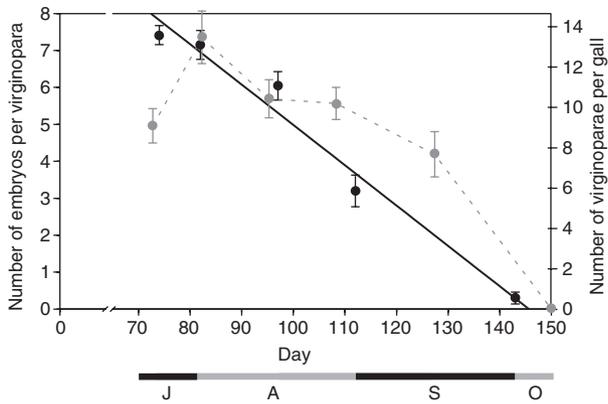


Fig. 4. *Pemphigus spyrothecae*: the average number of embryos (\pm SE, $n = 20$) contained in adult virginoparae indicates the change in birth rate during the galling phase of 1999. A line of regression is fitted ($r^2 = 0.98$, $P = 0.002$). The dashed grey indicates the average number of virginoparae per gall (\pm SE, $n = 50$).

Birth rate in *Pemphigus spyrothecae*

At the time when the first virginoparae reached maturity (23 July 1999), the average number of embryos contained in the ovarioles of a virginopara was 7.4 ± 0.25 . A steady decrease in this average occurred thenceforth, reaching a value of 0.3 ± 0.15 embryos per virginopara in the 30 September sample, the last to contain live virginoparae. This decrease in the number of embryos in the ovarioles of virginoparae coincided with a decrease in the average number of adult virginoparae found in galls (Fig. 4).

Discussion

Temporal instar extension in *Pemphigus spyrothecae*

When taken together, two observations denote that duration of the soldier instar of *P. spyrothecae* increases over time: (1) Although the ratio of soldiers in the mature colony remains approximately constant, the ratio of first-instar virginoparae to first-instar sexuparae increases at a statistically significant rate from mid-season onward. Investment in first instars is therefore biased toward soldiers during this later period. (2) The number of embryos found in adult virginoparae declines over the season, just as the number of adult virginoparae in galls declines. The colony birth rate therefore decreases and, in fact, approaches zero toward the end of the season.

There is no evidence that *P. spyrothecae* is able to adjust its morph allocation at birth; however, colony fecundity is so reduced toward the end of the season that, even if a colony produced nothing but soldiers, this shift would still be insufficient to account for their persistently high proportion. This is easily evidenced by the following calculation: from Fig. 4, it can be seen that, at day 100, each gall contained approximately 10 virginoparae each bearing five embryos.

This figure had dropped to eight virginoparae each bearing four embryos by day 120. The maximum possible embryo expenditure for this 20-day period is thus 18. A period of 20 days corresponds to the instar duration measured by Rhoden (1997) for *P. spyrothecae* soldiers at the beginning of the season. If all the soldiers present on day 100 had moulted at this early-season rate, the only soldiers present on day 120 would be those that were born during the period, their number constrained to being equal to or fewer than 18. The mean number of soldiers per gall, however, was always significantly higher than this figure (being 54 ± 8 on day 100 and 40 ± 9 on day 120 in the example of *Coton Footpath*). The continuing investment in soldiers thus cannot be explained by increased soldier production at birth and it can be concluded that it takes the form of instar extension.

The consistent temporal decline in the proportion of soldiers in colonies of *P. bursarius* contrasts with the constancy seen in *P. spyrothecae*. There is no indication that instar extension is present in *P. bursarius*.

Identifying instar extension

The current study used demography and birth rate (estimated from dissections) to identify instar extension. In many species, measuring the number of pharate individuals across the season can provide a more direct measure of temporal change in the moulting rate (Akimoto, 1992). The rarity of pharate individuals made such measurement impractical in *P. spyrothecae*. Another approach is to measure the instar duration by close laboratory observation of aphids over the days or weeks immediately following their birth (Rhoden, 1997). This method has the potential to provide considerable accuracy, but being laboratory-based, it may inadvertently deprive aphids of the cues that would affect instar duration under field conditions.

Proximate causes of instar extension

The proximate factors that may induce instar extension are not known. Akimoto (1992) suggested that, in *H. persimilis*, the maternal generation might provide an ideal cue by which the duration of the soldier instar can be set. Offspring of first-generation apterae would thus have a relatively shorter first-instar span than offspring of third-generation apterae. As two to three generations of virginoparae can occur in galls of *P. spyrothecae*, it is possible that the species uses such a maternal-generation mechanism; however, the modest amount of data that Rhoden (1997) was able to collect under laboratory conditions indicates that the instar duration of second- and third-generation soldiers may be equal.

It has also been suggested that temporal instar extension could be brought about by a reduction of feeding by soldiers (Akimoto, 1992). The mechanism may certainly be a nutritional one as the food quality provided by a tree can deteriorate over the season (Dixon, 1970; Wiktelius &

Chiverton, 1985; Akimoto, 1989). The age of the host might also influence instar extension. It has been demonstrated in *P. bambucicola* that the average ratio of soldiers to non-soldiers is lower in colonies on fast-growing young bamboo (Sunose *et al.*, 1991) and higher on older shoots coming from older stems (Sakata *et al.*, 1991). *Pseudoregma bambucicola* colonies also tend to have higher ratios of soldiers to early-instar non-soldiers at the end of the secondary-host phase. Unlike *P. spyrothecae* and *H. persimilis*, this higher than expected soldier ratio can not be attributed to instar extension because the soldiers of *P. bambucicola* are obligately sterile. A concluding demographic state in which a relatively large proportion of soldiers is retained while the rest of the colony continues to mature may thus be adaptive regardless of the route that is taken to reach it.

The adaptive nature of temporal instar extension

The strategy of having sufficient soldiers to defend those reproductives that have adequate time to reach maturity is clearly adaptive. Facultatively sterile soldiers that are produced at the end of the season will not have ample time to mature and are thus functionally sterile. Given that this is the case, the optimal use of a colony's resources may be to ensure that these soldiers remain soldiers rather than advancing to the later instars which would be incapable of adding, either directly or indirectly, to the overall reproductive success. By this strategy, those reproductives that can reach maturity may enjoy unprecedented protection against predation.

Instar extension may also provide a cheaper method of defence investment. Whereas the mature virginoparae of a colony may not have sufficient embryonic resources to produce more soldiers at birth, investment in soldiers may nevertheless be increased merely by lengthening the soldier instar.

Knowledge of the different morphologies of the various generations of *P. spyrothecae* soldiers indicates that instar extension is greatest in those soldiers that are best equipped to defend. Whitfield (1997) has demonstrated that, as the generations progress, distinctive soldier features such as curved claws and thickened hind femora become significantly more pronounced. It makes sound evolutionary sense that soldiers with increasingly specialised morphologies and extended instar durations may have been selected for in the latter portion of the galling phase. Protection of present and future sexuparae is likely to be particularly important during this period, as it is then that the galls open and predation rises markedly (Braendle, 1999; Pike, 2002).

Theoretical investigations of the ultimate factors likely to encourage instar extension are still scarce but one study predicts that lower birth rates will be unimportant except for those colonies which will undergo many generations (Akimoto, 1996). In aphid colonies in which the number of generations is limited by the duration of the galling phase (like those of *P. spyrothecae*), prolongation of the galling period is likely to be a more significant promoter of instar extension.

The general significance of instar extension to aphid sociality

In those aphid species with monomorphic instars, the possibilities for altruism to be manifested as physiological instar extension or morphological armour are limited. Defensive behaviour devoid of instar extension may thus be the first step that results in relatively simple sociality (e.g. the first-instar behavioural soldiers of *P. bursarius*). The evolution of instar dimorphism concomitantly facilitates differences in developmental rates. A further step toward sociality may then occur when morphologically specialised defensive instars evolve longer durations than other instars (e.g. the morphologically distinct soldiers of *P. spyrothecae*). The final step of relinquishing all direct fitness may occur when the soldier instar is extended so much that mortality always precedes the (perhaps obsolete) factors that induce moulting (e.g. the sterile soldiers of *P. bambucicola* and, perhaps, the sterile late-season soldiers of *P. spyrothecae*).

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References

- Abbot, P., Withgott, J.H. & Moran, N.A. (2001) Genetic conflict and conditional altruism in social aphid colonies. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 12068–12071.
- Akimoto, S. (1989) Gall-invading behavior of *Eriosoma* aphids (Homoptera: Pemphigidae) and its significance. *Japanese Journal of Entomology*, **57**, 210–220.
- Akimoto, S. (1992) Shift in life-history strategy from reproduction to defense with colony age in the galling aphid *Hemipodaphis persimilis* producing defensive first-instar larvae. *Researches on Population Ecology*, **34**, 359–372.
- Akimoto, S. (1996) Ecological factors promoting the evolution of colony defense in aphids: computer simulations. *Insectes Sociaux*, **43**, 1–15.
- Aoki, S. (1977) A new species of *Colophina* (Homoptera, Aphidoidea) with soldiers. *Kontyû*, **45**, 333–337.
- Aoki, S. (1980) Life cycles of two *Colophina* aphids (Homoptera, Pemphigidae) producing soldiers. *Kontyû*, **48**, 464–476.
- Aoki, S., Akimoto, S. & Yamane, S. (1981) Observations on *Pseudoregma alexanderi* (Homoptera, Pemphigidae), an aphid species producing pseudoscorpion-like soldiers on bamboos. *Kontyû*, **49**, 355–366.
- Aoki, S. & Kurosu, U. (1986) Soldiers of a European gall aphid, *Pemphigus spyrothecae* (Homoptera, Aphidoidea): why do they molt? *Journal of Ethology*, **4**, 97–104.
- Aoki, S. & Kurosu, U. (1992) Gall generations of the soldier-producing aphid *Pseudoregma bambucicola* (Homoptera). *Japanese Journal of Entomology*, **60**, 359–368.

- Aoki, S. & Miyazaki, M. (1978) Notes on the pseudoscorpion-like larvae of *Pseudoregma alexanderi* (Homoptera, Aphidoidea). *Kontyû*, **46**, 433–438.
- Braendle, C. (1999) *The ecology of polyphenism in a soldier-producing aphid*. M. Phil., University of Cambridge, Cambridge.
- Dixon, A.F.G. (1970) Quality and availability of food for a sycamore aphid population. *Animal Populations in Relation to Their Food Resources* (ed. by A. Watson), pp. 271–287. Blackwell, Oxford.
- Foster, W.A. (1990) Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). *Behavioral Ecology and Sociobiology*, **27**, 421–430.
- Lampel, G. (1960) Die morphologischen und ökologischen Grundlagen des Generationswechsels monözischer und heterözischer Pemphiginen der Schwarz- und Pyramidenpappel. *Zeitschrift für angewandte Entomologie*, **47**, 334–375.
- Pike, N. (2002) *Defence investment and altruism in Pemphigus aphids*. PhD, University of Cambridge, Cambridge.
- Rhoden, P.K. (1997) *The evolution of social behaviour in aphids of the genus Pemphigus*. PhD, University of Cambridge, Cambridge.
- Sakata, K. & Itô, Y. (1991) Life history characteristics and behaviour of the bamboo aphid, *Pseudoregma bambucicola* (Hemiptera: Pemphigidae), having sterile soldiers. *Insectes Sociaux*, **38**, 317–326.
- Sakata, K., Itô, Y., Yukawa, J. & Yamane, S. (1991) Ratio of sterile soldiers in the Bamboo Aphid, *Pseudoregma bambucicola* (Homoptera: Aphididae), colonies in relation to social and habitat conditions. *Applied Entomology and Zoology*, **26**, 463–468.
- Schütze, M. & Maschwitz, U. (1991) Enemy recognition and defence within trophobiotic associations with ants by the soldier caste of *Pseudoregma sundanica* (Homoptera: Aphidoidea). *Entomologica Generalis*, **16**, 1–12.
- Shibao, H. (1998) Social structure and the defensive role of soldiers in a eusocial bamboo aphid, *Pseudoregma bambucicola* (Homoptera: Aphididae): a test of the defence-optimization hypothesis. *Researches on Population Ecology*, **40**, 325–333.
- Shibao, H. (1999) Reproductive schedule and factors affecting soldier production in the eusocial bamboo aphid, *Pseudoregma bambucicola* (Homoptera: Aphididae). *Insectes Sociaux*, **46**, 378–386.
- Shingleton, A.W. & Foster, W.A. (2000) Ant tending influences soldier production in a social aphid. *Proceedings of the Royal Society of London B*, **267**, 1863–1868.
- Sunose, T., Tsuda, K. & Ohseko, S. (1982) Seasonal change in ratios of soldier in a population of the bamboo aphid, *Pseudoregma bambucicola*. *Bulletin of the Society of Population Ecology*, **35**, 59–61.
- Sunose, T., Yamane, S., Tsuda, K. & Takasu, K. (1991) What do the soldiers of *Pseudoregma bambucicola* (Homoptera, Aphidoidea) defend? *Japanese Journal of Entomology*, **59**, 141–148.
- Whitfield, J.A. (1997) *Studies of the diversity and evolution of soldier aphids*. PhD, University of Cambridge, Cambridge.
- Wiktelius, S. & Chiverton, P.A. (1985) Ovariole number and fecundity for the two emigrating generations of the bird cherry-oat aphid (*Rhopalosiphum padi*) in Sweden. *Ecological Entomology*, **10**, 349–355.
- Withgott, J.H., Abbot, D.K. & Moran, N.A. (1997) Maternal death relaxes developmental inhibition in nymphal aphid defenders. *Proceedings of the Royal Society of London B*, **264**, 1197–1202.

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