Research article

Direct and indirect benefits of cooperation in collective defense against predation

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ACKNOWLEDGEMENTS

This study was funded by the Research Council of Finland via the project no 330578 (CL) and 333482 (SW). We are grateful to Nina Immonen, Liina-Lyydia Jämsä, Venla Korhonen, and Jenni Sohlman for helping with the maintenance of lab population and collecting field data as well as local Forest Centers in Central Finland for providing information about the potential outbreak areas.

Authors declare no conflict of interests.

AUTHOR CONTRIBUTION STATEMENT:

CL conceived the ideas and CL, RR, RT, HH and SW designed methodology; RR and RT collected the data; RR, RT and SW conducted genetic and phenotypic analyses of the data; RR led the writing of the manuscript, with input from CL and HH. All authors contributed critically to the drafts and writing and gave final approval for publication.

DATA AVAILABILITY STATEMENT

When the paper will be accepted, all the data and final R-codes used in these analyses will be included in open access database in DRYAD.

Abstract

The evolution and maintenance of public goods cooperation, despite cheating, remains a key interest in social biology. Specifically, identifying how ecological factors determine the direct and indirect benefits that maintain cooperation has proven challenging, as these can vary significantly across species and environments. Here, we study this problem by using the social pine sawfly *Neodiprion sertifer* (Hymenoptera) as a model system. During their larval stage, *N. sertifer* live in groups and collectively secrete a defensive fluid against predators. This behavior comprises a public good as it is costly to exhibit and beneficial to others, and individuals vary in their contribution to group defense. We experimentally manipulated individual contributions to defense to assess how these influence individual survival. Our results indicate that defense has a group-level benefit as individuals were more likely to survive in cooperative groups that had a higher proportion of defending larvae. Moreover, being able to deploy defensive fluid confers direct survival benefits to individuals, regardless of group cooperativeness. Genetic and phenotypic analysis of natural populations further shows that kin selection promotes collective defense, as groups of larvae are often composed of full siblings. We also find that contribution to defense is female-biased and diminishes in male-biased and in larger groups, indicating that individuals adjust their contributions based on the social context. Overall, we find that cooperation in anti-predator defense provides both direct and indirect benefits and that individuals regulate their contributions mainly based on the social environment, resulting in variation within and among natural populations.

Key words: chemical defense, haplodiploids, group living, social environment, kin-selection, sex-biased cooperation

Introduction

Individuals can cooperate in public goods which are often costly to produce for an individual, but beneficial for all members of a local group or population (1). Public good systems are abundant across species with examples including social immunity in burying beetles (2), parasitoid wasps with cooperative brood care (3), siderophores and biofilms in bacteria (4, 5) and communal nesting in birds (6). The fundamental challenge of public good systems is their vulnerability to exploitation or cheating, i.e., benefiting from the public good without contributing to the production of it (1, 7–9). If contributing is costly, and benefits are shared, it is not a priori guaranteed that contributing to collective good leads to higher fitness than reaping the benefit without having contributed to its production (10, 11). This raises the question of the evolutionary stability of such systems over time.

Several mechanisms that select for cooperation have been identified (1, 11–15), affecting both direct and indirect fitness components of inclusive fitness (16, 17). For example, exploitation can be limited through negative frequency-dependence (11, 18, 19). In this case, the fitness of cheats will decrease when their frequency increases limiting their spread. The frequency-dependence can become stronger when at least some group members are related (20). In this context, an increase in cheating within a group would increasingly harm the relatives as well. Finally, if more cooperative individuals have better access to the public goods they have contributed to (i.e., direct benefits of cooperation), it should limit the expression of cheating (21–24). While significant progress has been made using microbial systems to study these mechanisms empirically (10, 18, 25–28), these predictions still need to be experimentally validated in more complex study systems where cooperation is facultative, experimental manipulation possible, and which show a higher degree of plasticity. This will help us to better determine the sources of diversity in cooperation and its maintenance in nature.

Here, we investigate solutions to the public good dilemma within a chemical defense framework (29). Many organisms defend against predators using defensive secretions, which are costly to produce and maintain (30–36). Their expression is often facultative

(29–31, 36), giving individuals the option to choose whether or not to deploy the defensive fluid when approached by a predator. In general, chemically defended prey are considered to contribute to public goods by educating predators to avoid prey with similar appearance in future encounters (37, 29, 38). These shared costs of predator education are then a collective production of benefits shared by local group members or the population, i.e., cooperation (29, 39, 40). They apply both to solitary and gregarious species and can also occur at an interspecific level in Müllerian mimicry, i.e., defended individuals belonging to different species share the same warning signal (41).

Group living by the prey has been shown to enhance avoidance learning by predators (42, 43). This is because, when attacking an individual that is part of a group, a predator encounters the chemically defended prey more frequently, making it easier for the predator to recognize and memorize the appearance of that prey type and avoid attacking such prey in future encounters (43–45). A higher potency of chemical defense (46), and a higher proportion of individuals participating in the defense within a group (47, 48) are also expected to increase defense success. Finally, when multiple individuals release secretions simultaneously, the magnitude of these stimuli is likely to increase protection (43, 49, 50), for instance by forming a barrier of defensive fluid that makes it difficult for a predator to reach a single prey without being exposed to the harmful effects of the secretion. In addition to these public goods-type mechanisms, displaying defensive secretions can reduce the risks associated with predation for an individual directly (i.e., providing individual benefits). This is because a predator may assess the unpalatability or toxicity of an individual prey using visual, olfactory or gustatory cues of the defensive secretion, without actually killing the prey (51). Being able to secrete the defensive fluid will also help an individual in “fighting” against the attacking predator.

To study empirically the mechanisms that maintain public goods cooperation in collective chemical defense, we used social haplodiploid European pine sawflies (*Neodiprion sertifer*) as a model system. They live solitarily during the adult stage, but feed and defend collectively during the larval stage in dense groups on pine branches (52–54) (Fig. 1a). When threatened by natural enemies, such as birds, ants or parasitoid wasps, the larvae perform a synchronous defensive display where they raise their head

and tail (U-posture). As a secondary line of defense, larvae release a defensive secretion from their mouths and attempt to apply it directly onto the predator if it approaches close enough (55). If larvae do not lose the fluid during such encounters, they can reabsorb and reuse it during subsequent attacks (54). In *N. sertifer*, survival against bird predation is higher when larvae are in a group in comparison to solitary larvae (43). Furthermore, being able to deploy the defensive fluid has been shown to improve survival in gregarious pine sawfly larvae against invertebrate predators when compared to depleted larvae (50, 56).

Pine sawfly larvae show individual and sex-biased variation in the likelihood of deploying the fluid as well as its volume and contributing to the collective chemical defense is costly (36). Additionally, some larvae do not produce the fluid even when provoked, and benefit from this via enhanced growth (36). Such non-defending individuals can be considered cheats in this system (8, 29, 36). Losing the fluid also decreases an individual's capacity to defend in future encounters with predators (36, 56). Due to these features, it is possible to (i) quantify how much individuals are contributing to the collective defense, i.e., whether an individual deploys the fluid and the volume of fluid they produce and if so (ii) manipulate the cooperativeness within a group by manually depleting defensive fluid reserves and thereby decreasing the level of defense that can be exhibited (36). However, even when not actively secreting the defense fluid, pine sawfly larvae remain unprofitable prey for avian and invertebrate (wood ants) predators (57–59).

We addressed five aims. First, we studied experimentally whether cooperation provides benefits through defense against predators. This was done by comparing the mean survival of individuals against wood ant predation between groups containing a high proportion of defending individuals (20% of individuals depleted, 80% non-depleted) and groups containing a low proportion of defending individuals (70% of individuals depleted, 30% non-depleted). We hypothesized that individuals are more likely to survive in groups with higher proportion of defending individuals than in groups with lower proportion of defending individuals.

Second, we identified potential direct benefits of contributing to the costly collective defense. This was done by color-marking depleted and non-depleted individuals within experimental groups, and thus identifying whether depleted individuals (i.e., those that do not deploy the fluid or only in smaller amounts) are more likely to be killed by predators compared to individuals that are not depleted. We hypothesized that contribution to the collective chemical defense will be promoted if predators are less likely to attack an individual secreting the defense fluid (direct benefit) (51).

Third, we investigated the importance of kin selection in maintaining cooperation in gregarious chemically defended prey. This was done by collecting phenotypic and genetic data from four natural populations of *N. sertifer* in Finland to quantify the kin structure and social characteristics of larval colonies and their defensive behavior. If individuals are more likely to occur in the same group with their siblings, we can expect that contribution to the costly collective defense provides indirect fitness benefits by increasing the survival of kin (16, 17). Similarly, in kin groups the relative cost of cheating increases if it decreases the survival of the cheat's relatives (11).

Fourth, we used data gathered above to quantify the extent of variation in cooperativeness in natural populations. Fifth, we explored how such variation correlates with the social structure, i.e., group size, sex ratio, and kinship of groups. We hypothesized that if larval colonies vary in their relatedness structure, and if individuals were able to estimate relatedness, there would be less cooperativeness in groups with low levels of kinship. Furthermore, since females are more likely to contribute to collective defense in pine sawflies (36), we expected the mean contribution to defense to be lower when there was a higher proportion of males within a group. We also expected the individual contributions to decline with increasing group size if benefits gained by contributing to the collective defense decrease in larger groups simply due to dilution effects and individuals optimizing their behavior accordingly (30, 42, 60). This local variation in the social conditions (e.g., group size (30)) could provide one mechanism maintaining differences in collective defense among populations.

Results

Individuals are more likely to survive predation in more cooperative groups and contribution to collective defense provides direct benefits in terms of survival

The mean survival of individuals under predation by wood ants (*Formica s.str.*) was significantly higher in high cooperation groups compared to low cooperation groups (model 1: $LR\chi^2_1 = 5.508$, estimate = -1.314, error = 0.560, p = 0.019, Fig. 1b). When the individual's defensive status (depleted or non-depleted) was included in the model we found that depleted individuals had significantly lower survival than non-depleted individuals (model 2: $LR\chi^2_1 = 6.04$, estimate = 1.932, error = 0.786, p = 0.014, Fig. 1c). Furthermore, we did not find support for frequency-dependence as proportion of defending individuals within a group (model 2: $LR\chi^2_1 = 0.182$, estimate = -0.331, error = 0.774, p = 0.669, Fig. 1c) or its interaction with the defensive status of an individual (model 2: $LR\chi^2_1 = 0.463$, estimate = -0.652, error = 0.958, p = 0.496, Fig. 1c) did not affect survival of depleted individuals significantly (Fig. 1c).

Contribution to collective defense provides indirect benefits

Kinship was calculated based on consanguinity values. Therefore, values for full siblings are expected to be 0.375 between sisters, 0.5 between brothers, 0.25 between sisters and brothers (61). Across all four populations, individuals show a mean kinship of 0.30 (+- 0.01 SEM) among females, 0.45 (+- 0.01 SEM) among males and 0.19 (+- 0.02 SEM) among females and males (Fig. 1d).

Social environment and collective chemical defense behavior vary locally

To compare within and among population variation in the defensive behavior of *N. sertifer* larvae, we first focused on the collective chemical defense behavior of larvae within a group. This was measured by attacking one randomly chosen individual in the middle of the group by poking it on the dorsal side with the tip of a pair of tweezers and measuring the number of individuals displaying the U-posture and deploying the fluid. Proportion of individuals displaying the U-posture varied significantly among populations (Table S1).

This was mainly driven by the significant difference between the populations Pöntiö and Puumala as well as Pöntiö and Pieksämäki. All the other populations did not differ from each other in pairwise comparisons (Table S1, Fig. S2d).

Next, we analyzed the proportion of individuals per group participating in the second and more costly stage of the defense (i.e., deployment of fluid). Similarly to U-posture, the proportion of individuals deploying the fluid varied significantly across populations (Table S1). Pairwise comparisons indicate that this variation was again driven by the Pöntiö population where the proportion of individuals deploying the fluid per group were lower compared to all other populations (Table S1, Fig. S2c).

We then analyzed how defensive behavior (whether larvae deployed the fluid or not) and the volume of fluid secreted varied among individuals and populations when larvae were attacked individually (i.e., defended themselves). Contribution to defense was sex-biased: males were less likely to deploy the fluid than females (model 11: estimate: -1.77, low 95% CI: -2.88, high 95% CI: -0.77, R-hat: 1) and produced lower volumes of it (model 12: estimate: 0.89, error: 0.09, low 95% CI: 0.73, high 95% CI: 1.07, R-hat: 1). Due to these sex biases, we analyzed the responses of females and males separately in all subsequent analyses for individually measured defense traits.

The individual's likelihood of deploying the fluid did not vary significantly among populations, either among female or male larvae (Table S1). However, the volume of fluid individuals contributed varied significantly among populations for females (Table S1), but not for males (Table S1). In general, female larvae deployed the highest volumes in Kaavi and lowest in Pieksämäki populations (Fig. S5). Size of the individual did affect the volume deployed in males (smaller male larvae deployed higher volumes), but not in females (Table S1). In general, individuals not deploying the fluid (i.e. cheaters) were less frequent than individuals deploying the fluid (Fig. S3). Across all populations, 92% of females deployed the fluid and 72% of males deployed the fluid when individually attacked.

Populations also differed significantly in terms of social characteristics of larval groups. The Pöntiö population had both the most female-biased sex ratios and largest group sizes (see Table S1 for pairwise comparisons, Fig. S2a and S2b,). However, the kinship of larval colonies did not differ significantly among populations for female-female, male-male or female-male kinships (Table S1, Fig. 1d). We excluded Kaavi from the analyses of male-male and female-male kinships because of the low number of males (N = 1 for male-male kinship and N = 2 for female-male kinship).

For the proportion of individuals which displayed U-posture synchronously when provoked, neither sex ratio nor group size had a significant effect (Table 1, Fig. S4a and S4b). Similarly, there were no significant correlations between any of the kinship variables and the proportion of individuals in a group that displayed the U-posture (Table 1, Fig. S4c).

However, when the secondary more costly stage of the collective defense was analyzed (i.e., proportion of individuals that deployed the fluid), cooperativeness of the group decreased significantly with an increasing proportion of males. Similarly, proportion of individuals that deployed the fluid decreased with the increase in group size (Table 1, Fig. 2a and 2b). Only male-male kinship was positively correlated with contribution to the collective chemical defense. None of the other two kinship comparisons (female-female, female-male) correlated with the proportion of individuals deploying the fluid within a group (Table 1, Fig. 2c).

Similar analyses were run for the individually measured defensive traits. For males neither sex ratio, group size nor any of the kinship comparisons affected their probability of deploying defensive fluid (Table 2) or the amount of fluid secreted (Table 2). This was also the case for females except for female-male kinship which increased with the amount of defensive fluid. Larval length did not affect how much fluid was deployed in both females and males in these models.

We analyzed how social characteristics of larval groups were correlated. According to these analyses, proportion of males increased in bigger groups, but was not associated

with any of the kinship estimates (Table S2). Similarly, female-female and female-male kinship decreased in larger groups. (Table S2, Fig. S6a, S6b and S6c).

Finally, populations were not genetically structured. First, we found that F_{ST} values were close to zero for all pairwise population comparisons and all female combinations (Fig. S7a). Second, F_{IS} values were slightly negative for most combinations of females (Fig. S7b). These results suggest panmixia, i.e., low differentiation across large distances and indicate lack of inbreeding within populations.

Discussion

Determining factors that drive cooperation and cheating in public goods is fundamental to predict variation in cooperative interactions within and between species (24). Based on Hamilton's inclusive fitness theory, we can expect selection to favor cooperation when the direct and indirect fitness benefits to the actor outweigh the fitness cost. Here, we have demonstrated experimentally that cooperation in collective chemical defense is beneficial through improving survival of individuals, and that this results in indirect benefits as most individuals within larval colonies are full siblings. We have also shown that cooperation confers direct benefits, as individuals that had maintained their capacity to defend had a higher probability of survival than those that lost it. Our phenotypic data from wild groups reveal significant variation in collective chemical defense across groups and populations. This variation among larval groups is partly explained by group size and sex ratio, with individuals becoming less cooperative in larger, more male-biased groups. Contribution to collective chemical defense was also sex-biased, with females more likely to deploy the defensive fluid and in greater volumes than males. Together, these results suggest that while contributing to defense improves individual survival, individuals adjust their contributions to the collective good based on their social environment and sex. Variation in these factors leads to within- and among-population variation in cooperative behavior.

Public goods systems are often vulnerable to cheating, but cheats might not outcompete cooperative phenotypes completely if cooperation has high direct benefits. Our data

323 from natural populations indicates that cheating in *N. sertifer* populations is rare among
324 females but not unusual among males (8% of females and 28% of male larvae do not
325 defend when individually attacked and measured; Fig. S3). However, in contrast to
326 frequency dependent benefits (11), cheats (experimentally represented by depleted
327 individuals) did not benefit from being rare, but instead had equally low survival chances
328 against predators both when they were rare (20% depleted) and when they were common
329 (70% depleted) (Fig. 1a and 1b). This suggests that at least under high predation intensity,
330 contribution to collective defense is promoted via high direct benefits of defense. It
331 remains possible that frequency-dependent costs and benefits could occur at more
332 extreme frequencies not included into our experimental design. Additionally, our
333 experiment included only one type of predator (wood ants). In nature, predator species
334 vary in their sensitivity to defense mechanisms of prey (reviewed in (62)) and in their
335 responses to visual cues and signals of prey individual's unprofitability (63, 64).
336 Therefore, the benefits of contributing to group defense can vary depending on the
337 intensity of predation as well as predator community structure. How cooperation is
338 selected in a more natural set-up offers therefore an interesting avenue for future
339 research.

340 The benefits of cooperation can also depend on group size (30). For example, studies on
341 public goods systems in microbes have shown that the fitness of cheats increases at high
342 population densities (60). Accordingly, our phenotypic data from natural populations
343 suggest that the proportion of individuals contributing to collective defense decreases
344 as group size increases. At larger aggregations, it might be more beneficial for a
345 gregarious prey to rely on the dilution effect and allocate energy and resources to growth
346 instead (32, 33, 42, 65). Group sizes also varied quite extensively within and among
347 populations (Fig. S2b). Part of this variation can be a result of merging of offspring from
348 several females (50, 66). This is supported by our observation that the within-group
349 kinship decreases in larger group sizes. In addition, the quality of the host plant can affect
350 both oviposition decision of females (67, 68) as well as the hatching success of eggs (67)
351 and mortality rates of larvae (69), further influencing larval group sizes.
352

Our results show that similarly to *Diprion pini* (36), also *N. sertifer* females contribute to collective defense significantly more often and in greater quantities than males. One possible explanation for this is that females are pre-adapted to help, given that they grow larger than males in the larval stage and are thus able to contribute to defending more effectively. This is similar to termites, where size could be a pre-adaptation promoting the sex biased division of labor among females and males (70). More generally, maternal behaviors as pre-adaptations (rather than haplodiploidy *per se*) explain the prominence of female helpers in social Hymenoptera (71, 72). The evolution of sex-biased helping can also be linked to haplodiploidy and the easy sex ratio adjustments it allows, given that sex of the eggs is determined by fertilization (73). As mothers may be under selection to produce the more helpful sex (i.e., local resource enhancement), and the reproductive value is lower for the individuals of the more common sex ("the rarer sex effect") (74, 75) female-bias may drive a positive feedback loop between sex ratio bias and sex-biased helping (73). Given the female-bias in *N. sertifer* larval groups and populations (see also (76–78) for female-biases in other sawfly species), and that sex-biased allocation to the collective defense results in a higher level of chemical defense at the group level (Fig. 2a), this coevolutionary feedback loop seems a promising topic for future research.

Kin selection plays an important role in promoting cooperation across many different species (79–81). Similarly, our results here show that pine sawfly larvae occur in kin groups, providing indirect benefits for cooperative individuals. Within-group kinship values suggest that single matriline groups where the mother is singly mated are the rule, but occasional all male groups from unmated females, multiple mating or multiple matriline groups may occur (77). Facultative adjustment of cooperativeness based on the variation in kinship within larval groups seems absent or very weak, however, and other components of the social environment are more important drivers of within-species variation in cooperativeness in natural populations. To better understand the cause-and-effect relationships between kinship, group size, and sex ratio on the contribution to collective chemical defense, controlled experiments are required in which their effects are manipulated and measured independently of each other.

Finally, our results also provide new insights into the evolution of costly chemical defenses. Although R. A. Fisher suggested already in 1930 (74) that kin aggregations could promote the evolution of prey distastefulness, the importance of kin selection in favoring the evolution of costly chemical defenses has received much less attention (39) compared to the theoretical and empirical work on the direct benefits it provides (42, 44, 82). Our results here suggest that kin aggregations can play an important role and should be considered when examining the early evolution of costly antipredator defenses. However, in the model first proposed by Fisher (1930) and later developed by Leimar et al. (82) and Best et al. (39) the chemical defenses were stored within the prey, and there were no cues or signals indicating their distastefulness to predators. This means that predators need to sample, taste and kill the prey to identify its defensive status before learning to avoid it. Our findings suggest that for gregarious prey that excrete defensive secretions externally, the direct benefits could be sufficient to promote the evolution of these traits, as defended prey do not necessarily all die before local predators learn to avoid them.

More generally, we demonstrate that ecological drivers of cooperation can play an important role in its maintenance, and that the facultative expression of cooperativeness due to social conditions can significantly contribute to the maintenance of local variation in its expression.

Material and methods

Predation experiment with ants

The predation experiment with ants was conducted in the summer 2023. The larval families used in this experiment originated from insect cultures established from the wild individuals originating from the larval colonies collected in different parts of Finland. Separate insect cultures per population were established with no fewer than the following number of colonies collected from the wild: Alavus (50 colonies in 2020), Kaavi (10 colonies 2021 and 40 colonies 2022), Pieksämäki (40 colonies 2021 and 20 colonies 2022), Pöntiö (20 colonies 2021), Seinäjoki (10 colonies 2021). Experimental individuals

originated from F1-F3 generations depending on the starting year of the insect culture. They were reared in a laboratory at the University of Helsinki, where they were kept at room temperature and fed fresh pine needles *ad libitum*. At approximately 10 days of age, larval groups were assigned to the depletion treatment or left to retain their defensive fluid. Depleted and non-depleted larvae were identified with fluorescent elastomer tags (83, 84). These were injected subcutaneously, remain through instars, and function well in pine sawfly larvae without affecting their survival (see Fig. S1). We used either fluorescent orange or blue dye (Visible Implant Elastomer Tags by Northwest Marine Technology, Inc.) and varied the colors for depleted and non-depleted treatment across groups to avoid the color markings to confound the effects of the experimental treatments. On the same day, we simulated an attack by gently pressing them with a cotton swab on the dorsal side. If they regurgitated defense fluid, it was swiftly removed with the same cotton swab. Our supplementary data (Fig. S8) suggests that this procedure should be repeated multiple times to ensure that most of the depleted larvae can no longer deploy the defense fluid. We therefore repeated this once more, two days later. Larvae were then placed in groups of 10 on a fresh pine twig, mixing depleted and non-depleted sibling individuals to create groups with 20% or 70% depleted individuals per group. The groups were then left undisturbed until the next morning. We did not mix individuals of different families i.e., larvae were presented to ants in full-sib groups.

In the field, we placed an approximately 40 cm long pine branch into the ground on an foraging trail in the vicinity of *Formica s.str.* mound and attached the twig bearing the group of larvae to it using green coated metal wire. Such replicates were carried out at 8 different ant nests. Altogether, we had 6 replicates for the 20% treatment and 10 replicates for the 70% treatment. We always had all the treatment combinations within the same nest, except for two nests where we only had one of the two treatment groups. We recorded the survival of the larvae over a seven-hour period.

Collection of *N. sertifer* specimen for measurements of defense behaviors and social environmental factors

We collected larvae in summer 2021 from different parts of Finland (Pöntiö, Pieksämäki, Puumala, and Kaavi) with only one group taken from each tree per study site. Each group

consisted of larvae found in close proximity to one another on the same branch of the tree, clearly forming a single group. These groups were subsequently reared in a greenhouse at the University of Jyväskylä, where the temperature matched natural outdoor conditions. Larvae were fed fresh pine branches ad libitum. Lighting conditions always included both natural sunlight and some level of electric light. To measure the defensive behavior of each group, we simply randomly selected one individual per group, poked it gently (non-injuriously) on its dorsal side with the tip of a pair forceps, and counted the number of larvae that responded by regurgitating defensive fluid and/or raising their heads in response to the simulated attack. When larvae formed subgroups within each aggregation, we simulated an attack on one individual randomly within each subgroup. We measured a total of 84 groups (Kaavi: N = 8, Pöntiö: N = 20, Puumala: N = 18, Pieksämäki: N = 38). We determined the size of each larval group immediately after these measurements. We sexed the individual larvae based on the adults that eventually emerged and when an individual pupated but failed to eclose, we used cocoon size as an estimator for sex (females being larger than males (36, 54)). The sex of 70 individuals were unknown as they died before pupation. For the individual defense measurements, we simply randomly chose 5 individuals from each group and simulated an attack on each one. We also sucked any fluid secreted into a glass capillary and measured the length of the tube occupied by the liquid (using calipers accurate to 0.1 mm) and converted the measure to a volume (mm^3). In total, we measured the defense behavior of 345 individuals from 69 different groups (Kaavi: 10 groups, 50 individuals, Pöntiö: 20 groups, 100 individuals, Puumala: 19 groups, 95 individuals, Pieksämäki: 20 groups, 100 individuals).

DNA extraction & preparations for sequencing

The majority of groups had at least 10 females and males, but due to mortality and variation in original larval group sizes and sex-ratios, some contained only of 5 females or 1 male per larval group. We included only groups that contained both females and males. We followed the Qiagen kit instruction with minor modifications. After extraction, we normalized the DNA concentration to approximately 20ng/µL and double-digested it with the enzymes Mspl and MluCl. To associate each sequence with a specific 96-well plate, we ligated double-stranded adapters to each DNA strand. We then conducted a

479 test-PCR, followed by gel electrophoresis to confirm successful DNA digestion and
480 adapter ligation, which led to the exclusion of 14 samples. The remaining samples were
481 pooled into a single 96-well plate and cleaned using an AMPure substitute. Next, we
482 attached index primers with unique dual indices (UDI) to associate each sequence with
483 a specific position on the 96-well plate. All samples were then pooled into a single
484 Eppendorf tube and run on a 1.5% agarose gel for size selection (320-500 kb). We excised
485 the DNA region from the agarose gel and purified it with the Monarch DNA Gel Extraction
486 Kit (New England Biolabs). The cleaned library was quantified via qPCR using the
487 NEBNext Library Quant Kit (New England Biolabs) and sequenced on the Illumina
488 NovaSeq platform at the Institute for Molecular Medicine Finland (FIMM) of the University
489 of Helsinki. Altogether, after the filtering steps described below, we sampled from Pöntiö
490 10 groups and 189 individuals, from Pieksämäki 10 groups and 186 individuals and from
491 Puumala 9 groups and 166 individuals, and from Kaavi 8 groups and 69 individuals.

492

493 **Bioinformatics (kinship analysis):**
494 We trimmed the 4bpIMI off the raw sequencing data from the left of each forward read
495 and then demultiplexed them based on the 5bp index allowing us to identify the sample
496 name for each read. Following this, we used the ipyrad pipeline to *de novo* assemble the
497 genome for each individual. For the parameter settings, we deviated from the default
498 settings as follows: Assembly method: pairddrad, restriction overhang: AATT, GC,
499 filter_adapters: 2. We then filtered the vcf file using *vcftools* (85) with the following
500 arguments: --remove-indels, --max-alleles 2, --max-missing 0.75. Pairwise kinship, i.e.
501 consanguinity, was calculated using the R package *HapDipKinship* (61). To account for
502 haplodiploidy (sawflies have haplodiploid genetics), we treated the "heterozygous" loci
503 the same as the alternative allele.
504
505 **Bioinformatics (population genetic data):**
506 For the population genetics, we randomly chose one female per group from each
507 population, resulting in a sample size of 37 individuals, and ran the analysis for 20
508 different combinations of females. To infer the population structure, we performed a
509 clustering analysis with ADMIXTURE (86), testing for different number of

clusters/populations (*K*) ranging from 1 to 10. We determined the most suitable value for *K* by using ADMIXTURE's cross-validation (CV) procedure, comparing the CV error values for each *K*. We further checked for population differentiation and inbreeding by calculating F_{ST} and F_{IS} values using the R packages *adegenet* (87, 88) and *hierfstat* (89), respectively.

Statistical analysis

We performed all statistical analyzes using R version 4.2.2 (R Core Team, 2024). We used *glmmTMB* v1.1.10 (90) to run all Frequentist models and the *DHARMa* package v0.4.7 (91) with the *simulateResiduals* function to test and confirm the model assumptions. When we could not confirm Frequentist model assumptions we constructed Bayesian models using the *brm* function of the *brms* package, v.2.22.0 (92–94). For all models, we used the default priors provided by the *brm* function, i.e., flat or weakly informative priors for the intercept, fixed and random effects. We checked for convergence and autocorrelation using the R-hat values as well as assessed visually trace and autocorrelation plots. All R-hat values were at 1, autocorrelation plots showed rapid decay, and trace plots indicated stable patterns. There were divergent transitions for the following models: model 19 (1 transition), model 21 (1 transitions), model 23 (1 transition), model 24 (1 transition), model 36 (2 transitions), model 37 (11 transitions), model 38 (11 transitions), model 39 (13 transitions). We checked model fits using pareto k diagnostics with the *loo* package 2.8.0 (95–97). Influential points (k > 0.7) were detected for model 12 (1 count), model 15 (1 count), model 17 (2 counts), model 18 (counts), model 22 (2 counts) model 23 (3 counts), model 24 (2 counts), model 33* (1 count), model 37 (1 count), model 38 (2 counts). Model specifications of each model are listed in Table S3. We obtained beta coefficients and their standard errors from the model summary, and chi-squared values and p-values from the Anova function of the *car* package, v3.1-3 (98).

We centered all numeric explanatory variables (i.e., predictors have a mean of zero) to improve interpretability of the results. For those models that included one of the kinship types and/or male individual traits (model 5, 6, 9, 10, 11, 12, 15, 16, 17, 18, 21, 22, 23, 24,

28, 32, 30, 31, 33, 38, and 39), we excluded the Kaavi population because of low sample size (n = 2 for female-male comparisons and n = 1 for male-male comparisons, n = 2 for individual-level traits). Sex ratio was calculated of groups that consisted of at least 10 larvae of known sex. All data wrangling and visualizations were done with the *tidyverse* v2.2.0 (99) and *ggpub* v0.6.0 package (100). We constructed separate models for each kinship type (female-female, male-male, female-male), because kinship data was not available for each group for all the kinship types. As *glmmTMB* and *brm* are only able to make use of those lines that have data for all variables included in the model, using all 5 variables in the same model would have led to loss of the majority of sex ratio and group size data.

Predation experiment with ants

We constructed generalized linear mixed models with a binomial distribution and a logit link function. In the first model, we examined whether survival differs between groups with lower (20%) and higher (70%) proportions of depleted individuals. We used individual survival at the end of the experiment as the binary response variable and included the proportion of depleted individuals as a fixed factor. To account for variation between ant nests (e.g., in number of individuals, activity and behavior), we included Ant nest ID as a random factor. To account for potential effects of shared environment (i.e., larval group and/or location of larvae on the ant trail) on survival, we additionally included Group ID as random factor. In the second model, testing for individual benefits of contributing to the defense, we included an interaction term between depletion treatment and the proportion of depleted individuals as fixed factor. We also included Ant nest ID and Group ID as a random effect.

Phenotypic and kinship data

U-posture display within a group

To test how displaying the U-posture in response to a simulated attack varies with the social environment, we used generalized linear mixed models. We modeled the response variable as binomial data. We constructed separate models for each kinship type (female-female, male-male and female-male) as well as group size and sex ratio to analyze how each of them impacts whether individual larvae display the U-posture. In all

four models, we included Population ID as a random effect to account for variation among populations in the display of the U-posture. As our data was overdispersed, we used a beta-binomial distribution with a logit link function.

Deployment of defense fluid within a group

To test how cooperative behavior (deploying the fluid) varies with the social environment, we used the same approach as when analyzing the U-posture with the difference that we used a binomial distribution as data was not overdispersed.

Differences between sexes

To analyze whether females and males differ in their defense behavior, we used Bayesian modelling. One model for the measure of fluid secreted or not, and one for the measure of the volume of fluid secreted. In both cases, we used sex as a fixed factor, and Group ID was nested under Population ID as a random factor to account for potential genetic and phenotypic variation among groups and populations in cooperativeness. For the binary data (fluid deployed or not), we used a binomial distribution with logit link function. For the volume data, we used a gamma distribution because of the strongly right skewed distribution of the response variable with a log link function. Because gamma distributions do not allow for zero values, two decimals more than the precision of the data (i.e., 0.001) was added to the values of the defensive fluid in all the analyses for the volume of defensive fluid (see also below). We included also those individuals that did not regurgitate any fluid in this analysis to take into account the whole extent of the observed variation.

Individual defense and social environment

We used Bayesian modelling to analyze whether the social environment of the larvae affects the defensive behavior (deployment of fluid and its volume) when individually attacked. Due to the sex-biased defense behavior (36), we analyzed females and males separately. We constructed separate models for each kinship type (female-female, male-male and female-male) to test whether group size, sex ratio and kinship impacts the individual defense. In all 12 models, we included Population ID and Group ID as a random effect to account for potential differences among populations and groups. In the

six models analyzing whether individuals secreted the fluid or not, we used a binomial distribution with a logit link function. In the other six models analyzing the amount of fluid that individuals regurgitated, we used a gamma distribution because of the strong right skewed distribution of the response variable with a log link function. To account for allometric effects, we additionally included larval length as a fixed factor. We included also those individuals that did not regurgitate any fluid in this analysis.

Local variation of social environment and collective chemical defense behavior

To analyze whether populations differ in cooperativeness and social environmental factors, we constructed 11 models, one for each trait: proportion of individuals deploying the fluid, proportion of individuals displaying the U-posture, individual defense (fluid deployed or not) of females, individual defense (fluid deployed or not) of males, individual defense (defense fluid volume) of females, individual defense (defense fluid volume) of males, group size, sex ratio, female-female kinship, male-male kinship, female-female kinship. For traits that were measured on the individual level (i.e., fluid deployment of females and males, volume of fluid produced by females and males), Group ID was added to the models as a random effect. Larval length was additionally included when modelling the amount of defense fluid deployed. For the binary data (deployed the fluid or not), we used a binomial distribution with a logit link function. Sex ratio and group size data was approximately normally distributed and we therefore modelled it with a gaussian distribution and identity link function. Secreted defense volume was strongly right skewed and contained zeros. We therefore used a tweedie distribution with a log link function. As we failed to confirm the model assumptions of model 31 (female-female relatedness) and model 33 (female-male relatedness), we re-ran them results with a Bayesian approach which confirmed the results we received from the Frequentist approach.

Correlations between traits

To test correlations between the social characteristics of groups, we applied a Bayesian modelling approach. To test how group size changes with the sex ratio of the group, we used the proportion of males as the fixed factor and Population ID as a random factor, accounting for potential within-population similarities. To test how kinship is affected by

group size and sex ratio, we constructed a model for each kinship type with Population ID as the random factor. We used a gaussian distribution with identity link function.

Figures

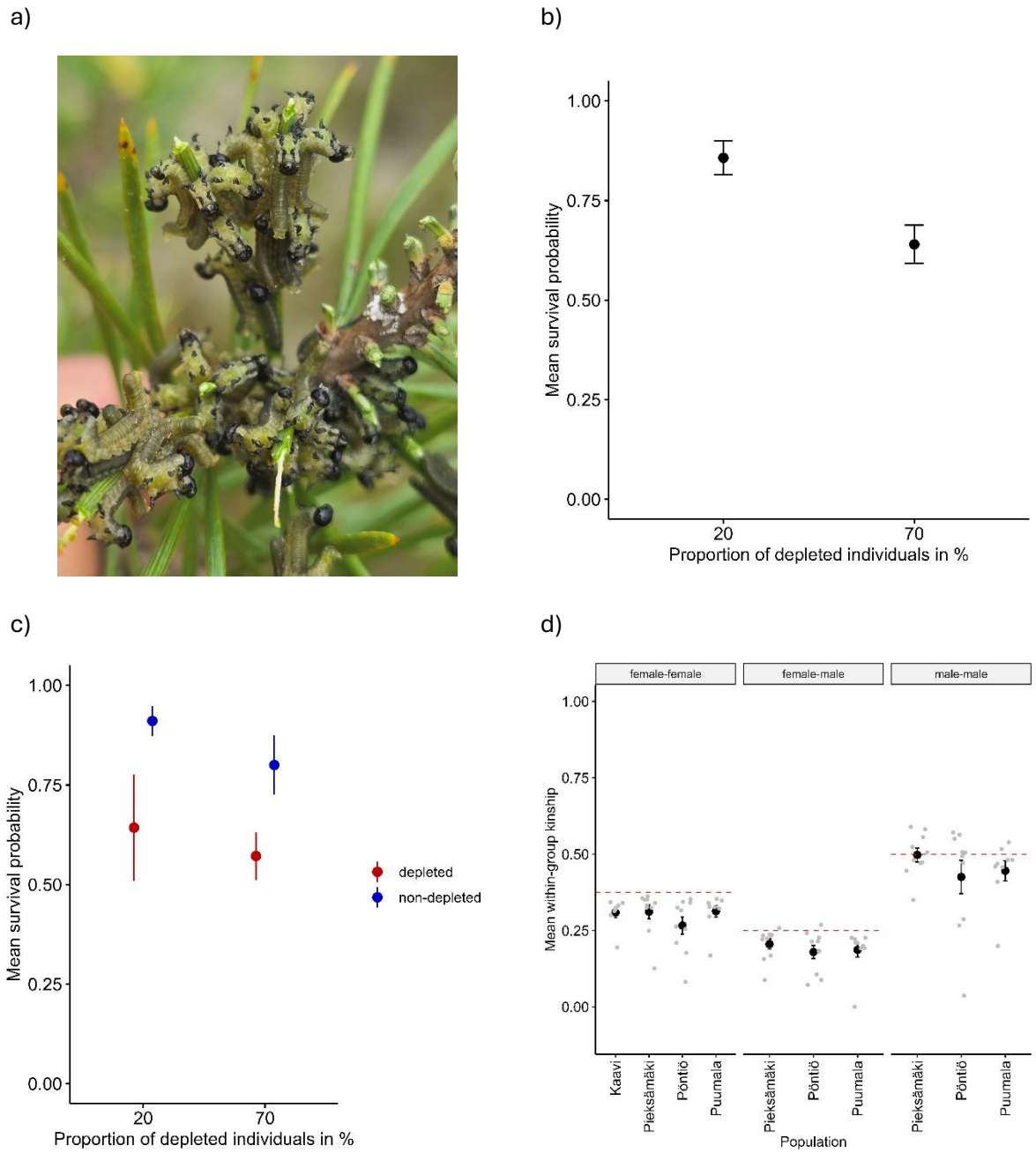

Fig. 1. a) Group of mid-instar *N. sertifer* defending in the wild. **b)** Mean survival of individuals was higher in more cooperative groups (individuals in 20% depleted group: N = 70, individuals in 70% depleted group: N = 100). **c)** Depleted individuals (red) were less likely to survive than non-depleted individuals (blue) (depleted individuals in 20% depleted group: N = 14, non-depleted individuals in 20% depleted group: N = 56, depleted individuals in 70% depleted group: N = 70, non-depleted individuals in 20% depleted group: N = 30). **d)** Within-group kinships levels for female-female, female-male, and male-male comparisons for each population. Kaavi was excluded from the female-male and male-male comparisons due to low sample size of males (n= 1, and n = 2, respectively). Error bars indicate ±1 SEM.

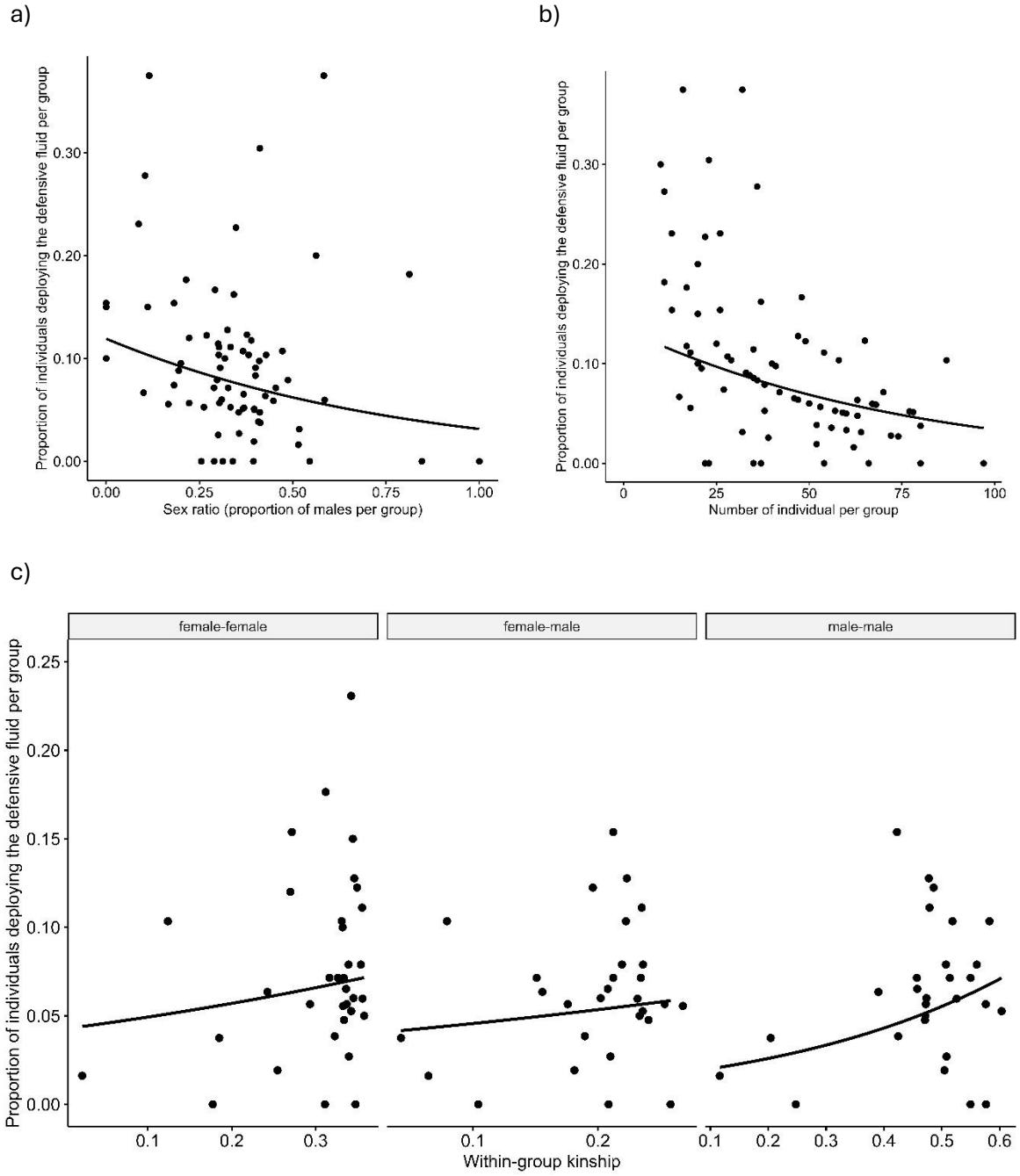

Fig. 2. Phenotypic association between the contribution to the collective defense (i.e., secretion of defensive fluid) and the a) sex ratio (proportion of males) b) size and c) kinship levels of *N. sertifer* larval groups.

Tables

Table 1. Effect of sex ratio, group size and kinship on the proportion of individuals per group performing the U-posture or deploying the fluid. Significant values in bold.

Model #	Fixed factor	Chi^2	Beta	Error	p-value
Proportion of individuals displaying the U-posture within a group					
3	Sex ratio	LR χ^2_1 = 2.002	-1.379	0.974	0.157
	Group size	LR χ^2_1 = 0.378	-0.005	0.008	0.539
4	Female-female kinship	LR χ^2_1 = 3.184	6.027	3.378	0.074
5	Male-male kinship	LR χ^2_1 = 0.238	1.008	2.067	0.626
6	Female-male kinship	LR χ^2_1 = 2.410	7.078	4.560	0.121
Proportion of individuals deploying the defense fluid within a group					
7	Sex ratio	LR χ^2_1 = 6.564	-1.428	0.557	**0.010**
	Group size	LR χ^2_1 = 13.766	-0.015	0.004	**<0.001**
8	Female-female kinship	LR χ^2_1 = 0.872	1.376	1.474	0.351
9	Male-male kinship	LR χ^2_1 = 3.878	2.737	1.205	**0.048**
10	Female-male kinship	LR χ^2_1 = 0.977	1.760	1.781	0.323

682

Table 2. Effect of sex ratio, group size, and kinship on individuals' defensive traits.

Model #	Fixed factor	Estimate	Low 95% CI	High 95% CI	Error	R-hat
Defense fluid deployed when individually attacked						
Females						
13	Sex ratio	-3.04	-7.52	1.39	2.26	1
	Group size	0.03	-0.01	0.07	0.02	1
14	Female-female kinship	6.41	-2.50	14.90	4.38	1
15	Female-male kinship	4.96	-0.76	10.61	2.87	1
Males						
16	Sex ratio	2.51	-3.34	9.27	3.21	1
	Group size	-0.0035	-0.0483	0.0419	0.0230	
17	Male-male kinship	-6.90	-32.94	12.44	11.51	1
18	Female-male kinship	-0.65	-15.75	11.21	6.73	1
Volume of defense fluid deployed when individually attacked						
Females						
19	Sex ratio	0.17	-1.23	1.58	0.72	1
	Group size	0.00	-0.01	0.01	0.01	1
	Length	0.02	-0.08	0.11	0.05	
20	Female-female kinship	2.24	-1.10	5.51	1.68	1
	Length	0.03	-0.11	0.15	0.07	
21	Female-male kinship	3.65	1.38	5.70	1.09	1
	Length	-0.04	-0.19	0.11	0.08	
Males						
22	Sex ratio	1.01	-2.95	5.41	2.12	1
	Group size	-0.02	-0.05	0.02	0.02	
	Length	-0.26	-0.56	0.05	0.15	
23	Male-male kinship	-3.21	-25.51	19.19	6.89	1
	Length	-0.22	-0.63	0.20	0.21	
24	Female-male kinship	-2.99	-14.73	6.14	5.28	1
	Length	-0.24	-0.68	0.17	0.21	

683

684

685

References

1. S. A. West, A. S. Griffin, A. Gardner, Evolutionary Explanations for Cooperation. *Current Biology* **17**, R661–R672 (2007).

2. A. Duarte, *et al.*, Social immunity of the family: parental contributions to a public good modulated by brood size. *Evol Ecol* **30**, 123–135 (2016).

3. M. K. Abdi, D. Lupi, C. Jucker, I. C. W. Hardy, Kinship effects in quasi-social parasitoids I: co-foundress number and relatedness affect suppression of dangerous hosts. *Biological Journal of the Linnean Society* **130**, 627–641 (2020).

4. M. A. Brockhurst, A. Buckling, D. Racey, A. Gardner, Resource supply and the evolution of public-goods cooperation in bacteria. *BMC Biol* **6**, 20 (2008).

5. K. Drescher, C. D. Nadell, H. A. Stone, N. S. Wingreen, B. L. Bassler, Solutions to the Public Goods Dilemma in Bacterial Biofilms. *Current Biology* **24**, 50–55 (2014).

6. R. E. Van Dijk, *et al.*, Cooperative investment in public goods is kin directed in communal nests of social birds. *Ecology Letters* **17**, 1141–1148 (2014).

7. S. A. Frank, A general model of the public goods dilemma. *J of Evolutionary Biology* **23**, 1245–1250 (2010).

8. M. Ghoul, A. S. Griffin, S. A. West, Toward an evolutionary definition of cheating. *Evolution* **68**, 318–331 (2014).

9. S. A. Levin, Public goods in relation to competition, cooperation, and spite. *Proc. Natl. Acad. Sci. U.S.A.* **111**, 10838–10845 (2014).

10. A. S. Griffin, S. A. West, A. Buckling, Cooperation and competition in pathogenic bacteria. *Nature* **430**, 1024–1027 (2004).

11. A. Ross-Gillespie, A. Gardner, S. A. West, A. S. Griffin, Frequency Dependence and Cooperation: Theory and a Test with Bacteria. *The American Naturalist* **170**, 331–342 (2007).

12. J. L. Sachs, U. G. Mueller, T. P. Wilcox, J. J. Bull, The Evolution of Cooperation. *The Quarterly Review of Biology* **79**, 135–160 (2004).

13. M. A. Brockhurst, A. Buckling, A. Gardner, Cooperation Peaks at Intermediate Disturbance. *Current Biology* **17**, 761–765 (2007).

14. L. Lehmann, L. Keller, The evolution of cooperation and altruism – a general framework and a classification of models. *J Evolution Biol* **19**, 1365–1376 (2006).

15. O. Leimar, P. Hammerstein, Cooperation for direct fitness benefits. *Phil. Trans. R. Soc. B* **365**, 2619–2626 (2010).

16. W. D. Hamilton, The genetical evolution of social behaviour. I. *Journal of Theoretical Biology* **7**, 1–16 (1964).

17. W. D. Hamilton, The genetical evolution of social behaviour. II. *Journal of Theoretical Biology* **7**, 17–52 (1964).

18. S. P. Diggle, A. S. Griffin, G. S. Campbell, S. A. West, Cooperation and conflict in quorum-sensing bacterial populations. *Nature* **450**, 411–414 (2007).

19. A. Jousset, *et al.*, Predators promote defence of rhizosphere bacterial populations by selective feeding on non-toxic cheaters. *The ISME Journal* **3**, 666–674 (2009).

20. O. M. Gilbert, K. R. Foster, N. J. Mehdiabadi, J. E. Strassmann, D. C. Queller, High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 8913–8917 (2007).

21. J. Field, E. Leadbeater, Cooperation between non-relatives in a primitively eusocial paper wasp, *Polistes dominula*. *Phil. Trans. R. Soc. B* **371**, 20150093 (2016).

22. P. Hammerstein, O. Leimar, Cooperating for direct fitness benefits. *Journal of Evolutionary Biology* **19**, 1400–1402 (2006).

23. N. Teunissen, S. A. Kingma, M. Fan, M. J. Roast, A. Peters, Context-dependent social benefits drive cooperative predator defense in a bird. *Current Biology* **31**, 4120–4126 (2021).

24. S. A. West, G. A. Cooper, M. B. Ghoul, A. S. Griffin, Ten recent insights for our understanding of cooperation. *Nat Ecol Evol* **5**, 419–430 (2021).

25. J. M. Biernaskie, S. A. West, Cooperation, clumping and the evolution of multicellularity. *Proc. R. Soc. B.* **282**, 20151075 (2015).

26. B. D. Connelly, E. L. Bruger, P. K. McKinley, C. M. Waters, Resource abundance and the critical transition to cooperation. *J of Evolutionary Biology* **30**, 750–761 (2017).

27. S. E. Kapsetaki, S. A. West, The costs and benefits of multicellular group formation in algae*. *Evolution* **73**, 1296–1308 (2019).

28. D. J. Sexton, M. Schuster, Nutrient limitation determines the fitness of cheaters in bacterial siderophore cooperation. *Nat Commun* **8**, 230 (2017).

29. M. P. Speed, G. D. Ruxton, J. Mappes, T. N. Sherratt, Why are defensive toxins so variable? An evolutionary perspective. *Biological Reviews* **87**, 874–884 (2012).

30. D. Daly, A. D. Higginson, D. Chen, G. D. Ruxton, M. P. Speed, Density-dependent investment in costly anti-predator defences: an explanation for the weak survival benefit of group living: Density-dependent defence. *Ecology Letters* **15**, 576–583 (2012).

31. A. D. Higginson, J. Delf, G. D. Ruxton, M. P. Speed, Growth and reproductive costs of larval defence in the aposematic lepidopteran Pieris brassicae: Costs of larval defence. *Journal of Animal Ecology* **80**, 384–392 (2011).

32. A. D. Higginson, G. D. Ruxton, Dynamic models allowing for flexibility in complex life histories accurately predict timing of metamorphosis and antipredator strategies of prey. *Functional Ecology* **23**, 1103–1113 (2009).

33. A. D. Higginson, G. D. Ruxton, Dynamic state-dependent modelling predicts optimal usage patterns of responsive defences. *Oecologia* **160**, 399–410 (2009).

34. M. Knapp, M. Řeřicha, D. Židlická, Physiological costs of chemical defence: repeated reflex bleeding weakens the immune system and postpones reproduction in a ladybird beetle. *Sci Rep* **10**, 9266 (2020).

35. C. Lindstedt, K. Suisto, J. Mappes, Appearance before performance? Nutritional constraints on life-history traits, but not warning signal expression in aposematic moths. *J Anim Ecol* **89**, 494–505 (2020).

36. C. Lindstedt, *et al.*, Ecological conditions alter cooperative behaviour and its costs in a chemically defended sawfly. *Proc. R. Soc. B.* **285**, 20180466 (2018).

37. L. Lindström, R. V. Alatalo, J. Mappes, Imperfect Batesian mimicry—the effects of the frequency and the distastefulness of the model. *Proc. R. Soc. Lond. B* **264**, 149–153 (1997).

38. M. Chouteau, M. Arias, M. Joron, Warning signals are under positive frequency-dependent selection in nature. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 2164–2169 (2016).

39. R. Best, G. D. Ruxton, A. Gardner, Intragroup and intragenomic conflict over chemical defense against predators. *Ecology and Evolution* **8**, 3322–3329 (2018).

40. M. P. Speed, G. D. Ruxton, M. Broom, Automimicry and the evolution of discrete prey defences. *Biological Journal of the Linnean Society* **87**, 393–402 (2006).

41. F. Müller, Ituna and Thyridia: a remarkable case of mimicry in butterflies. *Trans. Entomol. Soc. Lond* **1879**, 20–29 (1879).

42. M. Riipi, R. V. Alatalo, L. Lindström, J. Mappes, Multiple benefits of gregariousness cover detectability costs in aposematic aggregations. *Nature* **413**, 512–514 (2001).

43. B. Sillén-Tullberg, Do predators avoid groups of aposematic prey? An experimental test. *Animal Behaviour* **40**, 856–860 (1990).

44. R. V. Alatalo, J. Mappes, Tracking the evolution of warning signals. *Nature* **382**, 708–710 (1996).

45. J. Mappes, R. V. Alatalo, Effects of novelty and gregariousness in survival of aposematic prey. *Behavioral Ecology* **8**, 174–177 (1997).

46. J. Skelhorn, C. Rowe, Prey palatability influences predator learning and memory. *Animal Behaviour* **71**, 1111–1118 (2006).

47. R. S. Jones, S. Davis, M. P. Speed, Defence Cheats Can Degrade Protection of Chemically Defended Prey. *Ethology* **119**, 52–57 (2013).

48. J. Mappes, J. Tuomi, R. V. Alatalo, Do palatable prey benefit from aposematic neighbors? *Écoscience* **6**, 159–162 (1999).

49. D. F. Howard, M. S. Blum, H. M. Fales, Defense in Thrips: Forbidding Fruitiness of a Lactone. *Science* **220**, 335–336 (1983).

50. W. Tostowaryk, The effect of prey defense on the functional response of podisus modestus (hemiptera: pentatomidae) to densities of the sawflies neodiprion swainei and n. Pratti banksianae (hymenoptera: neodiprionidae). *The Canadian Entomologist* **104**, 61–69 (1972).

51. J. Skelhorn, C. Rowe, Predator avoidance learning of prey with secreted or stored defences and the evolution of insect defences. *Animal Behaviour* **72**, 827–834 (2006).

52. S. G. Codella, K. F. Raffa, "Defense Strategies of Folivorous Sawflies" in *Sawfly Adaptations to Woody Plants, M. R. Wagner, K. F. Raffa, Eds. (Academic Press, 1993), Pp. 261-294*.

53. R. A. Haack, W. J. Mattson, "Life history patters among North American tree-feeding sawflies" in *Sawfly Adaptations to Woody Plants, M. R. Wagner, K. F. Raffa, Eds. (Academic Press, 1993), Pp. 503-545*.

54. C. Lindstedt, K. Koskenpato, S. Van Meyel, Gregarious, chemically defended pine sawflies as a new model to study cooperative interactions in an ecological context. *Insect. Soc.* (2024). https://doi.org/10.1007/s00040-024-01012-y.

55. T. Eisner, J. S. Johnessee, J. Carrel, L. B. Hendry, J. Meinwald, Defensive Use by an Insect of a Plant Resin. *Science* **184**, 996–999 (1974).

56. S. G. Codella, K. F. Raffa, Individual and social components of wood ant response to conifer sawfly defence (Hymenoptera: Formicidae, Diprionidae). *Animal Behaviour* **52**, 801–811 (1996).

57. C. Lindstedt, R. K. Bagley, S. Calhim, M. Jones, C. R. Linnen, The impact of life stage and pigment source on the evolution of novel warning signal traits. *Evolution* **76**, 554–572 (2022).

58. C. Lindstedt, H. Huttunen, M. Kakko, J. Mappes, Disengtangling the evolution of weak warning signals: high detection risk and low production costs of chemical defences in gregarious pine sawfly larvae. *Evol Ecol* **25**, 1029–1046 (2011).

59. C. Lindstedt, J. Mappes, J. Päivinen, M. Varama, Effects of group size and pine defence chemicals on Diprionid sawfly survival against ant predation. *Oecologia* **150**, 519–526 (2006).

60. A. Ross-Gillespie, A. Gardner, A. Buckling, S. A. West, A. S. Griffin, Density dependence and cooperation: theory and a test with bacteria. *Evolution* **63**, 2315–2325 (2009).

61. Y.-W. Wang, C. Ané, KIMGENS: a novel method to estimate kinship in organisms with mixed haploid diploid genetic systems robust to population structure. *Bioinformatics* **38**, 3044–3050 (2022).

62. J. A. Endler, J. Mappes, Predator Mixes and the Conspicuousness of Aposematic Signals. *The American Naturalist* **163**, 532–547 (2004).

63. O. Nokelainen, J. Valkonen, C. Lindstedt, J. Mappes, Changes in predator community structure shifts the efficacy of two warning signals in A rctiid moths. *Journal of Animal Ecology* **83**, 598–605 (2014).

64. B. Rojas, J. Mappes, E. Burdfield-Steel, Multiple modalities in insect warning displays have additive effects against wild avian predators. *Behav Ecol Sociobiol* **73**, 37 (2019).

65. D. Daly, A. Higginson, D. Chen, G. Ruxton, M. Speed, Density-dependent investment in costly anti-predator defences: an explanation for the weak survival benefit of group living. *Ecology Letters* **15**, 576–583 (2012).

66. S. G. Codella, K. F. Raffa, Contributions of female oviposition patterns and larval behavior to group defense in conifer sawflies (hymenoptera: diprionidae). *Oecologia* **103**, 24–33 (1995).

67. C. Björkman, S. Larsson, R. Bommarco, Oviposition Preferences in Pine Sawflies: A Trade-Off between Larval Growth and Defence against Natural Enemies. *Oikos* **79**, 45 (1997).

68. H. H. Hattemer, W. R. Henson, F. Mergen, Some factors in the distribution of European pine sawfly egg clusters in an experimental plantation of hard pines. *Theoret. Appl. Genetics* **39**, 280–289 (1969).

69. S. Larsson, C. Björkman, R. Gref, Responses of Neodiprion sertifer (Hym., Diprionidae) larvae to variation in needle resin acid concentration in Scots pine. *Oecologia* **70**, 77–84 (1986).

70. K. Matsuura, A novel hypothesis for the origin of the sexual division of labor in termites: which sex should be soldiers? *Evol Ecol* **20**, 565–574 (2006).

71. N. G. Davies, L. Ross, A. Gardner, The ecology of sex explains patterns of helping in arthropod societies. *Ecol Lett* **19**, 862–872 (2016).

72. L. Ross, A. Gardner, N. Hardy, S. A. West, Ecology, Not the Genetics of Sex Determination, Determines Who Helps in Eusocial Populations. *Current Biology* **23**, 2383–2387 (2013).

73. A. Gardner, L. Ross, Haplodiploidy, Sex-Ratio Adjustment, and Eusociality. *The American Naturalist* **181**, E60–E67 (2013).

74. R. A. Fisher, *The genetical theory of natural selection*. (Clarendon Press, 1930).

75. A. Gardner, The rarer-sex effect. *Phil. Trans. R. Soc. B* **378**, 20210500 (2023).

76. H. C. Coppel, D. M. Benjamin, Bionomics of the Nearctic Pine-Feeding Diprionids. *Annu. Rev. Entomol.* **10**, 69–96 (1965).

77. T. P. Craig, S. Mopper, "Sex ratio variation in sawflies" in *Sawfly Adaptations to Woody Plants, M. R. Wagner, K. F. Raffa, Eds. (Academic Press, 1993), Pp. 61-92*.

78. W. R. Henson, L. C. O'Neil, F. Mergen, Natural Variation in Susceptibility of Pinus to Neodiprion Sawflies as a Basis for the Development of a Breeding Scheme for Resistant Trees. *Yale University School of Forestry Bulletin* **78** (1970).

79. L. J. Belcher, A. E. Dewar, M. Ghoul, S. A. West, Kin selection for cooperation in natural bacterial populations. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2119070119 (2022).

80. A. S. Griffin, S. A. West, Kin Discrimination and the Benefit of Helping in Cooperatively Breeding Vertebrates. *Science* **302**, 634–636 (2003).

81. T. Kay, L. Lehmann, L. Keller, Kin selection and altruism. *Current Biology* **29**, R438–R442 (2019).

82. O. Leimar, M. Enquist, B. Sillen-Tullberg, Evolutionary Stability of Aposematic Coloration and Prey Unprofitability: A Theoretical Analysis. *The American Naturalist* **128**, 469–490 (1986).

83. T. da Silva Melo, G. F. de Carvalho-Souza, M. C. L. Peres, H. C. B. Ribeiro, M. A. Dias, The utilization of visible implant fluorescent elastomers in spiders (Araneae: Theraphosidae). *Revista ibérica de aracnología* **23**, 99–101 (2013).

84. C. Moffatt, Using Visible Implant Elastomer to tag insects across life stages: a preliminary investigation with blow flies (Diptera: Calliphoridae). *Can Entomol* **145**, 466–470 (2013).

85. P. Danecek, *et al.*, The variant call format and VCFtools. *Bioinformatics* **27**, 2156–2158 (2011).

86. D. H. Alexander, J. Novembre, K. Lange, Fast model-based estimation of ancestry in unrelated individuals. *Genome Res.* **19**, 1655–1664 (2009).

87. T. Jombart, adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403–1405 (2008).

88. T. Jombart, I. Ahmed, adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics* (2011).

89. J. Goudet, Hierfstat, a package for R to compute and test hierarchical F-statistics. *Molecular ecology notes* **5**, 184–186 (2005).

90. M. Brooks E., *et al.*, glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* **9**, 378 (2017).

91. F. Hartig, DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.4.7. (2024). Deposited 2024.

92. P.-C. Bürkner, brms: An R Package for Bayesian Multilevel Models Using Stan. *Journal of Statistical Software* **80**, 1–28 (2017).

93. P.-C. Bürkner, Advanced Bayesian Multilevel Modeling with the R Package brms. *The R Journal* **10**, 395–411 (2018).

94. P.-C. Bürkner, Bayesian item response modeling in R with brms and Stan. *Journal of statistical software* **100**, 1–54 (2021).

95. A. Vehtari, *et al.*, loo: Efficient leave-one-out cross-validation and WAIC for Bayesian models. (2024). Deposited 2024.

96. A. Vehtari, A. Gelman, J. Gabry, Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Stat Comput* **27**, 1413–1432 (2017).

97. Y. Yao, A. Vehtari, D. Simpson, A. Gelman, Using Stacking to Average Bayesian Predictive Distributions (with Discussion). *Bayesian Anal.* **13** (2018).

98. J. Fox, S. Weisberg, *An R companion to applied regression* (Sage publications, 2018).

99. H. Wickham, *et al.*, Welcome to the Tidyverse. *JOSS* **4**, 1686 (2019).

100. A. Kassambara, ggpubr:'ggplot2'based publication ready plots. (2018). Deposited 2018.