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Report

Current Biology

Crozier's Effect and the Acceptance of Intraspecific Brood Parasites

Highlights

- Digger wasps parasitize parental care by replacing eggs in each others' nests
- Hosts sometimes reject foreign eggs, but not using intrinsic genetic cues
- Rejection involves more wastage of parental investment than acceptance
- Costly rejection causes selection against cue diversity required for discrimination

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In Brief

Despite its benefits, kin recognition is often absent in nature. Field et al. show that digger wasps often fail to reject foreign conspecific offspring from their nests. Rejection involves more wastage of parental investment than acceptance. Costly rejection leads to selection against allelic diversity required for discrimination using genetic cues.

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Report

Crozier's Effect and the Acceptance of Intraspecific Brood Parasites

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SUMMARY

Organisms can often benefit by distinguishing between different classes of individuals. An example is kin recognition, whereby individuals preferentially associate with or aid genetic relatives that bear matching recognition cues but reject others. Despite its potential benefits, however, kin recognition using genetically based cues is often weak or absent [1–4]. A general explanation, termed "Crozier's effect," is that when individuals interact randomly, rarer cue alleles less often match cues of other individuals, and so are involved predominantly in "reject"-type interactions. If such interactions are more costly, positive frequency-dependent selection will erode the cue diversity upon which discrimination depends [4, 5]. Although widely cited [1, 2, 4, 6–9], this idea lacks rigorous testing in the field. Here, we show how Crozier's effect applies to interactions between hosts and conspecific parasites, and measure it using field data. In the wasp we studied, conspecific parasitism fits a key assumption of Crozier's model: the same females act as both hosts and parasites. By exchanging offspring between nests experimentally, we find no evidence that females respond to genetically based cues associated with foreign offspring. Through measuring costs and benefits, however, we demonstrate a strong Crozier effect: because more parental investment is wasted when foreign offspring are rejected, interactions involving rejection have substantially lower payoffs than interactions involving acceptance. Costly rejection can thus eliminate cue diversity by causing selection against rare cue alleles, consistent with the absence of genetically based recognition that we observe. Females instead appear to rely on non-genetic cues that enable them to detect less than half of parasitic offspring.

RESULTS

We consider the evolutionary dynamics of genetically based cues used to discriminate kin from non-kin via self-matching mechanisms [5]. Note that cues need not be the result of selection for discrimination per se. We imagine situations in which individuals (or groups [10]) interact randomly, and where each individual takes the role of discriminator in some interactions but is the recipient in others. During interactions, discriminators act conditionally after comparing cues carried by recipients with equivalent cues carried by themselves. Conditional actions might include cooperation versus aggression [1], or somatic fusion versus rejection in tunicates and fungi [6, 7]. In the scenario we focus on here, parents act as discriminators, providing immature conspecifics with parental care if they bear matching cues but rejecting them if they bear non-matching cues. For example, individuals bearing "blue" cue alleles will categorize "red" cues as foreign and act accordingly (attack or reject), but will treat conspecifics that bear matching blue alleles as "self" (cooperate, fuse, or accept).

A key insight is that the fitness of an individual bearing a particular cue allele will depend on the frequency-dependent payoffs it obtains in both the recipient and the discriminator roles. Taking the example of conspecific parasitism, if the blue allele is rare, its bearers will encounter mainly non-matching red parasites. Blue hosts will therefore detect and reject a larger proportion of parasites than will individuals bearing common red cues. However, blue will also be detected more often itself when in the role of parasite, because it will fail to match the red cues borne by the majority of hosts. At first sight, it seems that these two effects cancel out: blue is successful in the host role to the same extent that it is unsuccessful as a parasite, whereas the opposite is true of red (Table 1, top). When calculating payoffs, however, we must also account for the possibility that different kinds of interactions incur different costs. Rare alleles such as blue will be involved in mainly "reject"-type interactions (both rejecting as host and being rejected as parasite), whereas alleles coding for common cues such as red are involved in mainly "accept" interactions (accepting and being accepted) (Table 1, top). A rare allele will therefore decrease in frequency if the net payoff, summed across both roles in the reject interactions it is predominantly involved in, is smaller than the payoff from the accept interactions that commoner alleles are predominantly involved in (Table 1, bottom). Summing across both roles assumes that on average, bearers of each allele take part in an equal number of interactions as host and parasite, so that payoffs in the two roles have an equal influence on fitness. Payoffs through reject may be smaller than through accept if, for example, rejection involves costs through fighting or wastage of parental investment (Table 1, bottom) that are not incurred

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Table 1. Frequency-Dependent Payoffs in Conspecific Parasitism

Cue Allele	Proportion of Parasitic Offspring Successfully Rejected When in the Host Role	Proportion of Own Offspring Successfully Accepted When in the Parasite Role
Blue	0.9	0.1
Red	0.1	0.9
Role	**Payoffs through Accept Interactions**	**Payoffs through Reject Interactions**
Host	−1	−0.25
Parasite	0.75	−0.25
Sum	−0.25	−0.5

Top: success rates for red and blue cue alleles borne by individuals that interact randomly in the roles of host or intraspecific parasite, assuming illustrative population frequencies of 0.9 (red) and 0.1 (blue). It is assumed that when parasite cues fail to match host cues, the parasitic offspring is rejected. Bearers of the rarer blue allele successfully reject 90% of parasitic offspring, but 90% of their own offspring are rejected by hosts. For bearers of the commoner red allele, both figures are 10%. Bottom: possible payoffs in intraspecific parasitism. Payoffs through an accept or reject interaction are expressed in terms of how the interaction changes the lifetime reproductive success of the interactants (offspring gained or lost). Within a single interaction, rejection (scoring −0.25) is better than acceptance (−1) for the host. But if rejection results from bearing a rare cue allele (allowing the host female to detect a mismatch with the parasite), it will lead to the same female being rejected herself when in the role of parasite (then scoring −0.25 instead of +0.75). The summed payoff from rejection via the two roles (−0.25 −0.25 = −0.5) is then smaller than for acceptance (−1 + 0.75 = −0.25), so that rare cue alleles, because they are involved in mainly reject interactions, will decrease in frequency. Specific payoffs in this example result from the behavioral sequences shown in Figure 1A using our payoff scores. In an accept interaction, the host wastes a full quota of investment on an unrelated offspring (payoff = −1 offspring), while the parasite obtains a fully provisioned offspring but invests at only 25% of the normal level (one egg and prey item; payoff = +0.75). In a reject interaction, the parasite wastes her 25% investment when the host rejects her egg (payoff = −0.25). The host produces a successful offspring, but has to invest in a replacement egg and prey (−0.25).

with acceptance. The resulting selection against rare alleles leads to cue monomorphism, and thus the absence of kin discrimination using genetically based cue diversity. We refer to this as "Crozier's effect" [5].

The above analysis suggests that costly rejection could result in the cue diversity required for kin discrimination being lost from the population. Within an interaction, rejection is likely to be better than acceptance for the host, but there may nevertheless be selection against hosts that are able to reject only because they bear rare cue alleles (Table 1, bottom). Such hosts will often reject parasites by detecting cue mismatches, but are correspondingly often rejected themselves when in the parasite role. Because costs and benefits are notoriously hard to measure, however, it is unclear whether payoffs in the field really are smaller for reject than for accept, or how strong any effect is [8, 11]. Here, we test for Crozier's effect using field data from conspecific parasitism. (1) We first test the key assumption that the same individuals take both host and parasite roles. (2) We then show that although some hosts can reject parasitic offspring, hosts do not appear to respond to genetically based cues, and (3) we measure payoffs from acceptance and rejection to assess Crozier's effect as an explanation for the absence of such cues. Finally, (4) we test for alternative, non-genetic cues that might enable hosts to reject some foreign offspring.

We focus on interactions between hosts and conspecific parasites [12, 13] in a progressively provisioning digger wasp, *Ammophila pubescens* (Figure 1D). During her lifetime, an *A. pubescens* female produces a series of spatially separate nest burrows, each containing a single offspring that she provisions with paralyzed insect prey (lepidopteran caterpillars) (Figure 1B). A typical nesting sequence proceeds as follows [15, 16]. On day 1, the mother digs a short burrow in the soil. Later that day, or on day 2, she places one prey item in the burrow and glues a single egg onto it. After an interval of 2–3 days, while the egg hatches and the wasp larva starts to feed on the prey, she re-enters the burrow, here termed an "assessment visit." Soon after this, the mother adds several (3 ± 0.09, range 0–8 in this study) further prey items one at a time, over a period of 1–7 days. She then permanently closes the burrow, and her larva consumes remaining prey and pupates.

During the interval between egg laying and the assessment visit, a nest may be entered by an unrelated conspecific (hereafter "the parasite"), which ejects the host offspring from the nest along with the prey item that bears it. The parasite soon returns to lay her own egg, usually on a new prey item that she brings. When the host carries out her assessment visit, she thus encounters a foreign immature, and in turn either (1) ejects the foreign prey item and egg ("reject"), later usually replacing it with a new egg of her own, or (2) shows no behavioral response and subsequently provisions the foreign immature ("accept") (Figure 1A). If the host replaces the parasite's egg, the parasite may return to accept or reject the host's second egg, with sometimes several successive eggs being replaced and one or both females provisioning the nest.

The Same Individuals Take Both Roles

As assumed in Crozier's [5] framework (Table 1), we found that the same *A. pubescens* females act as hosts in some interactions but as parasites in others (Figure 2). We observed 31 individually marked females that were involved in interactions at two or more different nests (range 2–9). Of these, 24 (77%) took both host and parasite roles at least once each. The remaining seven females interacted at only two nests each, so that their appearing to take only one role probably reflects limited sampling.

Hosts May Reject Foreign Offspring, but Not Using Offspring-Specific Cues

Hosts accepted foreign offspring at 17 (61%) of 28 unmanipulated parasitized nests, and rejected offspring at 11 (39%) nests, a much higher rate of rejection than for females' own eggs in unparasitized nests (12.8% following developmental failure or natural enemy attack [14]; n = 219 unparasitized nests, p = 0.004). However, there was no evidence that rejection involves genetically (or environmentally) based cues borne by offspring. In order to test this, soon after egg laying we replaced host eggs with foreign eggs of the same age, or carried out sham control manipulations where we removed, then replaced, host eggs (see STAR Methods). We thus separated cues intrinsic to

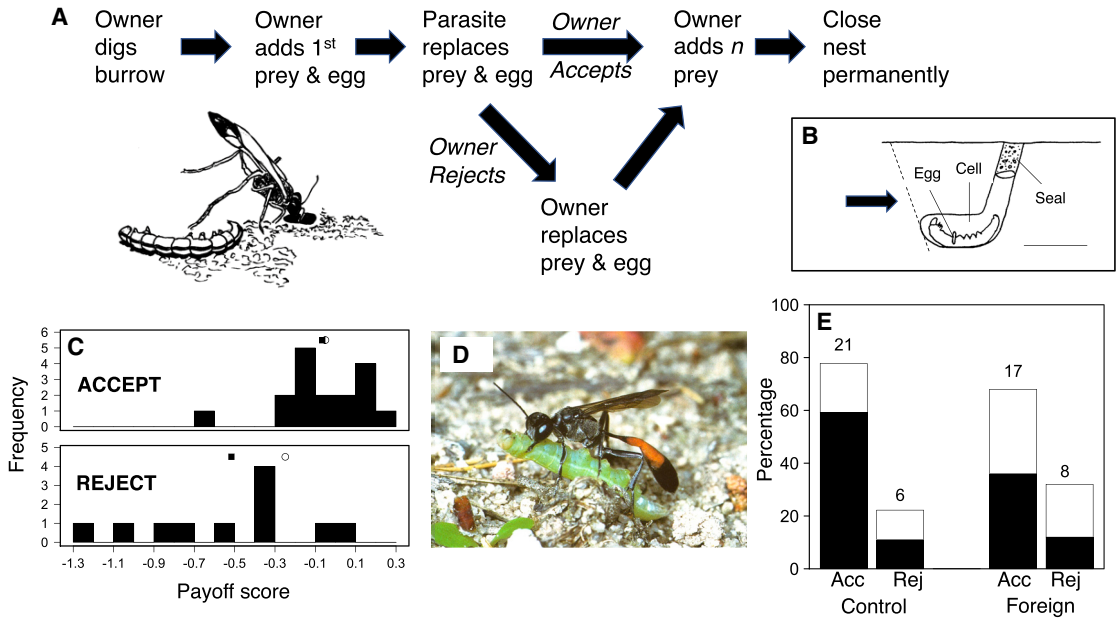

Figure 1. Intraspecific Parasitism and Crozier's Effect in *Ammophila pubescens*

(A) The simplest sequence of events when a nest owner accepts or rejects a parasitic offspring. The drawing shows an *A. pubescens* female that has just ejected a prey item from a nest. Drawing: Y. Field.

(B) *Ammophila* burrow containing the first prey item bearing an egg. The arrow indicates where soil to the left of the dashed line was excavated to allow contents to be replaced experimentally without damaging the burrow or its entrance [14]. Scale bar, 2 cm.

(C) Payoffs from accept interactions exceed those from reject interactions. Open circles above histograms indicate payoffs that would result from the specific behavioral sequences shown in (A); filled squares show observed mean payoffs across all sequences observed in the field. 10/18 accept nests and 5/12 reject nests indeed followed the sequences in (A). However, three reject nests failed to produce offspring, and the females at another two nests replaced each other's eggs three or more times. At three accept nests, the parasite laid her egg soon after the host had dug the burrow but before she had laid her own egg. Although the host accepted these eggs and subsequently provisioned the parasite's offspring, she did not pay the cost of laying an egg herself. Additional variation in costs reflects variation in the total number of prey provided to the offspring. See Table S1 for original data.

(D) *Ammophila* female carrying a prey caterpillar back to her nest. Scale: wasp length is 1.7 cm. Photo: M. Blosch.

(E) *A. pubescens* females are no more likely to reject foreign, same-aged offspring placed experimentally in their nests than controls.

Bars show the percentages of offspring accepted or rejected within foreign and control treatments. Shaded and unshaded portions of bars are data from artificial and natural nests, respectively. Numbers above bars are sample sizes (number of nests). The percentage rejected was almost the same for foreign and control offspring whether we used natural or artificial nests, suggesting that the result was not because artificial nests somehow mask chemical cues. (B) and (D) are modified from [14].

offspring from other, non-genetic cues that could potentially be associated with parasitism, such as hosts encountering foreign females at their nests. We replaced eggs either by carefully digging into the host cell (Figure 1B) [14] or by inducing hosts to use artificial nests that could be opened to allow manipulation of their contents (Figure S1) [16]. During their subsequent assessment visits, females were no more likely to reject foreign offspring than their own offspring (Figure 1E; χ^2 with Yates's correction = 0.23, degree of freedom [df] = 1, p = 0.63).

Crozier's Effect: Selection against Rare Cue Alleles

A. pubescens hosts sometimes reject foreign offspring, yet do not appear to respond directly to genetically based cues. Could there have been selection via Crozier's effect against rare cue alleles that entered the population, leading to the loss of genetic cue diversity and the observed lack of direct discrimination? For the effect to operate, recall that net payoffs (offspring gained or lost) during the reject interactions that rare cue alleles are predominantly involved in, summed across host and parasite roles, must be smaller than net payoffs during the accept interactions that common alleles are predominantly involved in (see above). In order to test this, we compared accept and reject in terms of their benefits (how often they led to successful offspring production), and in terms of parental investment costs incurred: the number of eggs laid, prey provisioned, and nests constructed, summed across host and parasite. We then combined costs and benefits into a single payoff score, which estimated how each interaction affected the lifetime reproductive success of the interactants. To understand how payoff scores were calculated, first note that an 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.F., C.A., and W.A.F., unpublished data). Previous experiments [15] indicate 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. Based on these findings, we assigned a payoff score to each interaction, calculated as the number of offspring produced (0 or 1) minus the parental investment costs

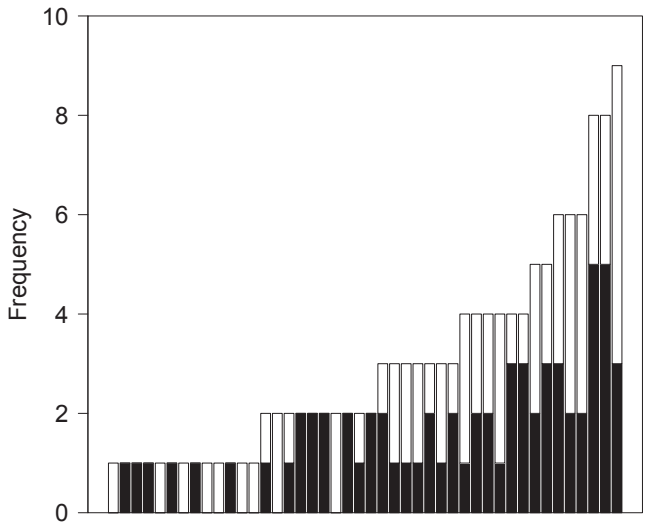

Figure 2. The Same Individuals Act as Both Host and Parasite

The number of interactions (frequency) where each female (n = 44) acted as host or parasite. Each bar represents a different female. Black shading represents interactions where a female took the host role, and white shading represents the parasite role. Additional females (n = 12) that were not involved in any observed interactions are not shown. Note that because each interaction must include one host and one (occasionally >1) parasite, the population-wide frequency of the two roles will approximate to a 1:1 ratio.

scaled in comparison with producing an offspring at an unparasitized 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, during the focal interaction. Our scaling meant that an interaction received a zero payoff score if it produced a successful offspring and had the mean values of E, P, and B required to produce an offspring at an unparasitized nest. A negative score indicated that more parental investment was required to produce an offspring than at unparasitized nests, thus reducing the summed lifetime reproductive success of the interactants, whereas a positive score indicated that less investment was required. The analysis revealed the effect suggested by Crozier: summed across both interactants, fitness payoffs were smaller at nests where the owner rejected the parasitic offspring than at nests where she accepted it (Figure 1C; Wilcoxon test, $p = 0.001$; generalized linear model [GLM], $p = 0.0004$; year and date both $p > 0.3$).

The difference between accept (mean payoff -0.07) and reject (-0.52) interactions was large, equivalent to 45% of the parental investment required to produce an offspring in an unparasitized nest [15]. The difference occurred for two main reasons. First, although all 17 "accept" nests produced an offspring, considerable parental investment was wasted at 3 of the 11 "reject" nests where no offspring were eventually produced. One of these nests was abandoned after four successive eggs had been laid; no further nesting activity occurred even though the interactants remained alive. At the other nests, one or both interactants disappeared (probably died) after two or three eggs had been successively rejected, and the remaining female abandoned the nest. Even if failed nests were excluded, however, payoff scores were still lower at reject nests (Wilcoxon test, $p = 0.01$; GLM, $p = 0.01$). This reflected a second underlying difference, which was that approximately one extra egg was laid during reject (2.9 ± 0.21, range 2–4) compared with accept (1.8 ± 0.1, range 1–2), as in Figure 1A (Wilcoxon test, $p = 0.0001$; GLM, $p = 0.06$). All interactions involved constructing a single nest burrow, and although more prey tended to be provisioned during reject (6.3 ± 0.92) than accept (5.6 ± 0.66), the difference was not significant.

Some Foreign Offspring May Be Rejected Based on Non-genetic Cues

The large Crozier effect we measured should select against rare cue alleles, leading ultimately to cue monomorphism and the loss of discrimination. This is consistent with the lack of differential rejection observed when we switched offspring experimentally (Figure 1E), but in turn raises the question of how unmanipulated hosts nevertheless succeed in rejecting nearly 40% of parasites. One possibility is that hosts can sometimes detect parasitism using non-genetic cues, such as that a parasitic offspring will necessarily be younger than the host offspring it replaced (age difference 48 ± 8 hr, median 29 hr at unmanipulated parasitized nests). Indeed, the age difference was larger at nests where a foreign offspring was rejected than at nests where it was accepted (binomial GLM, Wald test, $p = 0.02$). To test for causality, we manipulated the age difference experimentally. Two days after a female had laid her egg in an artificial nest, just prior to her assessment visit, we replaced her offspring either with a foreign egg that had only just been laid (age difference 51.0 ± 4.7 hr) or with a same-aged offspring taken from a different nest (age difference 0.08 ± 1.1 hr). All hosts thus encountered a foreign offspring, but its age relative to the host's original offspring differed between treatments. None of 8 tested females rejected same-aged offspring, but 6 out of 11 rejected offspring that were younger than expected (Fisher's exact test, $p = 0.018$).

DISCUSSION

Crozier [5] pointed out a problem with the evolutionary maintenance of allelic diversity at cue loci used in discrimination based on self-matching. Our results suggest that Crozier's effect will operate in *A. pubescens*. A rare cue allele that allowed females to discriminate foreign intruders, but also caused them to be categorized as foreign themselves, would tend to decrease in frequency. Its bearers would take part in predominantly reject interactions involving more wasted investment than in the accept interactions that occur between common alleles. This would lead, paradoxically, to the erosion of cue diversity and thus potentially to the evolutionary loss of discrimination. Crozier's effect appears to be large in our study system. Assuming a trade-off-based life history, where extra investment in one offspring results in fewer offspring produced in the future, a female bearing a rare cue allele that failed to match conspecifics would forfeit nearly half an offspring for each pair of nests where she acted as host and parasite, in comparison with a female bearing a common allele that was never discriminated by conspecifics. The negative effect on a real cue allele might be even greater: mutual detection could lead to interactions with repeated cycles of rejection and re-oviposition,

something that we did observe, but only occasionally. This might even lead to parasitism itself no longer being favored by selection.

Selection against rare cue alleles via Crozier's effect is consistent with the apparent absence of genetically based offspring recognition that we documented in *A. pubescens*. 60% of unmanipulated hosts failed to reject foreign offspring, and hosts did not differentially reject such offspring placed in their nests experimentally. This is despite parasitism being frequent during our study (11.5% of nests), and despite the fact that undetected parasitism leads hosts to waste their investment on unrelated offspring. Phylogenetic constraints on discrimination seem unlikely. The cuticles of *A. pubescens* adults, their eggs, and larvae bear complex hydrocarbon profiles of the kind routinely found throughout the Hymenoptera [17] (A. David, E. Hill, and J.F., unpublished data). Hydrocarbon cues are heritable, and are known to be involved in discrimination of nest mates, eggs of dominant versus subordinate individuals, relatives versus non-relatives, etc. [8, 18–22]. *A. pubescens* eggs therefore differ from "chemically insignificant" obligate parasites [23] that largely lack hydrocarbons, apparently as a form of concealment [24–27]. Hosts could potentially detect foreign immatures by learning the hydrocarbon profiles of their own eggs immediately after oviposition, for later comparison with offspring present in their nests during assessment visits [28]. Alternatively, because parasitic females clasp replacement prey items against their undersides during carriage to the host nest, hydrocarbons will most likely be transferred onto the prey cuticle. Hosts could compare these adult-derived cues with their own cues via mechanisms based on templates or sensory habituation ("self-referent phenotype matching" [29, 30]). Thus, although concealment or mechanistic constraints may act in concert with Crozier's effect in *A. pubescens*, there is no particular reason to expect them.

Given that genetically based discrimination cues do occur in some systems, Crozier [5] suggested that cue diversity might be maintained by counterbalancing negative frequency-dependent selection in other contexts, such as disassortative mating [8] or interspecific parasitism. Many wasps and bees are indeed attacked by heterospecific obligate cuckoo parasites, some of which mimic host odors in order to enter nests [23, 31, 32]. Hosts bearing rare cue alleles might then suffer reduced parasitism if parasites coevolve to match commoner cues, and interspecific parasitism does indeed appear to drive host hydrocarbon profile diversity [33, 34]. Whereas *A. pubescens* is not attacked by macro-parasites of this kind, chrysidid wasp parasites of some other *Ammophila* species would be interesting to investigate [35]. A further mechanism through which Crozier's effect could potentially be avoided is if parasites chose not to replace host eggs in the first place if they detected a cue mismatch that hosts could subsequently discriminate.

The failure of females to respond directly to cues borne by foreign immatures may force them to rely on other cues sometimes associated with parasitism. Parasitic offspring are younger than the host offspring that they replace, and we found experimentally that hosts were more likely to reject foreign offspring if they were 2 days younger than their own. However, the age difference in unmanipulated nests was often much less than 2 days, probably reducing the effectiveness of age as a cue, as evidenced by the 60% of unmanipulated hosts that failed to reject.

Our findings, obtained in the field, suggest that Crozier's [5] effect will act against the maintenance of genetically based cue diversity associated with detecting intraspecific parasitism. Analogous tests should be carried out in other systems. In birds, for example, where conspecific parasites add eggs to the host clutch, discrimination might involve hosts detecting a mismatch between genetically based patterning or color cues borne by their own versus foreign eggs. Testing for Crozier's effect would require measuring whether costs involved in rejection—damage to the host's own eggs, wastage of parasitic eggs, etc.—exceed costs of acceptance such as reduced fitness of individual offspring and reduced parental lifetime reproductive success, when rearing clutches larger than optimum [36].

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
  - Data from unmanipulated females
  - Host response to foreign eggs placed experimentally in nests
- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure and three tables and can be found with this article online at https://doi.org/10.1016/j.cub.2018.08.014.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.F.; Methodology, J.F., C.A., and W.A.F.; Formal Analysis, J.F.; Investigation, J.F., C.A., and W.A.F.; Writing – Original Draft, J.F.; Writing – Review & Editing, J.F. and W.A.F.; Supervision, J.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
Microsatellite primers	This paper	See Table S3 and accession numbers NCBI: MG951501-MG951519 at https://www.ncbi.nlm.nih.gov/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jeremy Field (j.p.field@exeter.ac.uk).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We studied *Ammophila pubescens* Curtis (Hymenoptera: Sphecidae *s.l.*) in the field. Unusually among digger wasps, *A. pubescens* is a so-called progressive provisioner [15, 16]: instead of a single mass of food being provided before the egg is laid, each offspring is provisioned more gradually as it develops, somewhat resembling the gradual provisioning seen in social wasps and altricial birds. Females typically maintain more than one nest simultaneously. Progressive provisioning means that mothers visit each nest repeatedly during larval development, and may thus encounter foreign immatures if their nests are parasitized by conspecifics. A nest is sealed with a plug of soil/stones whenever the mother departs (Figure 1B), but may be opened and re-closed by foreign females, as well as during the nest owner's visits. The nests of many different females are often intermingled spatially, so that by observing a relatively small nesting area, all of the events occurring at multiple nests can be recorded.

Most of our experiments and observations were conducted during 2012-2015 at Witley Common, Surrey, UK (51°09'04''N 0°40'53''W). One set of experimental replicates was carried out in 2010 at a site in North Norfolk, UK (52°56'19''N 1°06'44''E) [14]. The nesting areas observed were sections of bare sandy paths within heathland, adjacent to large areas of heather (*Calluna vulgaris*; *Erica* spp) on which *A. pubescens* hunts exclusively for prey.

METHOD DETAILS

Data from unmanipulated females

Marking and DNA sampling

During the few days before observations began each year, all females seen in the nesting area were captured and marked with unique color combinations of three paint dots on the thorax. Additional females were occasionally marked subsequently if they nested in the observation area. At the same time as marking, wing length was measured and a DNA sample obtained by removing the distal half of one antenna using micro-scissors and immediately placing it in 100% ethanol. DNA samples were not taken in 2010 (Norfolk field site). There was no evidence, from comparison with unsampled females, that DNA sampling affected behavior, reproductive investment or lifespan (Table S2).

Observation methods

Data concerning the costs, benefits and natural frequency of Accept and Reject, and whether the same individuals behave as both host and parasite, came from a 10 × 3 m nesting area at the Witley site during 2012-2014. Once marking was complete each year, the nesting area was observed continuously by 2-3 people simultaneously between approximately 09.00 and 19.00 on all days with weather suitable for wasp activity during July 23-August 18, 2012 and 5-30 July, 2013. In each year, the observation period began a few days after nesting had started. In 2012 there were 7 full days of warm sunny weather with continuous wasp activity, plus 13 part-days. Thirty females nested in the observation area and we obtained data from 69 provisioned nests. In 2013, the weather was almost continuously warm and sunny throughout the observation period, and we obtained data from 57 females and 359 provisioned nests. Data from three additional parasitized nests were obtained in 2014.

Whenever a female was observed digging a new burrow during observations, we placed a numbered marker 4cm from the entrance. All activities observed subsequently at the burrow were recorded, along with the color marks of the females involved. Whenever a prey caterpillar was taken into a burrow, we recorded prey type/color, and how long the female spent in the burrow before exiting: egg-laying involves being inside for > 25-30sec, whereas the time inside is normally < 10 s when provisioning subsequent prey. As soon as a burrow had been re-closed after a wasp immature was rejected from a nest during intraspecific parasitism,

the immature was placed in 100% ethanol. At the end of each year's observation period, we excavated all burrows where there had been intraspecific parasitism, recording presence/absence of an offspring, normally a cocoon. Cocoons were opened and prepupae placed in 100% ethanol.

Genotyping

DNA was extracted from offspring and adult antennal or wing samples, then 17 microsatellite loci were amplified and scored (Table S3). We then determined which of the females that had been active at each burrow matched the offspring genotype. Because potential mothers were normally unrelated to each other, offspring assignment was straightforward. We successfully amplified from all but one of the offspring excavated.

Primers were labeled using fluorescent dyes and divided into three multiplex panels (see Table S3). Each 5 μL PCR reaction contained approximately 10 ng of air-dried genomic DNA, 0.2μM of each primer and 4 μL PEQLab PCR mix (VWR). PCR amplification was performed using the same profile for all three multiplex reactions: 95°C for 15 min, followed by 44 cycles of 94°C for 30 s, 57°C for 90 s, 72°C for 90 s and finally 60°C for 30 mins. PCR products were genotyped on an ABI 3730 48-well capillary DNA Analyzer using ROX size standard (Applied Biosystems). Alleles were scored using GENEMAPPERv3.7 software (Applied Biosystems).

Observed and expected heterozygosities and estimated null allele frequencies were calculated with CERVUS v3.0.3 [37]. Tests for deviation from Hardy–Weinberg proportions and linkage disequilibrium between loci were performed using GENEPOP web version 4.2 [38]. Observed levels of heterozygosity ranged from 0.54 to 0.96 with 5-28 alleles per locus (Table S3). There were no significant deviations from HWE, and no pair of loci displayed linkage disequilibrium. Sequences were confirmed to be unique using BLAST software.

Do the same individuals act as both host and parasite?

To investigate this, we used our 2013 dataset, because the larger number of nests and parasitism events in that year provided the best opportunity to determine whether individuals will take both roles. Females that were active for longer during our observations (probably reflecting variation in lifespan) were involved in more interactions in total (Negative binomial GLM: $p = 0.001$; Pearson's $r = 0.42$, $p = 0.002$).

Costs and benefits through Accept and Reject

We aimed to measure the number of eggs laid, the number of prey provisioned, and determine whether an offspring was eventually produced during each Accept or Reject interaction. Nests were included in the analysis if five criteria were met: (1) the identity of the nest owner (the female that had dug the burrow) was known; (2) a foreign female was observed laying an egg in the nest; (3) the owner's response was directly observed when she next visited the nest; (4) if a successful offspring was produced, the nest contained a cocoon or (occasionally) large larva when excavated. Nests containing eggs or small larvae when excavated were excluded, because more prey might eventually have been provisioned, and the offspring could subsequently have been ejected. Large larvae were never ejected from nests, and nests containing large larvae were included when it was clear from our observations that no further prey would be added. (5) there was no indication that we had missed key events. Wasp immatures (usually eggs) that were ejected from nests during parasitism events were often damaged in the process, but where microsatellite amplification was possible, we checked that the ejected egg had been laid by the female implied by our observations. Using these inclusion criteria, we ended up with 28 analyzable nests. Two females were each the owner at two different nests, and three females were each the parasite at two nests, but no two nests had the same pair of interactants.

Host response to foreign eggs placed experimentally in nests

Response to own offspring versus same-aged foreign offspring

Soon after wasp activity had ceased on the day that the host laid her egg, we switched egg-bearing prey items between nests. We used two different methods. In 2010 at the North Norfolk field site, we carefully dug away soil until we reached the edge of the underground cell furthest from the nest entrance, not disturbing the entrance itself (Figure 1B [14]). We removed the egg-bearing prey item from the cell, then carried out one of two treatments on alternate nests. At control nests, we replaced the prey item in its original cell. At experimental nests, however, we placed a foreign egg-bearing prey item from another female's nest in the cell. Prey items were placed in the natural positions at the back of the cell. The soil we had removed was carefully replaced and gently compressed. When the nest owner next entered the nest for her assessment visit, we recorded whether she re-closed it as usual (Accept) or pulled the offspring-bearing prey out and discarded it (Reject).

Similar experiments were carried out in 2014 and 2015 at the Witley site, but this time using artificial nests made from plaster of Paris that could be opened and re-closed as required (Figure S1). As previously described by Baerends [16], a cast of an *A. pubescens* nest of natural dimensions was made in a 3 cm high x 5.5 cm diameter block of plaster of Paris constructed in two halves, so that the contents of the nest cell were exposed by lifting off the top half (Figure S1). A nest block was embedded in the soil, and in the evening after a female had laid her egg in the cell, we opened the block and carried out an experimental or control manipulation, as above.

Each individual female was used for at most one control and one experimental treatment. Control and experimental females assessed offspring of the same age (control offspring: 69.4 ± 8.9 hr old; foreign offspring: 70.1 ± 14.0), similar time intervals after they had laid their original eggs (Controls: 69.4 ± 8.9 hr after originally ovipositing; foreign: 69.7 ± 7.6). At experimental nests, the foreign egg was approximately the same age (age difference = 2.1 ± 0.43 h) as the host egg that it replaced. Nests were observed continuously by 3-4 observers from the time when the host first oviposited onward, to ensure that there was no unrecorded natural parasitism. As a check on observation accuracy, we also genotyped 11 immatures that were accepted (6 Controls, 5 experimentals),

and 5 that were Rejected (3 controls, 2 experimentals). Genotyping showed that all 16 of these immatures were offspring of the females expected from our behavioral observations. We excluded from analysis nests that were entered by foreign females or ants, and two nests that females appeared unable to fully enter during their assessment visits, probably because we had not lined up the two halves of the plaster blocks correctly after the treatment. Nests where the female never returned for her assessment visit, almost certainly because she had died, were also excluded.

Response to same-aged versus younger foreign offspring

For this experiment, we used only the artificial nests method described above. Offspring were switched on the second day after focal hosts had laid their eggs. Same-aged offspring were obtained from foreign nests where the egg had been laid two days before the switch, at about the same time as the focal host had laid. Younger offspring were obtained from foreign nests where the egg was laid just before the switch, on the second day after the focal host had laid. Accept and Reject responses were recorded as described above, during host assessment visits following the treatments. Each female was used at most once in each treatment.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data were analyzed using R version 3.3.2 [39]. Means are reported ± SE and statistical significance is assessed at the $p = 0.05$ level. We used a linear model to test whether the Payoff scores (y-variable) differed between Accept and Reject interactions. We added +2 to each score to remove negative values, and then used the function *powerTransform* from the R-library *car* to identify the transformation (+2.78) that best satisfied model assumptions. When the y-variable was the number of eggs laid or prey provisioned, we used Poisson errors. In all cases, we also carried out non-parametric Wilcoxon tests. We used GLMs with binomial errors when analyzing the frequency of rejection at unmanipulated nests (binary y-variable) in relation to whether the nest was parasitized, or in relation to the age difference between host and parasite offspring. We included year and date of focal egg-laying as covariates in all of these models, but these were never significant at the $p < 0.05$ level. Assumptions were checked by inspecting plots of residuals and by testing for normality using Shapiro-Wilks tests. With binary y-variables, we used Hosmer and Lemeshow goodness of fit tests.

We used a χ^2 test with Yates's continuity correction to test whether females differentially reject same-aged foreign offspring placed in nests experimentally (Figure 1E). With our sample sizes, foreign offspring would have had to be rejected at a rate 30% higher than the Control rate for a difference to be detected at the $p = 0.05$ level.

DATA AND SOFTWARE AVAILABILITY

The data used to compare payoffs at Accept and Reject nests are provided in Table S1. The accession numbers for the microsatellite primer sequences reported in this paper are NCBI: MG951501-MG951519.