Antimicrobial strength increases with group size: implications for social evolution

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We hypothesize that aggregations of animals are likely to attract pathogenic micro-organisms and that this is especially the case for semisocial and eusocial insects where selection ultimately led to group sizes in the thousands or even millions, attracting the epithet ‘superorganism’. Here, we analyse antimicrobial strength, per individual, in eight thrips species (Insecta: Thysanoptera) that present increasing innate group sizes and show that species with the largest group size (100–700) had the strongest antimicrobials, those with smaller groups (10–80) had lower antimicrobial activity, while solitary species showed none. Species with large innate group sizes showed strong antimicrobial activity while the semisocial species showed no activity until group size increased sufficiently to make activity detectable. The eusocial species behaved in a similar way, with detectable activity appearing once group size exceeded 120. These analyses show that antimicrobial strength is determined by innate group size. This suggests that the evolution of sociality that, by definition, increases group size, may have had particular requirements for defences against microbial pathogens. Thus, increase in group size, accompanied by increased antibiotic strength, may have been a critical factor determining the ‘point of no return’, early in the evolution of social insects, beyond which the evolution of social anatomical and morphological traits was irreversible. Our data suggest that traits that increase group size in general are accompanied by increased antimicrobial strength and that this was critical for transitions from solitary to social and eusocial organization.

Keywords: antimicrobial; evolution; sociality; thrips

1. INTRODUCTION

When animals live together in increasing numbers and proximity, it is likely that they provide increasingly attractive substrates for pathogenic micro-organisms. This would seem to be the case especially for social animals such as social insects. Indeed, since Darwin wrote that the existence of social insects was his ‘one special

difficulty’, potentially fatal to his theory, the scientific literature has addressed a variety of explanations for their evolution [1–3]. There were probably many factors that contributed to it [3,4]; however, because eusocial insect colonies are composed of relatively closely related individuals that co-occur in high densities, they represent ideal environments for pathogenic micro-organisms and are highly vulnerable to microbial attack [5,6]. Thus, entomopathogens may be strong selective agents wherever group size increases and in response, effective front-line antimicrobials may have been selected for, especially in social insects. Our previous research showed that the strength of antimicrobials in bees increased with the level of sociality [6]. However, the increase was nonlinear, with the greatest increment being between the solitary and semisocial species, that is, among the smallest group sizes, strongly suggesting that the very emergence of social traits required defences against microbial pathogens [6]. Thrips provide an ideal group for investigating this transition further.

Thrips belong to the insect order Thysanoptera, which includes 5500 species globally, with many more awaiting descriptions [7]. They include species that are solitary, many of them living in flowers and some well known because they are horticultural pests. Others exhibit a range of group sizes, frequently through the construction of more or less enclosed domiciles made of leaves, or other plant structures. A further assemblage of species induces a variety of plant galls, frequently on *Acacia* phyllodes, often inhabited by several hundred tightly packed individuals. Some of these colonies are not eusocial, consisting of a single female and one generation of her offspring. Other species show eusocial traits, especially the presence of a soldier caste [8,9].

2. MATERIAL AND METHODS

We examined eight species and the group sizes shown (group size $\bar{x} \pm \text{s.e.}$) were those from freshly collected specimens: The non-eusocial gall makers, *Kladothrips arotrum* (385 ± 70 , $n = 8$) and *Kladothrips antennatus* (130 ± 9 , $n = 15$), were species where the group consists of a single female with large numbers of her offspring, there being no castes or other eusocial traits. In sociobiological terminology, such single females surrounded by hundreds of offspring are regarded technically as ‘solitary’, but here our interest is in the group size. *Dunatothrips vestitor* forms loose associations of individuals within ‘domiciles’, formed when the animal pulls leaves (phyllodes) together with silk for shelter (46 ± 7 , $n = 9$). These three species, plus the solitary *Haplothrips froggatti* (one), were all collected from the same habitats near Alice Springs, Northern Territory, Australia. The eusocial gall-maker *Kladothrips intermedius* (146 ± 6 , $n = 15$) was collected from near Tomingley, New South Wales, Australia. All these sites were semiarid and selected to minimize differences in vegetation type. The activity of both *Teuchothrips ater* (23 ± 4 , $n = 20$) and *Klambothrips oleariae* (45 ± 8 , $n = 9$) deforms leaves, which curl or roll to form a tube that may or may not be closed. These species, which form small aggregations, were collected from the Canberra area. The solitary *Odontothripella aloba* (one) was collected from coastal sclerophyll near Sydney (table 1). In summary, the provenance of the groups was: large, non-eusocial groups living within galls; large, eusocial groups within galls; smaller aggregations within leaf domiciles and, finally, solitary animals. These groups then fell within one of three innate group-size classes: hundreds, tens or solitary. These classes were derived from both our observations and the literature (see table 1 and references therein).

There were no significant differences in the size of individuals among species.

Cuticular antimicrobials were obtained from living animals and bioassays were carried out according to established methods [5,6,10]. Thrips were washed in 90 per cent ethanol for 5 min followed by three rinses to ensure maximum extraction. The solvent was then removed by a rotary evaporator and the residual extract re-suspended in LB broth. Assays against *Staphylococcus aureus*

Figure 1. Concentration–growth response curves showing activity of extracts from eight thrips species against *S. aureus*. Axes *x*, number of thrips; *y*, ratio of test well to control well growth. The highest extract concentration was equivalent to 80 individuals with a three-fourth dilution series, except for *O. aloba* where the highest concentration was equivalent to 150 individuals with a one-half dilution series. Following incubation at 37°C for 18 h, growth was measured as an increase in optical density (OD) and expressed as: increased OD test well/increased OD *S. aureus* control. The curves were compared by functional analysis of variance [16] and differed significantly between group-size classes ($F = 55.108$, d.f. = 2, $p < 0.005$). (a) Open circles, *Kladothrips arotrum*; filled diamonds, *Kladothrips antennatus*. (b) Open circles, *Teuchothrips ater*; filled diamonds, *Klambothrips oleariae*. (c) Open circles, *Haplothrips froggatti*; filled diamonds, *Odontothripiella aloba*; open triangles, *Dunatothrips vestitor*. (d) Open circles, *Kladothrips intermedius*.

Table 1. Group sizes and sampling locations.

species	group sizes			GPS	references
	mean/s.e.	maximum	innate		
<i>Kl. arotrum</i>	385 ± 70	770	hundreds	23°42'26" S; 133°50'11" E	750 adults + 500 juveniles [14]; 433 [12]
<i>Kl. antennatus</i>	130 ± 9	176	hundreds	same as <i>Kl. arotrum</i>	313 [12]
<i>Kl. intermedius</i>	146 ± 6	190	hundreds	32°45'06" S; 148°22'30" E	57 ± 1.74 to 101 ± 20 [14]
<i>T. ater</i>	23 ± 4	50	tens	33°44'28" S; 151°02'11" E	L. A. Mound 2010 (personal communication)
<i>Km. oleariae</i>	45 ± 8	80	tens	35°27'39" S; 149°00'59" E	L. A. Mound 2010 (personal communication)
<i>D. vestitor</i>	46 ± 7	98	tens	23°45'59" S; 133°52'36" E	21, 50 [15]
<i>H. froggatti</i>	n.a.	1	solitary	23°42'19" S; 133°53'19" E	n.a.
<i>O. aloba</i>	n.a.	1	solitary	33°39'12" S; 151°13'27" E	n.a.

were performed, using opposing gradients of microbial extract concentration and bacterial cell numbers across rows of 12 wells in 96-well microtitre plates. This produced concentration–growth response curves for each species. There were three controls: broth alone, *S. aureus* with broth and extract with broth. In all cases but one, the greatest concentration of thrips extract used was equivalent to 80 thrips, with more than 320 insects being used to produce a three-fourth dilution series and 80 thrips equivalents within the first well, 60 in the second and so on. For *O. aloba*, the concentration of thrips extract used was equivalent to 150 thrips using a one-half dilution series. Following incubation at 37°C for 18 h, growth in the treatments and controls was measured as increase in optical density (OD) and expressed as: increase in OD test well/increase

in OD of *S. aureus* control. We performed additional tests for species that showed no activity in the dilution series as follows: in the solitary species, and *D. vestitor*, which showed no activity, additional wells containing artificial aggregations of 150 individuals ($n = 5$) were tested for activity. In the eusocial *Kl. intermedius*, the additional wells contained 120 individuals ($n = 5$).

3. RESULTS

Staphylococcus aureus was most strongly inhibited by *Kl. arotrum* and *Kl. antennatus* (figure 1a), the species with the highest innate group sizes. Extracts from

20 individuals inhibited bacterial growth. Tissue collected from *Acacia aneura* that constituted the gall were included as controls and showed no activity. The leaf-rollers *T. ater* and *Km. oleariae*, with smaller group sizes, showed only weak antimicrobial activity, and then only at the highest aggregations (figure 1b). The leaf-spinner *D. vestitor* and the solitary species *O. aloba* and *H. froggatti* showed no activity at all (figure 1c), even when artificial concentrations of 150 individuals ($n = 5$) were assayed separately. The eusocial gall-maker, *Kl. intermedius*, also showed no activity (figure 1d) but, in additional assay wells containing extract from 120 individuals ($n = 5$), representing the largest galls, there was some activity: a decline of 20 per cent in bacterial growth (not shown in figure 1d as individual wells rather than dilution series were used; see §2).

4. DISCUSSION

Our data show that the innate group size of these species is a predictor of its antimicrobial strength. Thus, when it was large, as in *Kl. arotrum* and *Kl. antennatus*, antimicrobials were strongest. This may be especially important when within-colony genetic diversity is low, as in these species [11]. For species with smaller innate group sizes, as in the leaf-rollers, antimicrobial activity was detectable only in the largest groups, suggesting a threshold for activity for any species where group size increases beyond a critical level.

The same applied in the eusocial *Kl. intermedius*, which showed no activity until group size approached 120. This apparent threshold was unexpected as, in a previous study, eusocial bees showed a very strong activity [6]. However, while it has a soldier caste, placing it squarely in the eusocial category [8], *Kl. intermedius* exhibits an intermediate group size with small brood numbers, small galls and small numbers of soldiers [9,12,13] and, therefore, possesses modest antimicrobial strength, appropriate to its group-size class. Group size probably sets this species apart from other eusocial insect species, such as the stingless bees *Trigona*, whose colonies contain thousands of individuals and secrete very strong antimicrobials [6].

Our data show that once group size reaches a critical point, there is powerful selection for an increase in the strength of antimicrobials. We see this especially in *Kl. arotrum* and *Kl. antennatus*, but also in *Km. oleariae* and *T. ater*. In this context, it is interesting to consider the case for species with eusocial traits that, by definition, drive increase in group size. The results for the eusocial *Kl. intermedius* are consistent with the others as antimicrobial strength increases at larger group sizes. Together, the three *Kladothrips* species represent a range of group sizes within a single genus and demonstrate evolutionary lability for antimicrobial defence, militating against phylogenetic constraints. Further, the possibility that the plant *A. aneura* generates a special requirement for antimicrobial production seems unlikely because species of thrips collected and analysed from this host show both the strongest

antimicrobials (*Kl. arotrum* and *Kl. antennatus*) and a complete lack of antimicrobial activity (*D. vestitor*).

Wilson [3] presented evidence that social insects descended from species adapted to well-protected microhabitats that could be defended against enemies, especially predators, parasites and competitors. We note that this list did not include microbial pathogens, which is ironic in this context, as they may have been the most important and ubiquitous class of enemy and one that could take advantage of the very defences assembled against all the other types of enemy. Our experimental data may shed light on the early stages of such social evolution, suggesting that species evolving social or eusocial traits may have experienced group sizes at which pathogenic micro-organisms were the most important selective agents. Further, strong antimicrobials may have been necessary to traverse the 'point of no return' early in social evolution, raising the question as to how far it would have got in their absence.

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