

# Brood parasites that care: alternative nesting tactics in a subsocial wasp

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## **Abstract**

Hosts and brood parasites are a classic example of conflict. Parasites typically provide no offspring care after laying eggs, imposing costs on hosts. Female subsocial wasps, *Ammophila pubescens*, alternated between initiating their own nests and an ‘intruder’ tactic of replacing eggs in nests of unrelated conspecifics. Hosts could respond by substituting new eggs of their own, with up to eight reciprocal replacements.

Remarkably, intruders usually provisioned offspring in host nests, often alongside hosts. We used field data to investigate why intruders provision and to understand the basis of interactions. We found that intruders could not increase their fitness payoffs by using the typical brood parasite tactic of not provisioning offspring. Intruders using the typical tactic would benefit when hosts provisioned in their stead, but their offspring would starve when hosts failed to provision. Although some hosts obtained positive payoffs when intruders mistakenly provisioned their offspring, on average utilizing a conspecific nest represents parasitism: hosts pay costs while intruders benefit. Both females used the same tactic of egg replacement, but intruders more often laid the final egg. Selection should favour better discrimination of offspring, which could lead to repeated cycles of costly egg replacement.

## Introduction

Whenever parents provide costly parental care, selection is likely to favour parasites that exploit that care. Intraspecific brood parasitism, where females lay eggs in the nests of conspecifics, is a widespread alternative tactic in taxa that provide care (Tallamy, 1985; Van den Berghe, 1988; Field, 1992; Yom-Tov, 2001). Brood parasitism has been best studied in birds (Lyon & Eadie 2008; Andersson et al. 2019), but is widespread among the more than 25,000 species of subsocial wasps and bees, which have been little studied (Field 1992). In birds, parasites typically add eggs to the host clutch (Lyon & Eadie 2008; Andersson et al. 2019). In wasps and bees, where only one offspring is reared in each nest cell, brood parasites replace the single host egg with an egg of their own (Field, 1992).

Conspecific brood parasites, like interspecific parasites such as the common avian cuckoo (*Cuculus canorus*), normally leave hosts to pay the usually substantial costs of caring for their offspring (Field, 1992; Davies, 2000; Loeb, 2003): avoiding these costs is generally seen as the principal benefit of parasitism. Here, we use field data to investigate interactions between females of the subsocial ammophiline wasp *Ammophila pubescens* (Curtis). Unexpectedly, we find that after replacing host eggs, conspecific intruders often provision the offspring, frequently alongside hosts (Field et al., 2007). We use our data to test hypotheses that could explain provisioning by intruders and to understand the basis of interactions. First, we investigate whether provisioning is maladaptive: would intruders obtain larger payoffs if they followed the classic brood parasitic strategy of not provisioning? We then explore the possibility that interactions are actually cooperative. We use genetic markers to determine whether hosts and intruders obtain indirect fitness benefits through being

related, as in some insects and birds (Loeb, 2003; Zink, 2003; Andersson et al. 2019). We use fitness estimates to evaluate whether one party is parasitizing the other: do hosts pay costs when intruders utilize their nests, and do intruders benefit, or vice-versa? Finally, because the outcomes of interactions and the costs paid by each party are unusually transparent in our study system, we can examine quantitatively the extent to which hosts or intruders optimize the outcome of interactions.

## Methods

### *Study system*

*A. pubescens* is a univoltine digger wasp in which a female produces a series of spatially separate nest burrows. Each contains a single offspring which the mother provisions progressively with paralysed insect prey (lepidopteran caterpillars). In a typical nesting sequence (Baerends, 1941; Field et al., 2007, 2018), on Day 1 the mother digs a short burrow in the soil then closes it temporarily with a plug of small stones and soil. Later that day, or on Day 2, she places one prey item in the burrow, glueing a single egg onto it. After an interval of typically 2-3 days, she re-enters the burrow, here termed an ‘assessment visit’. If her offspring is healthy, the mother adds several ( $3 \pm 0.09$ , range 0-8 in this study) further prey items singly over a period of 1-7 days. At each visit, she removes then replaces the closure plug. Having completed provisioning, she closes the burrow permanently and her larva consumes remaining prey and pupates. Mothers usually have more than one nest active simultaneously.

Before provisioning is complete, a nest may be entered by a conspecific ‘intruder’ that typically replaces the host egg and first prey item with ones of her own (Fig. 1). When the host next visits the nest, she ejects the intruder’s offspring (40% of occasions), later usually replacing it with a new egg and prey item of her own, or shows no behavioural response (60%; Fig. 1; Field et al., 2018).

### *Study site and field methods*

Field techniques have been described previously (Field et al., 2018). Fieldwork was conducted during 2012-2013 at Witley Common, Surrey, UK (51°09'04"N 0°40'53"W) on a 10 x 3 m section of a blind-ended sandy path. All females were marked with unique colour combinations of paint dots, their wing lengths measured, and an antennal sample taken. Once marking was complete, a few days after nesting had started each year, the nesting area was observed continuously by 2-3 people simultaneously from 09.00-19.00 on all days with weather suitable for wasp activity during July 23-August 18, 2012 (30 females, 69 nests) and 5-30 July, 2013 (57 females, 359 nests). Data from three additional unmanipulated nests were obtained in 2014 when the focus was a manipulative study not reported here.

Whenever a female dug a new burrow, we marked the entrance and recorded all subsequent activities. When a prey caterpillar was provisioned, we recorded how long the female spent inside: >25sec for oviposition on the first prey item; <10 sec for subsequent prey. At the end of each observation period, we mapped nest entrances and excavated all burrows where intruders had ejected offspring (hereafter referred to as 'multifemale nests'), recording presence/absence of an offspring, normally a cocoon. Cocoons were opened and prepupae placed in 100% ethanol after being weighed. We excavated an equal-sized sample of nests where ejection had not been observed (hereafter referred to as 'single-female nests'), matched with the dates of egg-laying at multifemale nests.

### *Among-female differences in tendency to use the intruder tactic*

Individual females act as hosts at some nests but as intruders at others. Thus, when we talk about ‘intruders’ or ‘hosts’ below, we are referring to behavioural tactics that all females alternate between, rather than to different sets of females. We used our larger 2013 dataset to test for individual repeatability in the tendency to use the intruder tactic (*rptR* package; Stoffel et al., 2017)

### *Provisioning at multifemale nests*

At nests where both host and intruder remained alive throughout offspring provisioning, we tested whether different categories of female (intruders versus hosts; mothers versus non-mothers of offspring) were equally likely to provision after the egg hatched (Fisher’s Exact tests). When females did provision, we compared the number of prey that they provided (Poisson GLMs). We also tested whether the number of times the two females met at the nest before the second prey item was provisioned affected whether both females subsequently provisioned (Binomial GLM with relatedness between the two females as a covariate). Finally, we used a linear model to compare fully fed (prepupal) offspring weight at nests provisioned jointly after egg hatch versus nests provisioned by one female alone (host or intruder alone, or single females), with offspring sex as a covariate.

### *Calculating payoffs*

We calculated a Payoff Score to estimate the approximate effect that each reproductive attempt in the role of host, intruder or single female had on the focal individual's lifetime reproductive success (Field et al., 2007, 2018). Payoff Scores combined benefits obtained (whether the female produced a successful offspring) with investment costs incurred: the number of eggs the focal female laid, the number of prey she provisioned and burrows she constructed. The relative magnitudes of these three facets of investment have been estimated in *A. pubescens* (Field et al., 2007). A negative payoff score indicated that a reproductive attempt was expected to reduce the focal female's lifetime reproductive success in comparison with single-female nesting: she invested resources but failed to produce an offspring, or produced an offspring but invested more than was required to produce an offspring at single-female nests. A positive score indicated that the focal female produced an offspring with less investment than single-female nesting, thus increasing her lifetime reproductive success through the interaction. For example, capturing an initial prey item and laying an egg on it represents 20% of the costs of producing an offspring at single-female nests. An intruder that successfully substitutes her egg for a host egg but avoids the costs of burrow-digging and further provisioning therefore scores 0.8. A similar intruder whose egg is then replaced by the host, however, scores -0.2. The host in the first example fails to produce an offspring and, assuming she carries out the same amount of provisioning as the average single female, scores -1. Further details and examples are in Appendix A



## *Payoffs from hypothetical alternative tactics*

### *No-Return tactic*

To test how intruders might perform if they behaved like classic brood parasites and did not return to provision offspring after laying eggs, we calculated a hypothetical 'No-Return' payoff score for every intruder that laid an egg. Each No-Return female was assumed to capture a single prey item, lay one egg on it then abandon the host nest. The payoff she obtained then depended on the behaviour of the observed host. In nine out of 52 instances, the observed host had died before the offspring was 3 days old (when it would normally start to receive further provisions). In these cases the No-Return female's offspring was assumed to starve and she obtained a payoff of -0.2 (as above and see Table A1). At nests where the observed host was alive, however, the host was assumed to carry out all the provisioning, and the No-Return female obtained one of two payoffs. If the observed host had failed to replace the intruder's egg and the offspring had survived to the pupal stage, the No-Return female's payoff was 0.8 (as above). Alternatively, if the observed intruder's first egg had failed to reach the pupal stage, nearly always because the host had replaced it, the No-Return female scored -0.2 (this included three instances where observed intruders had replaced hosts' second eggs. No Return females were assumed to be unable to do this).

### *Conditional Provision*

The second hypothetical tactic was a conditional one. A possible cue indicating the host's availability to provision was whether the nest contained an offspring when the intruder first entered. Some empty nests were simply awaiting provisioning, but 56% of hosts at empty nests were dead, compared with only 20% of hosts at nests containing offspring (n=52; Fisher's Exact P=0.016). Conditional-Provision females were assumed to target the No-Return tactic at nests where the host was more likely to be available. They used No-Return when the nest contained an offspring when first entered but were assumed to behave as observed (obtaining the observed payoff) when nests were initially empty.

#### *Mistaken provisioning*

Instead of ejecting the offspring found when they first entered conspecific nests, some females re-closed the nest and later provisioned it, here termed 'mistaken provisioning'. To investigate whether mistaken provisioning might represent nest orientation errors, we tested whether the distance between the conspecific nest and the nest of her own where she had last been active was shorter when an intruder mistakenly provisioned than when she ejected the host offspring (Binomial GLMM). Additional covariates were host offspring age (hours) and genetic relatedness between host and intruder.

#### *Genotyping and Genetic Relatedness*

We extracted DNA from immature offspring and adult antennal samples then amplified 17 microsatellite loci (5-28 alleles per locus: Field et al. 2018). Tests for

deviation from Hardy–Weinberg proportions and linkage disequilibrium were performed using GenAlEx 6.52 (Peakall & Smouse, 2012) after removing all but one randomly chosen female from any groups of close relatives ( $r > 0.5$ ), as recommended by Wang (2018). Pair-wise relatedness was estimated using Relatedness 5.0.8 (Queller & Goodnight, 1989) excluding three loci (C07, F06 and D10) that deviated from Hardy–Weinberg proportions ( $P < 0.05$ ) in more than one year. We also determined which female active at each multifemale nest matched the offspring genotype and checked whether offspring from single female nests matched the adult nest owner. Matching occurred when an offspring shared at least one allele at every locus with an adult female. Because hosts and intruders were normally unrelated (see Results), offspring assignment was straightforward. Immature offspring were sexed by recording whether only one allele amplified at each locus (haploid male) or two different alleles amplified at two or more loci (diploid female; no offspring was heterozygous at only a single locus).

To test whether relatedness between interactants differed from random expectation, we used a Monte Carlo method. Our observed value was the mean relatedness between 88 pairs of females, each pair comprising a nest owner and an intruder that entered her nest in 2013 (the year with by far the most data). For each observed pair, we picked a pair randomly (with replacement) from the females alive on the observed date. We then calculated mean relatedness for these 88 randomized pairs. We repeated this procedure 1000 times and calculated the proportion of randomized means that was more extreme than the observed mean. We also tested whether relatedness predicted whether a female entering a conspecific's nest ejected the host offspring or simply closed the nest without ejecting (Binomial GLM).

*Does utilizing a conspecific nest represent parasitism?*

If utilizing a conspecific nest represents parasitism, we expect individuals using the host tactic to lose and those using the intruder tactic to gain through the interaction. Alternatively, if interactions are cooperative, we expect both parties to gain. To test how interactions affect hosts, we compared payoff scores for hosts versus single females (Wilcoxon tests). To test how interactions affect intruders, we compared their payoff scores with those of females that initiated their own nests (single females and hosts combined). To be conservative, we counted mistaken provisioners as intruders, and owners of mistakenly provisioned nests as hosts.

*How successfully do hosts and intruders optimize interactions?*

We investigated the extent to which hosts and intruders optimize the outcome once an intruder has laid an egg (thus excluding mistaken provisioners). Some costs are unavoidable: the host has already constructed the nest when an intruder arrives; and to produce an offspring, neither party can avoid capturing a prey item and laying an egg on it. However, successfully laying the final egg is contestable, through rejecting conspecific eggs and re-ovipositing; and each party could manipulate the other into paying the provisioning costs. Each party's optimum would be to produce the final offspring but do none of the post-hatch provisioning, thus scoring +1 in terms of our payoff score (ignoring the unavoidable costs). The worst outcome would be to carry out all of the provisioning but not produce an offspring, scoring -0.22 (the mean provisioning investment to produce an offspring at single female nests, excluding egg-bearing prey). We plotted mean payoffs for hosts and intruders in this window of

conflict, restricting the analysis to interactions where both females remained alive so that each was available to be manipulated by the other.

### *Data analysis*

Data were analysed using R version 4.0.3 (R Core Team, 2020). Means $\pm$ SE are reported, and statistical significance was assessed at the  $P=0.05$  level. Sample sizes varied between analyses, but we included all possible data in every analysis. For example, genetic relatedness could not be calculated for a few interactions where one of the interactants was not genotyped. For datasets initially analysed using GLMMs or LMMs, nest owner identity was a random effect, with year and date of egg-laying, where date zero was the first day of nesting, as covariates along with the explanatory variables mentioned above. In some instances where there were singular fit errors, probably reflecting the low level of replication for the random effect, we instead report results from GLMs. The significance of fixed effects was assessed via stepwise backwards elimination until further removals led to a significant decrease in explanatory power. Significance levels for covariates are reported only if they were retained in the final model. As a check, we also carried out Wilcoxon tests using just the main variable of interest. In all analyses, GLMMs, GLMs and Wilcoxon tests produced the same qualitative results in terms of significance, and  $P$ -values were similar. Model assumptions were checked by inspecting plots of residuals and with binary response variables, Hosmer and Lemeshow goodness of fit tests. Payoff Scores were strongly bimodally distributed (see Fig. 3 below). When the response variable was the Payoff Score, we used Wilcoxon tests (unpaired unless indicated) and our data points were means (across interactions) for individual females.

## Results

During our two-year study, 15% of 395 provisioned nests were utilized for reproduction by females using the intruder tactic and/or had offspring ejected by intruders. Except in one instance, intruders were marked wasps that also had their own nests within our observation area (cf Pfennig & Reeve, 1989). Sometimes a nest was empty when an intruder first entered, but 70% contained an offspring which the intruder typically ejected, along with the prey item bearing it. The intruder usually (84%) then returned to lay her own egg, almost always on a new prey item that she brought. The host could then accept or reject the intruder's offspring, in the latter case usually then replacing it with a second egg of her own (Fig. 1). The intruder, in turn, could accept or reject the host's second offspring. Exceptionally, up to 8 eggs were successively replaced, with a few nests utilized by more than one intruder. Only one offspring ever reached pupation in each nest, referred to below as the 'final' offspring.

There was no evidence that some females specialized on the intruder tactic. Every female that made multiple nesting attempts (whether as host or intruder) constructed and provisioned at least one nest of her own, and we detected no among-female differences in the tendency to behave as an intruder (repeatability= $0.019 \pm 0.022$ ,  $P=0.19$ , 48 females and 425 nesting attempts). Date and female size did not have significant fixed effects on the tendency to use the intruder tactic, and including them in the model did not alter repeatability.

### *Provisioning by intruders*

After laying an egg in a conspecific's nest, intruders usually (72%, n=29) provisioned the final offspring further. In fact, intruders were no less likely to provision than hosts (Fisher's Exact  $P=1.0$ ). Of 25 nests where both females remained alive throughout provisioning, 7 were provisioned by just the intruder, 10 were provisioned jointly by both females, while only 8 were provisioned by the host alone. Whether a female provisioned was also independent of whether she was the mother of the final offspring: 19/25 mothers and 17/26 non-mothers provisioned (Fisher's Exact  $P=0.55$ ). The proportion of living females observed provisioning was smaller across all multifemale nests (76%) than single-female nests (93%, binomial GLMM,  $P=0.0008$ ). At multifemale nests, females were more likely to provision if the other female did not (including nests where the other female had died: hosts - Fisher's Exact  $P=0.02$ , n=29; intruders -  $P=0.03$ , n=29). Host and intruder met at only 33% of nests where both females remained alive. There was usually aggression, but the number of meetings did not influence whether both then provisioned (Binomial GLM,  $X^2_1=0.6$ ,  $P=0.44$ , n=25).

When they did provision, intruders provided the same number of prey as hosts, and mothers provided the same number of prey as non-mothers (hosts:  $2.68 \pm 0.34$  prey, n=22; intruders:  $3.25 \pm 0.35$  SE, n=20, Poisson GLM,  $X^2_1=1.14$ ,  $P=0.28$ ; mothers:  $3.33 \pm 0.37$  prey, n=21; non-mothers:  $2.57 \pm 0.31$ , n=22, Poisson GLM,  $X^2_1=2.07$ ,  $P=0.15$ ). Mothers at single female nests provided  $2.87 \pm 0.09$  prey (n=273). Offspring at joint-provisioned nests (n=18) were significantly heavier at the end of feeding than offspring with only a single provisioner (n=67, LMER,  $X^2_1=19.5$ ,  $P=0.000001$ ; offspring sex  $X^2_1=3.8$ ,  $P=0.05$ ; Figure S1).

*The classic No-Return tactic performs no better than the observed tactic*

Our first hypothetical tactic for intruders, No-Return, performed no better than the observed tactic (Fig. 2). Approximately 40% of intruders would have made small-to-moderate gains by following the No-Return tactic through hosts provisioning in their stead (Fig. 2B). However, these gains would be cancelled out by larger losses at nests where the host was dead so that unprovisioned intruder offspring would starve (Fig. 2B). No-Return females would also be unable to carry out further egg replacements at a few nests where hosts were observed to eject intruders' first eggs. Overall, payoffs from the No-Return tactic did not differ from observed payoffs (paired Wilcoxon test,  $V=286$ ,  $P=0.64$ ). The second hypothetical tactic, Conditional-Provision, where intruders provision only if the host nest is empty when first discovered, performed slightly better than the observed tactic (Fig. 2A,C; paired Wilcoxon test,  $V=111$ ,  $P=0.04$ ).

*Mistaken provisioning*

There were 19 instances (two at the same nest) of 'mistaken provisioning', where an intruder provisioned a larva in a conspecific's nest despite never having laid an egg there. Thirteen of these offspring were also provisioned by hosts, and in all cases genotyping revealed that the offspring belonged to the host. The distance between the conspecific nest and the nest of her own where she had last been active was shorter when an intruder mistakenly provisioned ( $152\pm 32$  cm, range 1-348) than when she ejected the host offspring ( $240\pm 34$  cm, range 18-552; Binomial GLMM:  $\chi^2_1=4.9$ ,  $n=33$ ,  $P=0.028$ ). There was also a marginal tendency for mistakenly provisioned



offspring to be older than ejected offspring ( $X^2_1=3.4$ ,  $P=0.067$ ; genetic relatedness and date both  $P>0.1$ ). Our data were inconsistent with mistaken provisioning being a result of observer error (Supplementary Material).

### *Genetic relatedness*

Relatedness between nest owners and intruders was close to zero ( $-0.0025 \pm 0.018$ ,  $n=88$  pairs) and did not differ significantly from the Monte Carlo simulation (35% of 1000 randomized values were larger than the observed value). When entering a conspecific's nest, an intruder's relatedness to the nest owner did not differ when she ejected the offspring ( $n=39$ ,  $r=0.008 \pm 0.03$ ) versus when she did not ( $n=24$ ,  $r=-0.005 \pm 0.03$ ;  $X^2_1=0.10$ ,  $P=0.75$ ; or  $X^2_1=0.19$ ,  $n=48$ ,  $P=0.67$  excluding mistaken provisioners). Relatedness was greater than 0.5 in only three instances. Mistaken provisioners were unrelated to their hosts ( $r=-0.022 \pm 0.026$ ,  $n=16$ , range -0.2 to 0.18).

### *Are intruders parasites?*

On average, hosts had significantly lower payoffs when their nests were utilized by intruders, forfeiting approximately half an offspring ( $n=46$  females that acted as hosts versus  $n=66$  that had unparasitized nests; Wilcoxon test,  $W=503$ ,  $P=1.9 \times 10^{-9}$ ). Hosts obtained positive payoffs when intruders mistakenly provisioned their offspring, or occasionally when they successfully replaced an intruder's egg and the intruder helped to provision (Fig. 3). However, replacement eggs represent a fitness cost for hosts (Field et al., 2007), and hosts were relatively unsuccessful at replacing intruders' eggs so that they were the mothers of final offspring in only 37% of cases

(or 20% excluding hosts of mistaken provisioners). In contrast, intruders never had to pay costs of nest construction and usually did produce a successful offspring (49% of cases or 67% excluding mistaken provisioners). On average, they gained one quarter of an offspring by avoiding parental care costs (or 40% of an offspring excluding mistaken provisioners). Females thus obtained significantly higher payoffs as intruders than when they initiated their own nests (Fig. 3,  $n=41$  females that acted as intruders versus  $n=73$  single females/hosts combined, Wilcoxon test,  $W=2240$ ,  $P=0.00001$ ).

#### *Intruders optimize interactions better than hosts*

In the window of conflict, hosts lie only 22% of the way towards the optimum from the worst-case scenario where they carry out all the post-hatch provisioning but fail to produce an offspring (Fig. 4A: payoff  $0.046 \pm 0.07$ ,  $n=26$ , interquartile range 0.22). In contrast, intruders lie 65% of the way towards the optimum, scoring  $0.57 \pm 0.07$  ( $n=28$ , interquartile range 0.95; Wilcoxon test,  $W=114.5$ ,  $P=0.001$ ). How are intruders achieving this? If we subtract observed provisioning costs from payoff scores, thus assuming that the other party is manipulated into doing all the provisioning, intruders are still doing much better than hosts (Fig. 4B; Wilcoxon test,  $W=135$ ,  $P=0.002$ ). However, if we retain observed provisioning costs, but assume that the focal party (host or intruder) is always the one that produces the final offspring, the difference almost disappears (Fig. 4C; Wilcoxon test,  $W=206.5$ ,  $P=0.21$ ). Thus, intruders are closer to the optimum mainly because they are more likely to produce the final offspring.

## Discussion

Individual *A. pubescens* females constructed and provisioned their own nests, but also used an alternative 'intruder' tactic. All females used both tactics, and we found no evidence of variation in the tendency to behave as an intruder. The outcome of intrusion varied: sometimes the final offspring was the host's, but more often it was the intruder's, and provisioning was carried out by one or both females. On average, however, intruders were parasites. Although some hosts benefitted when intruders mistakenly provisioned their nests, or occasionally when they successfully replaced an intruder's egg and the intruder provisioned their offspring, the negative payoffs that resulted when intruders laid the final eggs were larger and more frequent (Fig. 3). Intruders, on the other hand, benefitted by obtaining free nests, often together with help provisioning their offspring. Females appear to come across conspecific nests opportunistically, while searching for sites to dig new burrows of their own. Parasitism is then effectively a conditional tactic (Field 1989a, 1992), although the higher payoff through utilizing conspecific nests suggests that searching specifically for them could be favoured by selection under some conditions (e.g. high density).

Intruders also appear to be parasites in two of the three other digger wasps where payoffs have been estimated (Brockmann & Dawkins, 1979; Field 1989a, b; Field & Foster, 1995). In *Cerceris arenaria* and *Sphex ichneumoneus*, intruders start provisioning host nests, along with hosts if present. Joint-provisioning is less

remarkable than in *Ammophila*: the egg is laid at the end of provisioning rather than on the first prey item, so that either female has the potential to lay the final egg. Calculations suggest that intruding *C. arenaria* females are effectively parasites, and indeed they prefer to utilize nests that have owners (Field 1994; Field & Foster, 1995). In *S. ichneumoneus*, however, intruders utilize mainly unoccupied nests, and both parties appear to lose out when there is a co-occupation (Brockmann & Dawkins, 1979).

These previous studies were carried out before genetic markers became widely available, so that relatedness was not measured and parentage was assigned behaviourally. All three compared different tactics entirely in terms of offspring produced per unit time. This could lead to misinterpretation if parental care has physiological costs that are proportionately larger than time costs suggest. For example, digging a burrow may be disproportionately costly energetically in *A. pubescens* (Field et al. 2007), so that the benefits of utilizing even an occupied nest might be underestimated based on time costs alone. In addition, time assumed to have been spent on one component of care (e.g. provisioning) may in reality have been spent partly on another (e.g. maturing eggs). The approach in this paper, where costs are based on direct estimates of parental investment, avoids many of these pitfalls.

### *Provisioning of host nests by intruders*

After laying eggs in conspecific nests, most *A. pubescens* intruders exhibited the seemingly counterintuitive behaviour of provisioning the offspring. We found that if intruders instead used the classic brood parasite tactic of not returning to host nests following oviposition, and even if living hosts then took on all remaining

provisioning, the average payoff for intruders would not increase (Fig. 2). This reflects two features of wasp ecology. The first is uncertainty over whether the host is available to resume provisioning after the egg hatches: nearly 20% of hosts were dead by then. Intruders could not reliably detect living hosts by meeting them at the nest, and intruder offspring would starve at nests where hosts had died. This contrasts with birds, where adult mortality rates are likely to be lower and hosts are frequently present on nests. A second key feature is that provisioning is only one facet of parental investment (Rosenheim et al., 1996; Visser & Lessells, 2001; Field et al., 2007). At nests where their offspring starved, intruders following the classic tactic would waste eggs and miss opportunities to utilize free nests, which are costly to produce (Field et al., 2007). These losses tended to be larger than the potential gains at nests where hosts took over provisioning (Fig. 2B).

The second hypothetical tactic we tested, Conditional Provision, involved intruders targetting the No-Return tactic at hosts that were more likely to be alive. This performed slightly better than the observed tactic, but the difference was small (Fig. 1A). We can of course imagine more sophisticated conditional tactics. For example, intruders might repeatedly visit nests to ascertain whether hosts were provisioning. However, visits could attract interspecific parasites (Rosenheim, 1987; Field & Brace, 2004) and conditional tactics could be cognitively demanding, especially given that intruders must simultaneously schedule provisioning at multiple active nests of their own.

Although a female's provisioning effort was independent of whether she was the offspring's mother, females were more likely to provision if the other female did not. This suggests that females have some, albeit imperfect, information about each others' presence. When both females provisioned, offspring were significantly heavier

than usual. This could be maladaptive if the relationship between total provisions and offspring fitness is convex, so that extra food would be better utilized feeding extra offspring (Smith & Fretwell, 1974).

### *Mistaken provisioning*

Mistaken provisioning, where females fed offspring at nests where they had never laid eggs, was surprisingly frequent. At least some mistakes may have resulted from orientation errors. Females are unable to discriminate the offspring of conspecifics using cues intrinsic to the offspring themselves (Field et al., 2018), and mistakes tended to happen when the host nest was closer to the intruder's own previous nest. Mistakes also tended to occur when the host offspring was older (always a larva rather than an egg). Females may be less likely to eject or abandon older, more valuable offspring that might be their own. Alternatively, perhaps larvae can sometimes manipulate intruders into feeding rather than ejecting them. Mistaken provisioning might then effectively represent a cost of entering conspecific nests.

### *Genetic relatedness*

In some taxa, intraspecific egg-dumping may represent altruism or mutualism, if hosts and dumpers are related or clutches benefit from augmentation (Tallamy, 1985; Loeb, 2003; Zink, 2003; Andersson et al. 2019). In *A. pubescens*, interactions are not driven by indirect fitness benefits: interactants are unrelated, and relatedness did not predict offspring ejection. Intruders replace the host's single egg rather than adding eggs to a

clutch. The maximum possible relatedness between a host and an intruder's offspring is 0.375 (niece/nephew), so that hosts always lose out if their eggs are replaced.

*Intruders optimize interactions better than hosts*

Once an intruder has laid an egg, females might manipulate each other into provisioning, and use egg replacement to ensure that they produce the final offspring. Intruders are clearly more successful in this sense (Fig. 4). They provision slightly (but not significantly) less than hosts after the final egg hatches, but more importantly, they are three times more likely than hosts to produce the final offspring. This is despite the fact that hosts and intruders use the same tactic of egg replacement, and reflects an informational asymmetry. When an intruder first discovers a host nest, she can safely eject the offspring: it cannot be her own. In contrast, the host must rely on less clearcut cues and risks mistakenly ejecting her own egg. Conspecific eggs that hosts fail to eject represent immediate successes for intruders. This asymmetry has a larger effect when the overall ejection rate is low. For example, the observed 40% host ejection rate for the first intruder egg (Field et al., 2018) means that 60% of hosts are deceived in the first round of ejection.

In studies of avian brood parasitism, one emphasis has been on traits that increase a host's ability to detect and reject parasite offspring, via finer discrimination ability or through hosts laying more distinctive eggs (Lyon & Eadie, 2008). In the same way, mutations might increase an *Ammophila* host's ability to discriminate and eject intruders' offspring (Field et al., 2018). However, such mutations would also increase their bearer's success as intruders, through discriminating replacement host eggs. As they spread in the *Ammophila* population, mutations would result in more

and more costly reciprocal egg replacements (Table 1), weakening selection favouring parasitism itself, something that could be explored in future modelling. This differs from most avian brood parasitism, where repeated reciprocal egg replacements do not occur (but see De Mársico et al. 2013). A comparable scenario, however, might be reciprocal egg-tossing in some communally-nesting birds (Koenig et al., 1995; Riehl, 2010). In Greater Anis (*Crotophaga major*), for example, where multiple, unrelated pairs breed in the same nest, each bird ejects eggs laid asynchronously or before she has laid herself (Riehl, 2010). Discrimination based on egg phenotype, which could destabilize communal nesting, appears to be absent.

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### **Statement of Authorship**



J.F. conceived the ideas and, with W.A.F., developed the methodology and collected the data. C.S. and J.F. analyzed and visualized the data. J.F. wrote the first draft of the manuscript and, with C.S. and W.A.F., edited it. J.F. obtained funding for this work and supervised it. All authors contributed significantly to the article, which they approved.

### **Data and Code Accessibility Statement**

Data sets and R analysis scripts have been deposited in the Dryad Digital Repository (<https://datadryad.org/stash/dataset/doi:10.5061/dryad.rn8pk0pcb>).

## **Appendix A**

### **Calculation of payoff scores**

To understand how payoff scores were calculated (Table A1), first note that a single (unparasitized) female in our study population must construct an average of 1.2 nests, lay 1.3 eggs and provision 4.2 prey in order to produce one successful offspring (n=371 nests; J. Field and W.A. Foster, unpublished data). Previous experimental manipulations, where females in different treatments were allowed to carry out only some aspects of parental care, suggest that parental investment through egg-laying:provisioning:nest construction is approximately in the ratio 0.16:0.31:0.53 so that, for example, provisioning an offspring reduces future reproduction by twice as

much as laying an egg (Field et al., 2007). Based on these findings, our Payoff Score was calculated as the number of offspring the focal female produced (0 or 1) minus the parental investment costs, for convenience scaled in comparison with producing an offspring at a single female nest:  $-(0.16E/1.3)-(0.31P/4.2)-(0.53B/1.2)$ . E, P and B are the numbers of eggs laid, prey provisioned and burrows constructed respectively by the focal female at the focal nest.

**Table A1**

Example calculations of Payoff Scores<sup>1</sup>

	Single female	Host A	Host B	Host C	Intruder D	Intruder E
Burrows dug	1.2	1.2	1.2	1.2	0	0
Eggs laid	1.3	1.3	2.3	2.3	1	1
Prey provisioned	4.2	4.2	5.2	2	1	1
Offspring produced	1	0	1	1	1	0
PAYOFF SCORE	0	-1	-0.2	0.04	0.8	-0.2

<sup>1</sup>The table shows payoff scores derived from hypothetical examples of numbers of burrows dug, eggs laid, prey provisioned and offspring produced by an individual

during an interaction. These would normally be whole numbers (e.g. a single burrow, or four prey) but for illustrative purposes, we base examples on the average resources required to produce an offspring at single female nests in our population. A single female uses the average resources required to produce a successful offspring, so scoring zero (baseline). Host A uses the same resources as a single female, but fails to detect that her egg is replaced by an intruder, and so scores -1 (we expect her to produce one fewer offspring in her life than she would have done if her egg had not been replaced). Host B detects an intruder's egg and has to use extra resources in capturing an extra prey item and laying a replacement egg on it, thus scoring somewhat negatively. Like Host B, Host C replaces an intruder's egg, but this time the intruder does all of the remaining provisioning so that Host C gets a slightly positive payoff (she will produce slightly more offspring in her life than she would have done without the intruder). Intruder D replaces a host egg undetected and does no further provisioning and no burrow-digging, thus producing almost one extra offspring through the interaction. Intruder E does the same, but the host replaces her egg, or dies without further provisioning so that her offspring starves.

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**Table 1.** The average number of rejections expected during an interaction before an *A. pubescens* offspring is accepted, under different rejection rates.

Rejection rate	Mean number of rejections expected per nest
0.9	8.7
0.7	2.3
0.5	1.0
0.4 <sup>1</sup>	0.67
0.3	0.43
0.1	0.11

NOTE: The number of rejections expected is calculated making the following assumptions: (1) a constant rejection rate; (2) a new egg is substituted after each rejection; the new egg is then itself rejected with the probability set by the rejection rate; (3) this continues until there is an acceptance. For example, with a rejection rate of 0.1, the first intruder egg is rejected at 10% of nests, a second rejection occurs before acceptance at 10% of these etc.

<sup>1</sup>Observed rejection rate for the first intruder egg.



## Figure Legends

**Figure 1:** Example nesting sequences where the host either accepts or rejects an intruder's offspring. The drawings show a female ejecting an egg-bearing prey item from a nest (left, credit Y. Field), and a nest containing the first prey item bearing an egg (right; scale bar represents 2cm).

**Figure 2:** Payoffs obtained by observed intruders, and payoffs calculated for intruders using two hypothetical alternative tactics, No-Return and Conditional-Provision (see Methods). *A.* Boxplots showing the median, interquartile range and maximum/minimum payoffs. Superimposed points show the payoff differences for individual females (No-Return or Conditional-Provision minus Observed), with the horizontal dashed line representing a zero difference. Points are slightly jittered for clarity; *B.* and *C.* Difference in payoffs between observed and alternative tactics for the 52 individual events ordered along the x-axis. Solid lines: positive values are events where the alternative tactic gains when hosts are assumed to provision instead of intruders; negative values are where the alternative tactic loses out when intruder offspring are assumed to starve because the host is dead and so cannot provision; Dashed lines: alternative tactic either gains by not laying a second egg that was observed to be replaced, or loses out by failing to lay an egg that was observed to be successful. Zero differences (shown as dots) are intruders that were observed not to provision, including some that died before provisioning was complete. Events are in the same order along the x-axis in *B.* as in *C.*

**Figure 3:** Payoff Scores for single females, hosts and intruders in *A. pubescens*.

White bars are instances where focal females produced a successful offspring; grey bars are where they did not. Cross-hatched shading represents mistaken provisioners and their hosts. Note the different y-axis scale for single females.

**Figure 4:** Average $\pm$ SE Payoff Scores for intruders (open circles) and hosts (filled circles) plotted in a window of conflict from the worst possible outcome (carrying out all post-hatch provisioning but not producing an offspring) to the optimum outcome (carrying out none of the provisioning and producing an offspring). *A.* observed means; *B.* excluding observed provisioning costs; *C.* including provisioning costs but assuming that focal females always produce the final offspring.







