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Reproductive constraints, direct fitness and indirect fitness benefits explain helping behaviour in the primitively eusocial wasp, *Polistes canadensis*

Seirian Sumner[1,*], **Hans Kelstrup**[2] **and Daniele Fanelli**[3]

[1]*Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK*
[2]*Department of Biology, University of Washington, Seattle, WA 98195, USA*
[3]*Institute for the Study of Science, Technology and Innovation, The University of Edinburgh, Old Surgeons' Hall, Edinburgh EH1 1LZ, UK*

A key step in the evolution of sociality is the abandonment of independent breeding in favour of helping. In cooperatively breeding vertebrates and primitively eusocial insects, helpers are capable of leaving the group and reproducing independently, and yet many do not. A fundamental question therefore is why do helpers help? Helping behaviour may be explained by constraints on independent reproduction and/or benefits to individuals from helping. Here, we examine simultaneously the reproductive constraints and fitness benefits underlying helping behaviour in a primitively eusocial paper wasp. We gave 31 helpers the opportunity to become egg-layers on their natal nests by removing nestmates. This allowed us to determine whether helpers are reproductively constrained in any way. We found that age strongly influenced whether an ex-helper could become an egg-layer, such that young ex-helpers could become egg-layers while old ex-helpers were less able. These differential reproductive constraints enabled us to make predictions about the behaviours of ex-helpers, depending on the relative importance of direct and indirect fitness benefits. We found little evidence that indirect fitness benefits explain helping behaviour, as 71 per cent of ex-helpers left their nests before the end of the experiment. In the absence of reproductive constraints, however, young helpers value direct fitness opportunities over indirect fitness. We conclude that a combination of reproductive constraints and potential for future direct reproduction explain helping behaviour in this species. Testing several competing explanations for helping behaviour simultaneously promises to advance our understanding of social behaviour in animal groups.

Keywords: social evolution; *Polistes canadensis*; inclusive fitness; insurance-based advantages

1. INTRODUCTION

In the animal kingdom, a wealth of behavioural strategies have evolved to maximize the reproductive success of individuals. Understanding why these strategies have evolved and how they are maintained is a key area of research in evolutionary and behavioural ecology. The evolution of helping behaviour (whereby some individuals forgo reproduction in order to help others reproduce) in animal groups is arguably the most difficult of reproductive strategies to explain, and was one of Darwin's greatest challenges to his theory of natural selection (Darwin 1859). It remains an issue of debate today (West *et al*. 2007).

Choice of reproductive strategy is influenced by a range of different factors (e.g. ecological, social, physiological and genetic factors; Keller & Reeve 1994; Cockburn 1998; Clutton-Brock 2009*a*). For helping behaviour to evolve, individuals may be constrained from independent nesting such that they stay at home rather than leave. Ecological constraints such as lack of an available territory or there being high costs of nesting alone are thought to be important (Emlen 1997;

Gunnels *et al*. 2007; Zammit *et al*. 2008). Helping might also be explained if some individuals are reproductively constrained (or subfertile), making helping the best (or only) option (West-Eberhard 1975; Craig 1983). Alternatively, individuals may help while waiting for a breeding opportunity on their natal nest or elsewhere (Field *et al*. 2006). Finally, helpers may gain indirect fitness benefits from helping by raising non-descendant kin (Hamilton 1964; Stacey & Ligon 1991; Foster *et al*. 2006; Hughes *et al*. 2008), with or without the above constraints.

Many studies on why helpers help have focused on ecological constraints in vertebrates (reviewed in Dickenson & Hatchwell 2004; Clutton-Brock 2009*b*) and indirect fitness in insects (Hughes *et al*. 2008; Ratnieks & Helantera 2009). But biological systems are rarely governed by single factors of large effect, and a more realistic approach is to consider multiple factors and their interactions simultaneously (Hatchwell & Komdeur 2000; Bergmuller *et al*. 2007). Few studies have done this, as it is difficult to separate the relative importance of different proximate and ultimate factors.

In this paper, we consider how constraints on reproduction and genetic fitness benefits explain helping behaviour in the primitively eusocial wasp *Polistes canadensis*. In *Polistes*, all group members have the option of independent

* Author for correspondence (seirian.sumner@ioz.ac.uk).

Table 1. Predictions for hypotheses 2 (direct fitness from helping) and 3 (indirect fitness from helping). Observed results for each prediction are indicated in the 'obs.' columns, where Y indicates support for the prediction and N indicates a lack of support.

prediction	direct fitness (hypothesis 2)	obs.	indirect fitness (hypothesis 3)	obs.
(i) ex-helpers stay on nest	young ex-helpers stay	Y	all ex-helpers stay	N
	old ex-helpers leave	Y		
(ii) foraging effort	forage little (young ex-helpers)	Y	forage heavily (all ex-helpers)	N
(iii) fate of non-descendant kin	mainly old brood survive	Y	all brood survive	N

reproduction (Reeve 1991), making them comparable to social vertebrates and therefore of general relevance to social evolutionary questions (Brockmann 1997; Field & Cant 2007). Typically, *Polistes* paper wasp females emerge from hibernation in the spring, build nests out of plant material and raise the brood of one or a few dominant queens (Reeve 1991; Turillazzi & West-Eberhard 1996). Tropical species of *Polistes* (like *P. canadensis*) are little studied, but provide unique opportunities to explore mechanisms for helping behaviour as brood are reared all year round. To nest independently a female must survive long enough for brood to reach adulthood. In *P. canadensis*, survivorship of single foundress nests is very low, with 97 per cent of single female nests failing before brood reach adulthood (West 1967; Ito 1995), suggesting that ecological constraints are strong. There has been little evidence for reproductive constraints in primitively eusocial insects (e.g. Röseler *et al.* 1985; Shakarad & Gadagkar 1997; Field & Foster 1999), but recent studies in *Polistes* suggest there may be pre-imaginal caste differentiation that would preclude some females from being queens, rendering them 'sub-fertile' helpers (Hunt *et al.* 2007). Moreover, age may influence the reproductive potential of helpers in tropical polistines, including *P. canadensis* (West-Eberhard 1969, 1986; Jeanne 1972). *Polistes canadensis* helpers forage, rear brood and defend the nest. They have the potential to obtain high indirect fitness from helping, as they are closely related to the brood they raise (helpers to brood relatedness = 0.56 ± 0.135; Sumner *et al.* 2007). Moreover, helpers can gain direct fitness by taking over from the queen if she dies (S. Sumner & S. Drier 2009, unpublished data).

We relaxed ecological constraints for *P. canadensis* helpers, giving them a head-start in independent nesting by providing them with the opportunity to become queen on a mature nest full of related brood. We removed all nestmates except one helper from 31 nests and observed whether the ex-helper (i) remained on the nest, (ii) laid eggs and (iii) continued raising non-descendant kin. Then, we tested three hypotheses for helping behaviour. Hypothesis 1: if there are no constraints on reproduction, we expect all ex-helpers to become egg-layers. However, if age constrains reproduction younger ex-helpers are predicted to become egg-layers more readily than older ex-helpers (West-Eberhard 1969, 1986; Jeanne 1972). Hypothesis 2: if helping is explained by direct fitness benefits, ex-helpers will prioritize personal reproduction and avoid risky tasks such as foraging. Specifically, we expect ex-helpers to stay on their nests (especially if they are young), invest little in foraging and care only for the older brood, if any, as they represent the ex-helper's future worker force (Rabenold 1985; Ligon & Ligon

1987; see table 1). Hypothesis 3: if indirect fitness is important, ex-helpers will raise non-descendant brood at the expense of personal reproduction. Specifically, we expect all ex-helpers to stay on their nests, irrespective of age, and to invest heavily in foraging and care for all brood (see table 1).

2. MATERIAL AND METHODS

(a) *Study site and data collection*
Polistes canadensis nest in large aggregations in trees and buildings in Central and South America. Our experiments were conducted between 1st June and 20th July 2004, on 31 post-emergence nests (where young adults have already started emerging) in a scattered aggregation of 100+ nests on the undersides of buildings at Hospital Estancia Larga, 5 km outside Panama City, Republic of Panama. All wasps from the 31 nests were given unique paint marks and their forewings were measured. Nests were monitored every other day for one month prior to the manipulation experiment in order to mark new females of known ages emerging on their nests.

(b) *Manipulation experiment*
We removed all nestmates except a single helper (henceforth called 'ex-helper') from each nest, and monitored the behaviour of ex-helpers over a two-week period. To test the age component of hypothesis 1, ex-helpers were either 'young' (10 days old) or 'old' (30 days old; age of 'old' females was defined as the age at which approx. 50% of foragers had died). At the time of manipulation there were 15.48 ± 1.42 (mean $\pm$ s.e.) females per nest. On days 1–3, censuses of wasps present on all nests were taken in the mornings and afternoons to determine nest membership; on days 2 and 3, nests were also observed for 1 hour per day to record foraging, aggression and egg-laying. On day 4 all wasps were removed from their nests before dawn. After sunrise, brood were mapped and a single 10-day-old (young, $n = 16$) or 30-day-old (old, $n = 15$) ex-helper was placed directly back on her nest. Treatment (age) was random, as far as possible. Removed wasps were frozen at -20°C for dissection.

Nests were monitored every 30 min on the manipulation day (day 4) until nightfall. New nestmates that emerged on the nest after the initial manipulation were marked and allowed to remain on the nest. The experiment was repeated on three cohorts of nests at three different times during June–July 2004 in order to facilitate the handling of large numbers of nests (table 2). All experiments were terminated 14 days after the manipulation day. Ex-helpers and their nestmates were collected at dusk and frozen at -20°C for dissection.

Table 2. Summary of nest characteristics used in manipulation experiment. Means ± standard errors are reported.

removal	date	young ex-helpers (10 days old)			old ex-helpers (30 days old)		
		no. of nests	mean no. of brood	mean wing length of ex-helper	no. of nests	mean no. of brood	mean wing length of ex-helper
1	11 June	6	100.6 ± 19.3	19.2 ± 0.3	4	188.3 ± 25.2	19.2 ± 0.2
2	30 June	8	90.8 ± 16.4	17.9 ± 0.2	5	107 ± 52.8	18.5 ± 0.2
3	4 July	2	61 ± 46	18.2 ± 0.8	6	116.7 ± 26.1	18.6 ± 0.4
all		16	90.8 ± 11.7	18.4 ± 0.2	15	140 ± 0.22	18.8 ± 0.2

(c) *Hypothesis testing*

Hypothesis 1: Ex-helpers can lay eggs; young females are more able to lay eggs than old.

We compared ovarian development and insemination status of ex-helpers with similarly aged females that we removed. Eggs were considered mature if they were greater than 2 mm in length (West-Eberhard 1969). We report the mean largest egg size ± standard errors. For the ex-helpers that we could not collect, we detected egg-laying by mapping the brood every 2 days. We used a general linear model (GLM) with binomial errors to determine why females laid eggs. Response variable was 'laid eggs'/'did not lay eggs'. Full model included nest variables (days alone, presence of new nestmates, number of cells, number of empty cells, number of brood, presence of nest parasites and manipulation date) and ex-helper variables (age and size).

Hypotheses 2 and 3: Direct and/or indirect fitness benefits to helping (table 1).

(i) *Ex-helpers stay*

Censuses of ex-helpers and new nestmates on their nests were conducted twice a day for 3 days after manipulation, and then once every 2 days for two weeks. We also scanned all accessible nests in the aggregation every 2 days for the presence of marked wasps to determine whether ex-helpers were founding or joining other nests. To determine why ex-helpers left, we used linear models (LM) with normal errors: response variable was number of days an ex-helper remained on her nest, and the explanatory variables were as for the egg-laying GLM (above).

(ii) *Ex-helpers forage*

Wasps must leave the nest to forage and so the relative time spent away from the nest provides a good indication of foraging (helping) effort in primitively eusocial insects (e.g. Field *et al.* 2006; S. Sumner, H. Kelstrup & D. Fanelli 2004, personal observation of *P. canadensis*). We compared time spent on nest by ex-helpers before and after manipulation in order to detect any change in foraging effort. We also compared the time spent on the nest by ex-helpers relative to their new nestmates to assess foraging effort of ex-helpers relative to other nestmates. We used parametric (Student's *t*-test) or non-parametric tests (Chi-square (χ^2), Wilcoxon signed-rank test, sign test, Kolmogorov–Smirnov test) as appropriate.

(iii) *Ex-helpers raise non-descendant kin*

Brood were mapped every other day in order to determine the proportion of eggs, small larvae and large larvae that survived. We tested for differences in survivorship among brood categories using a GLM with proportion of brood surviving as the response variable. We then identified factors explaining brood survivorship using a second GLM with quasi-binomial

errors to correct for overdispersion (Crawley 2007). Three models were analysed with proportion of eggs, small larvae and large larvae that survived as response variables. Full models consisted of nest factors (emergence of new nestmates, whether new nestmates stayed, number of cells, removal date) and ex-helper factors (ex-helper stayed, ex-helper laid, body size and age).

(d) *Statistics*

Means ± standard errors are reported. For all GLMs and LMs the significance of each term was assessed by stepwise elimination from the full model, with the significance of the contribution of each term being assessed by the change in deviance with $p = 0.05$ as a significance cut-off. Minimum adequate models in which only significant terms remained are reported. All analyses were conducted in the statistical package R v2.9.0 (R Development Core Team 2009).

3. RESULTS

All ex-helpers were observed foraging before manipulation. They spent on average 63 ± 5.7 per cent (mean ± s.e.) of their time on their nests in the 3 days prior to manipulation. There was no significant difference between the amount of time (Wilcoxon signed-rank: $p = 0.87$) or distribution of time (Kolmogorov–Smirnov: $p = 0.99$) spent on the nest by young or old females (old = $64 \pm 7.7\%$, $n = 15$; young = $61 \pm 8.7\%$, $n = 16$). This indicates that young and old ex-helpers invested equally in foraging. Moreover, ex-helpers were not at all aggressive to other nestmates before manipulation, indicating that they were low-ranked foragers.

Before manipulation, ex-helpers were unlikely to be reproductively mature. The young and old wasps that were removed had little ovarian development (mean size of largest egg = 0.6 ± 0.08 mm, $n = 55$), and only 2 females (3.6%) had mature eggs. These two wasps were 8–11 days old, but in contrast to the young ex-helpers used in our experiment, they were not observed foraging and they *had* behaved aggressively towards other nestmates. Young females had significant reproductive development relative to old females (mean largest egg (mm): old = 0.16 ± 0.055, young = 0.84 ± 0.09; Wilcoxon signed-rank: $p < 0.0001$). Fifty-two per cent of all females were mated (62% and 42% of old and young females, respectively, were mated; NS difference between age groups $\chi^2 = 0.91$, d.f. = 1, $p = 0.34$).

After manipulation, only nine (29%) ex-helpers stayed on their nests until the end of their monitoring period (two weeks). This is not significantly different from the expected disappearance rate for foragers over 14 days $(1 - (1 - 0.07)^{14} = 64\%$: based on 7 per cent chance of
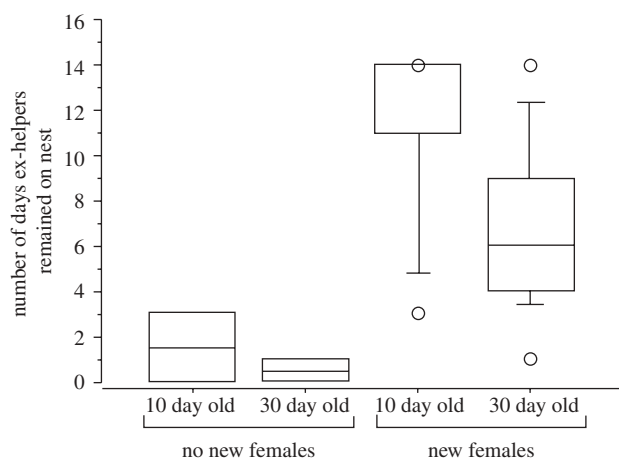
Figure 1. Time (in number of days) that ex-helpers remained at their nests after manipulation for nests that produced new nestmates during the experimental period ('new females') compared with those that did not ('no new females').

disappearing per day; Sumner *et al.* 2007). Of the wasps that left their nests, three could be accounted for. One old ex-helper joined another nest. Another two ex-helpers founded new nests with other (unmarked) females within the monitored area. Some ex-helpers acquired new nestmates through the emergence of new adults ($n = 26$), or by other ex-helpers joining from other experimental nests ($n = 1$ old ex-helper). Average group sizes by the end of the manipulation experiment were 6.23 ± 0.73 females per nest, range $1-15$.

Hypothesis 1: Ex-helpers can lay eggs; young females are more able to lay eggs than old.

Eight of the nine ex-helpers that were present on their nests at the end of the experiment were collected. All eight were inseminated, had mature eggs and had larger eggs than their nestmates (ex-helper mean largest egg (mm) = 2.28 ± 0.066, $n = 8$; new nestmate mean largest egg = 0.69 ± 0.12, $n = 33$; t-test: $t_{8,33}$ $p < 0.0001$), indicating that ex-helpers had become the dominant egg-layers on their nests. Egg size in ex-helpers was significantly different from that of removed females (Wilcoxon signed-rank: $p < 0.0001$). Thus, ex-helpers had developed their ovaries in response to the manipulation. The two ex-helpers who founded new nests with other (unmarked) females had also started to develop their ovaries (largest eggs = 0.91 and 1.04 mm). These data suggest that ex-helpers are capable of developing their ovaries.

Brood maps revealed that 42 per cent of ex-helpers (13 out of 31) laid eggs on their natal nests (includes two females who laid before disappearing). Variability in egg-laying ability is explained by age: old females were less likely to become egg-layers than young females (62.5% (11/16) of young females laid eggs; 13% (2/15) of old females laid eggs: $\chi^2 = 4.25$, GLM: $p = 0.039$). This suggests there is an age-related decline in reproductive ability.

Hypotheses 2 and 3: Direct and/or indirect fitness benefits.

(i) *Ex-helpers stay*
Two variables explained why ex-helpers stayed. On nests that did not acquire any new nestmates during the experiment, all ex-helpers disappeared from their nests within

3 days (mean = 1 ± 0.71 days, $n = 4$) of manipulation. In contrast, on nests that did acquire new nestmates, ex-helpers stayed on their nests for 9.29 ± 0.84 days ($n = 27$). This difference was significant (LM: $p < 0.0001$; figure 1). Ex-helpers were also likely to stay if they were young: 50 per cent of young and 6.67 per cent of old ex-helpers were present on their nests at the end of the experimental period (LM: $p < 0.0001$). Thus, ex-helpers were more likely to stay on their nest if they acquired new nestmates and if they were young, providing support for direct fitness benefits (see table 1, prediction (i)).

(ii) *Foraging effort*
After manipulation, ex-helpers spent on average 85.6 ± 3.5 per cent of their time on their nests. This is significantly different from before manipulation ($63 \pm 5.7\%$, Kolmogorov–Smirnov: $p < 0.01$), but was due only to a change in foraging effort by young ex-helpers, who spent more time on their nests after removals than before ($61 \pm 8.7\%$ before versus $87.5 \pm 5.0\%$ after, sign test: $p = 0.023$). Old ex-helpers did not differ in the time they spent on the nest ($64 \pm 7.7\%$ before versus $68.9 \pm 9.1\%$ after, sign test: $p = 0.77$). Thus, young females reduced their foraging effort in response to manipulation, but old females did not, supporting the predictions for direct fitness (see table 1, prediction (ii)).

We compared the time that ex-helpers spent on their nests relative to their new nestmates in order to determine relative investment in foraging. Young ex-helpers spent more time on their nests than their new nestmates ($t_{16,16}$(paired) = 2.46, $p = 0.026$), but old ex-helpers did not ($t_{15,15}$(paired) = 0.705, $p = 0.25$). Thus, old ex-helpers invested similar foraging effort to new nestmates, therefore maximizing indirect fitness, but young ex-helpers invested less than their new nestmates, as expected if they are investing in direct reproduction (see table 1, prediction (ii)).

(iii) *Fate of non-descendant kin*
On average, survivorship of brood across all nests was low ($11.9 \pm 2.6\%$, $n = 31$), although variation in survival among the different brood classes was high, with significantly more large larvae surviving ($40.1 \pm 6.89\%$) than small larvae ($12.3 \pm 4.01\%$) or eggs ($5.75 \pm 1.98\%$; GLM: $t = 5.6$, d.f. = 87, $p = <0.0001$). Survivorship of eggs and small larvae did not differ (GLM: $t = 1.16$, d.f. = 87, $p = 0.25$). Ex-helpers and their new nestmates therefore raised older brood at the expense of the younger brood. Large larvae survivorship was positively associated with the presence of new helpers (GLM: $F = 5.40$, $p = 0.028$). Small larvae survivorship was positively associated with egg-laying by ex-helpers (GLM: $F = 30.0$, $p < 0.0001$) and size of ex-helpers (GLM: $F = 13.1$, $p < 0.001$; see table 3 for details). These results suggest that ex-helpers who become egg-layers on their nests invest primarily in the older brood, as predicted if they are seeking a worker force, but that ex-helper quality (size) and presence of nestmate helpers determine brood survival.

4. DISCUSSION
Determining the factors that constrain independent reproduction allows us to understand what limits an

Table 3. Brood survivorship: average percentages $\pm$ standard errors of brood that were still alive at the end of the experiment in nests grouped by the two categorical variables that significantly explained brood survivorship. Bold script highlights significant explanatory variables for a particular brood category, as revealed by the GLM model. See text for details.

explanatory variables (no. of nests)	brood category		
	eggs	small larvae	large larvae
new helpers left (10)	0.96 $\pm$ 0.96	4.81 $\pm$ 3.10	**25.3 $\pm$ 12.7**
new helpers stayed (21)	8.03 $\pm$ 2.78	15.5 $\pm$ 5.56	**46.5 $\pm$ 8.01**
ex-helper did not lay eggs (18)	1.93 $\pm$ 0.99	**2.08 $\pm$ 1.5**	21.9 $\pm$ 6.99
ex-helper laid eggs (13)	11.0 $\pm$ 4.18	**27.7 $\pm$ 7.79**	63.92 $\pm$ 10.02

individual's reproductive decision, while determining the relative importance of indirect and direct fitness reveals how an individual might benefit from helping. We tackled these two factors by relaxing the ecological constraints on independent breeding for helpers of a primitively eusocial wasp. We removed all nestmates except one helper and provided the remaining ex-helper with the opportunity to become a breeder, but without the constraints of establishing a new nest alone. We predicted that ex-helpers would mature their ovaries and become egg-layers if there are no reproductive constraints. We found large but explicable variation in the behaviour of ex-helpers: in general, those who laid eggs were young and those who did not lay were old. However, a surprisingly large proportion (71%) of ex-helpers disappeared from their nests with or without egg-laying. This rate was comparable to that expected of foragers on unmanipulated nests (see §3). However, the young ex-helpers foraged little and so we cannot attribute their disappearance solely to forage-related mortality. Old ex-helpers continued foraging and so forage-related mortality may explain their high disappearance rate. Ex-helpers therefore appear to be deciding their strategy based on personal reproductive constraints and expected direct and indirect fitness benefits. Until now, few studies have been able to separate direct and indirect fitness benefits in this way (Clutton-Brock *et al.* 2001). We were able to go some way towards achieving this because of the unusual patterns of reproductive constraints.

(a) Constraints on independent nesting
Most studies on helping behaviour have focused on how ecology may constrain independent breeding (e.g. where the high costs of solitary nesting may explain helping behaviour; Emlen 1997; Cockburn 1998). Ecological constraints appear to be very strong in *P. canadensis* as only 2 to 3 per cent of single female nests survive until brood emergence (West 1967; Ito 1995). Failure is probably due to the prolonged period of care prior to the emergence of the first helpers (egg to adult development period is approx. 40 days; S. Sumner 2002, personal

observation), when the single foundress must leave her nest unprotected while she forages, which in itself carries high mortality risks. Constraints on independent nesting are evident in cooperatively breeding vertebrates (Clutton-Brock 2002; Heg *et al.* 2006) and other primitively eusocial insects, where high nest density or the presence of nestmates reduces predation risk (Gunnels *et al.* 2007; Zammit *et al.* 2008).

There is little evidence that helpers in primitively eusocial insects are reproductively constrained, since helpers usually adopt their vacant nests as egg-layers when given the opportunity (Bull & Schwarz 1996; Field & Foster 1999; Langer *et al.* 2004). Our study is therefore unusual in demonstrating that reproductive constraints can exist in primitively eusocial insects and are important in shaping an individual's decision on choice of reproductive strategy. The age-dependent decrease in reproductive potential in *P. canadensis* supports the observations of West-Eberhard (1969) and Jeanne (1972) on tropical polistine wasps, but contrasts with the gerontocratic patterns observed in temperate *Polistes*, where the oldest helpers are most likely to become egg-layers (Strassmann & Meyer 1983; Hughes & Strassmann 1988). It is possible that the ex-helpers who did not lay eggs were biased to be non-reproductive helpers through pre-imaginal caste differentiation (Hunt *et al.* 2007). Once we account for the age effect, pre-imaginal caste biasing would only account for 3/14 ex-helpers who were young and had new nestmates, but still left their nests. This low proportion (21%) is unlikely to explain why the vast majority of females become helpers in this species, indicating that any pre-imaginal caste biasing is mild and unlikely to play a major role in determining helping behaviour (see also Solis & Strassmann 1990). The age-determined reproductive constraints may serve to reduce conflict over reproduction among potentially long-lived individuals and serve as a mechanism for maintaining helping behaviour. Studies on other tropical polistines (e.g. *Mischocyttarus drewensii*; Jeanne 1972) will reveal whether our observations are of a phenomenon specific to tropical species.

(b) Direct or indirect fitness drives helping behaviour
Recent evidence suggests that direct fitness benefits may be important in primitively eusocial wasps, where helpers are hopeful inheritors of the dominant position (Field & Cant 2007). Once we account for the age constraints on reproduction, the majority of our ex-helpers (11/16) gained direct fitness by laying eggs (table 1, prediction (i)). Young ex-helpers foraged little, as predicted if direct benefits are important, as foraging is risky (table 1, prediction (ii)). Where direct benefits are important, care of non-descendant brood serves to augment group size, which has survival and productivity benefits (Shreeves & Field 2002). Our data support this as the presence of new nestmates enhanced brood survival, probably because new nestmates foraged. Raising the oldest brood provides a new worker force quickly (Rabenold 1985; Ligon & Ligon 1987; table 1, prediction (iii)). In support of this, 63 per cent of large larvae survived on nests where the ex-helper laid eggs. Moreover, ex-helpers left if they were unlikely to acquire new nestmates, suggesting that ex-helpers can assess the potential for a future group from the brood

(Queller 1996; Field *et al.* 1998). Interestingly, brood also survived better with large ex-helpers. Queens are on average larger than helpers in this species (S. Sumner, H. Kelstrup & D. Fanelli 2004, unpublished data), and so size is likely to be an indicator of female quality. In conclusion, direct benefits appear to be of paramount importance in explaining why young *P. canadensis* helpers help. Any brood-rearing is likely to be for group augmentation rather than indirect fitness *per se*.

If indirect fitness were of prime importance, all females (old and young) would have stayed on their nests to raise non-descendant kin, especially on nests where new nest-mate helpers (and replacement queens) emerged (table 1, prediction (i)). Our data do not support this, which was particularly surprising for old ex-helpers who are reproductively challenged: since indirect fitness from raising non-descendant kin is the best old females could hope for, they should have remained on their nests as helpers. The old ex-helpers that did stay invested the same foraging effort before and after manipulation, which may in part explain why so many disappeared (i.e. through forage-related mortality). If they did survive, though, they gained indirect fitness as well as potentially insuring the fitness of their removed nestmates by providing continued care to part-reared brood (Queller 1989; Gadagkar 1990). Life insurance-based models are particularly relevant to helping behaviour in eusocial insects, where adult lifespan is short relative to the time taken for offspring to become independent such that solitary nesting has a very low success rate (Queller 1996). To date, there has been mixed evidence for insurance-based advantages in primitively eusocial insects (Field *et al.* 2000; Shreeves *et al.* 2003; Smith *et al.* 2003; Tibbetts & Reeve 2003; Nonacs *et al.* 2006). Considering that survivorship of single foundress nests is poor in *P. canadensis*, even a very low level of insurance could theoretically select for helping behaviour in this species. We were not able to assess this directly from our study as the experiments were terminated before most of the brood reached adulthood. However, given the high survivorship of the larger brood, insurance-based advantages could be an important component of helping behaviour in *P. canadensis* (table 1, prediction (iii)). Future work should explore this by allowing brood on manipulated nests to mature to adulthood, and by comparing productivity in group-size-matched manipulated and unmanipulated nests (e.g. Field *et al.* 2000; Shreeves *et al.* 2003).

5. CONCLUSIONS

Determining the relative importance of constraints on independent breeding and the benefits of helping is a major stumbling block in understanding helping behaviour. *Polistes canadensis* females are constrained by both ecological and reproductive constraints. Because reproductive constraints vary with age, we were able to make specific predictions about the relative importance of direct and indirect benefits to young and old helpers. Our results suggest that direct fitness (personal reproduction) may be more important than indirect fitness (raising non-descendant kin) benefits for young females: they have the option of waiting to become a breeder on their natal nest and meanwhile gaining indirect fitness by helping raise non-descendant kin, or they can leave and

co-found a new nest but be unsure of the indirect and/or direct fitness payoffs. For old females, maximizing indirect fitness by undertaking risky foraging on their natal nest is likely to be the best (only) option as they are reproductively constrained.

In conclusion, helping behaviour in *P. canadensis* can be explained primarily through direct fitness benefits, but the relative importance of direct fitness benefits to individual helpers varies because of age-dependent reproductive constraints. Let us imagine that we had considered only the direct fitness hypothesis in this study. Without knowing how age constrains reproduction, we would have concluded that direct fitness benefits were not strongly driving helping behaviour, as 71 per cent of the ex-helpers appeared to abandon their nests. Our study therefore highlights the importance of using an integrated approach to studying helping behaviour, where several competing explanations are considered simultaneously in order to disentangle interactions between the different driving forces.

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