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REPORT

Social life-history response to individual immune challenge of workers of *Bombus terrestris* L.: a possible new cooperative phenomenon

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Abstract

Parasites typically reduce host survival or fecundity. To minimize fitness loss, hosts can make temporal adjustments of their reproductive effort. To date such plastic shifts of life-history traits in response to parasitism are only known from solitary organisms where infected individuals can react by themselves. In the case of social insects, where brood care and reproductive effort is shared between reproductive individuals (typically the queen) and workers, adjustments of the reproductive effort would depend on collective decision-making. We tested for this possibility by experimentally activating the immune response of individual workers in colonies of the bumblebee, *Bombus terrestris* L. This induction resulted, in combination with environmental conditions, in a reduction of fitness of the social unity (i.e. colony success, measured by number and biomass of offspring) and a collective response towards earlier reproduction. As both phenomena are expressed at the level of the colony, the result suggests that key elements of the use of immune defence have been maintained through the evolutionary transition to sociality.

Keywords

Immunity, life history, parasite resistance, reproduction, social insect.

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INTRODUCTION

Parasitic infection typically affects, to the detriment of the host's fitness, one or several of the three key parameters of host life history, growth, survival and fecundity, which, together, determine host life history (Roff 1992; Stearns 1992). If infection is unavoidable, the host can reduce its loss of fitness by controlling the parasite through its immune system. Although this may keep infections at low levels, the immune response comes at a cost (Moret & Schmid-Hempel 2000). Alternatively, the host could reduce the magnitude of adverse effects by facultative adjustment of its life-history parameters (Minchella 1985; Hochberg *et al.* 1992; Forbes 1993; Perrin *et al.* 1996; Agnew *et al.* 2000). For example, freshwater snails that become infected by castrating parasites alter their life history and accelerate the time of maturity. In this way, the snails manage to produce at least some offspring before the effect of castration sets in. These life-history changes can also be elicited by exposing the snails to water that has only contained the parasite but without actual infection (Minchella 1985).

Until at present, such facultative modification of host life history in response to parasitism is only known from solitary organisms, such as snails, where the individual itself is infected and reacts accordingly (Minchella 1985; Forbes 1993; Adamo 1999). No such response is known from social animals, although socially living organisms share many similarities in their life-history traits with solitary organisms. However, fitness in social animals is the result of cooperative effort (Oster & Wilson 1978). Therefore, any shift in the reproductive schedule in response to parasitism in a social system would depend on some kind of collective decision-making, which must be a considerably more complex phenomenon. We here investigate whether, despite this added complexity, social animals have the ability to cooperatively vary life-history parameters in response to parasitism in similar ways as known from solitary organism (Minchella 1985; Forbes 1993; Adamo 1999). In particular, we use colonies of the primitively eusocial bumblebee, *Bombus terrestris* L., as a model system and immune elicitors to mimic parasitic infections.

In *B. terrestris*, colony survival, growth and reproduction are based, both, on the success of the founding queen and on the efforts of her workers (Oster & Wilson 1978). Queens of this species are typically singly mated (Schmid-Hempel & Schmid-Hempel 2000), and found their colony in

spring. Then colonies grow by a cumulative production of new workers until reproduction takes place in late summer. At this point, sexual offspring, i.e. the males and young daughter queens, are produced and leave the colony to mate. Bumblebees have annual colonies and only the daughter queens enter hibernation. Bumblebees are known to be host to a wide range of parasites that mostly infect the workers (Shykoff & Schmid-Hempel 1991; Schmid-Hempel 1998). As it is the case in other insects, bumblebees can respond to such infections by activating their immune system (Rees *et al.* 1996; Schmid-Hempel 1998). Experimentally, an immune response can be elicited, for example, by injecting a small dose of bacterial surface molecules (lipopolysaccharides, LPS), which results, among other things, in the measurable production of antibacterial peptides (Moret & Schmid-Hempel 2000). LPS are non-pathogenic and non-living surface molecules extracted from *Escherichia coli*, which are highly immunogenic (Söderhäll 1982; Rattclife *et al.* 1985; Jomori *et al.* 1990). Here, we have used this technique to experimentally mimic an environment with a high and persistent level of parasitism to test for eventual life-history variation. In particular, we immune challenged the workers with LPS, administered over prolonged periods of time during the colony cycle. As we have shown previously, this immune challenge has no direct pathogenic effects but activates the immune system at a cost of individual survival (Moret & Schmid-Hempel 2000). In social insects, such an increase in worker mortality is akin to the effect of castration in solitary animals, because without the help of her workers, the queen will not manage to reproduce. Under the hypothesis of a facultative response to parasitism, similar in kind to that observed in solitary animals, we expected to observe a shift in reproductive timing. In particular, immune challenged colonies should reproduce earlier than control colonies, as this could reduce the anticipated loss of reproductive capacity associated with increased worker mortality rates. We also expected that such facultative responses would primarily emerge under harsh conditions, where worker survival is paramount to eventual colony fitness.

MATERIAL AND METHODS

Queens of *B. terrestris* collected in the field around Zurich in spring 2000 were stimulated to start a colony in the laboratory. We then used a split-colony design (Schmid-Hempel & Schmid-Hempel 1998) to test the hypothesis as follows. As soon as the workers of the first brood had emerged, the colonies were transferred into a nest divided into two equal sides with a fine wire mesh. Half of the workers and the brood were each carefully placed into one of the two nest sides. The wire mesh prevented exchange of food, workers and brood but allowed effective pheromonal and other communications between the two sides. The queen was placed randomly in one side of the nest and then swapped from one side to the other every 24 h to ensure egg laying in both halves of the nest and colony cohesion through the physical presence of the queen. Bumblebees of each side were fed *ad libitum* with sugar water and pollen. Treatment started as soon as the colony was transferred into the split nest.

Harsh and favourable environmental conditions were experimentally generated by keeping the colonies at ambient temperature of 18 and 24 °C, respectively, for their entire life cycle. Due to practical limitations, the study was conducted in two successive experiments: first at 18 °C and then at 24 °C. For this reason, data were analysed within temperature groups only. Previous experience suggests that the lower temperature is a rather marginal condition for *B. terrestris* colonies, as it forces workers to spend additional time and energy to heat the brood (Plowright & Pendrell 1977; Heinrich 1979; E. Benelli and P. Schmid-Hempel, unpublished data). This demanding activity highly constrains resource acquisition by workers. The higher temperature is clearly more benign and quite typical for the natural habitat and during a normal season. For each colony used in this study, one side of the experimentally split nest was randomly assigned to the treatment 'challenged', while the other was kept as the control. Every week, and for the whole life cycle of the colony, 70–80% of the workers in both sides were randomly selected to be injected with 5 μl of insect Ringer. While the control side workers received Ringer only, the injection of the challenged workers additionally contained a small dose (0.5 mg ml^{-1}) of bacterial surface molecules (LPS; Sigma L-2755, St. Louis, MO, USA). LPS elicits a persistent response of production of antibacterial peptides over many hours (Söderhäll 1982; Rattclife *et al.* 1985; Kato *et al.* 1994). The challenge of 70–80% of the workers aimed to mimic a severe microbial infection that spread rapidly by affecting most of the workers. Note that for this experiment the mother queen was never injected. For the injections, the workers were chilled and immobilized on ice. Injection was through the pleural membrane between the second and the third tergite, using a sterilized glass capillary that had been pulled out to a fine point. Hence, in this experimental paradigm, the two halves of each nest represented the same colony headed by the same mother queen and sister workers but which experienced a different environment (challenged vs. control). In addition, the whole colony lived either in a harsh or favourable environment.

Every week, 4 days after injections, a random sample of 10% of workers from both sides was removed and tested for the antibacterial activity of their haemolymph. The workers were chilled on ice and 10 μl of haemolymph per worker was taken using a sterilized glass capillary. Each

haemolymph sample was put into a 0.5 ml Eppendorf tube containing 50 µl of cold Ringer's solution. The tubes were stored at −80 °C until the antibacterial test could be carried out. Antibacterial test plates (diameter 9 cm; Sterilin, Stone, UK) were prepared by adding 0.05 ml of live *Arthrobacter globiformis* bacteria suspension (10^7 cells ml^{-1}) to 5 ml of sterile broth medium (10 g bactotryptone, 5 g yeast extract, 10 g NaCl, 1000 ml of distilled water, pH 7.5), with 1% of bacto-agar at 45 °C. Plates were swirled to disperse the bacteria and left to settle at room temperature. Ten holes (diameter: 2 mm) per plate were made in the agar, and 2 µl of the haemolymph solution was added per hole for the test. The plates were then incubated at 28 °C overnight. The areas of these zones of inhibition were used as a measure of the strength of antibacterial activity in the haemolymph. We measured the mean of the minimum and maximum diameters (in mm) of each zone of inhibition and calculated the corresponding area [with the formula: $\pi(\text{mean diameter}/2)^2$] and used it as our data point.

Body condition of the weekly sampled workers was estimated by measuring the size of their fat body, using an ether extraction method (David *et al.* 1975; Ellers 1996). The fat body of insects is considered to be an important storage organ and the principal site of synthesis of immune proteins (Zachary & Hoffmann 1984; Hultmark 1993). Percentage values are given as dry mass of fat body/dry mass of the abdomen. The size of the colonies was estimated by counting the total number of workers in both sides of the nest. In addition, the number of young males and queens produced from the mother queen on each side and their fresh mass were recorded as an indication of the reproductive success. The date of emergence of sexuals was always evaluated in days since the start of the treatment for the colony (i.e. after the first brood had hatched).

Within each environment (harsh vs. favourable), the data obtained from each of the two nest sides were treated as a paired sample (i.e. one pair per colony) and therefore treatment effect on measures such as average antimicrobial activity of worker haemolymph was analysed with a Wilcoxon's matched pairs sign test validated with Bonferroni's correction for repeated tests within the same data set (here for worker's antibacterial activity and fat body). The timing of production of sexuals between the two sides was compared within each environmental condition with a time-dependent Cox regression analysis. Cox regression allows comparing the timing of the production of sexuals between treatments by calculating the expected cumulative probability function of an event to happen at time t as $f(t) = [f_0(t)]^p$. In the current case, such an event is the emergence of a sexual at time t during the colony cycle. Furthermore, the term, $f_0(t)$, represents a baseline probability function (in the model being typically calculated as to decrease with time) that is calculated from the observed time course of events (i.e. the observed emergence of sexuals). While $f_0(t)$ depends only on time, the term $p = e^{(a+bt)}$ is estimated so as to take into account the effect of treatment. In particular, the term a reflects the main effect of treatment whereas b is the term for the statistical interaction of treatment with time. The statistical test compares the observed time course of producing sexuals with that predicted under the appropriate null model of no difference among treatments (for more details, see Norusis 1994). We could furthermore use these estimates to plot the fecundity functions, $m(t)$, where $m(t) = 1 - f(t)$ in order to reflect the biologically given increase in the accumulation of sexuals over time, with these coefficients produced by the statistical model (see Fig. 1). Differences were further analysed by testing the distribution of time to maturity,

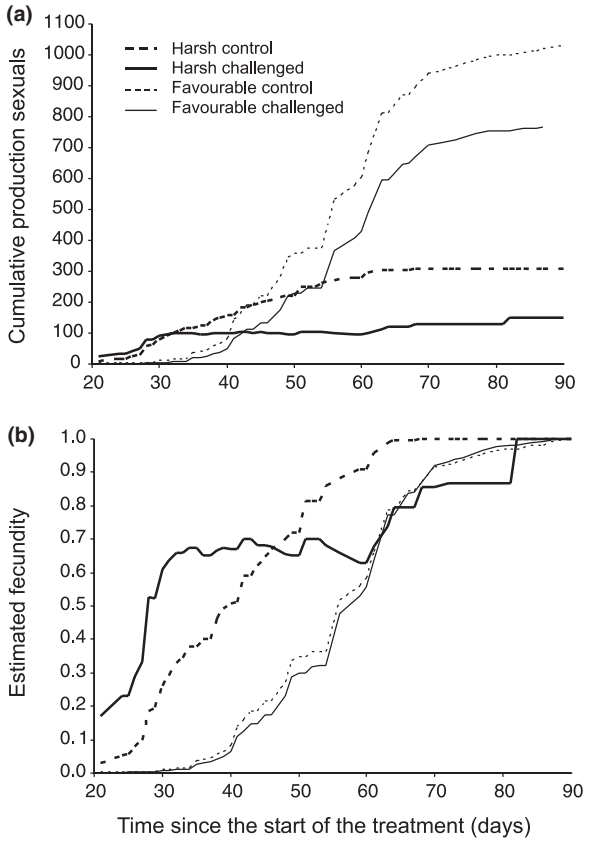

Figure 1 Reproductive effort of all the colonies together according to environmental conditions and immune treatments. (a) The cumulative production of sexuals (males and young queens) over time for all colonies in a particular environment and treatment, and (b) the estimated fecundity functions (see Methods) over time for all colonies in a particular environment and treatment estimated from the time-dependent Cox regression model. The functions represent the probabilities at which sexuals are produced at time t (days after start of treatment). The two functions for the harsh environment are significantly different (see text for statistical details).

defined as the emergence of the median sexual, for the colony as a whole with a two samples Kolmogorov–Smirnov test. All the analyses were carried out using SPSS 6.1.1 (SPSS Inc., Chicago, IL, USA).

RESULTS

Eleven and 15 colonies were, respectively, exposed to favourable and harsh temperature conditions. While we could use data from all the colonies exposed to the favourable condition, data from 13 colonies exposed to the harsh condition could be used. The other two colonies were excluded from the data set because the mother queens of these colonies died within the 21st day of the experimental challenge.

As expected from the immune-challenge treatment, workers in the challenged halves showed higher antibacterial activity than workers in the control halves over the entire colony cycle under both environmental conditions (Table 1). The challenge therefore resulted in workers activating and utilizing their immune system for the duration of the experiment. However, within each colony, the number of workers produced was similar in the challenged and the control side (Table 1). Surprisingly, the body condition of workers, estimated by the relative size of their fat body (percentage fat), did not show any difference because of the treatment (Table 1).

Harsh condition *per se* (i.e. independent of treatment) reduced the reproductive output of colonies as expected (Table 1). In addition, immune challenging the workers had an effect on reproductive success of the entire colony as already reported elsewhere (Moret & Schmid-Hempel 2001). In particular, under harsh conditions, fewer males plus queens were produced in the challenged sides as compared with the control sides (Table 1). Using only the number of queens produced as a fitness measure, there is a non-significant effect probably because of the small number of queens produced overall (Table 1). Immune challenge resulted in a comparable effect under favourable conditions, although, as expected, the magnitude of the effect was not as strong. Only the number of queens produced in the challenged sides was reduced compared with the control sides while the number of males and the biomass of sexuals did not differ (Table 1).

With respect to the hypothesized shift in life history, environmental conditions alone had an effect, because the colonies in harsh conditions produced their males and young queens earlier than colonies in favourable condition (Fig. 1a; comparing the two environments, Kolmogorov–Smirnov $\chi = 1.878$, d.f. $= 1$, $P = 0.002$). In addition, and as expected from the hypothesis, the immune challenge also caused an environment-dependent change in life history, because the immune challenge led to earlier reproduction in the harsh but not in the favourable environment (Fig. 1a; see statistics below). For colonies under harsh conditions, time-dependent Cox regression analysis estimated the parameters for the predicted function $f(t)$ (see Methods) as $a = -1.62$ (+1.62) and $b = 0.035$ (−0.035) for the control

Table 1 Colony characteristics (means, SE) calculated from data collected during the whole life cycle of the colonies placed in harsh (18 °C) and favourable (24 °C) temperature conditions. *P*-value refers to the effect of the immune treatment (Wilcoxon's matched pairs sign test). *n* is the number of colonies (sample size)

Temperature	Variables	Control	Challenge	*n*	*P*-value*
18 °C	Number of workers†	25.54 (2.81)	25.15 (3.39)	13	0.780
	Worker ZI (mm^2) [172]	8.24 (0.57)	95.73 (0.31)	11	**0.003**$^{(1)}$
	Worker fat body (%) [172]	9.13 (0.21)	8.80 (0.26)	11	0.182$^{(1)}$
	Males [440]	22.92 (8.39)	10.92 (4.99)	13	**0.036**
	Queens [17]	0.69 (0.47)	0.61 (0.26)	13	0.715
	Biomass (mg)‡ [457]	6367.82 (2307.46)	2907.80 (1152.61)	13	**0.028**
24 °C	Number of workers	204.18 (19.63)	186.18 (22.00)	11	0.307
	Worker ZI (mm^2) [198]	36.21 (0.20)	77.60 (0.48)	11	**0.016**$^{(1)}$
	Worker fat body (%) [198]	12.02 (0.40)	12.40 (0.86)	11	0.328$^{(1)}$
	Males [1754]	89.45 (15.93)	70.00 (12.23)	11	0.109
	Queens [54]	4.18 (1.58)	0.73 (0.63)	11	**0.012**
	Biomass (mg) [1808]	22 952.45 (4219.18)	17 473.54 (3398.34)	11	0.109

*P-values with $^{(1)}$ evaluated with Bonferroni-correction for repeated measurements ($P_{crit} = 0.025$). Values in bold highlight significant effects of the treatment.

†'Number of workers' is the total number of workers produced by the colonies.

‡'Biomass' is the cumulative fresh mass of males and queens together.

Values in square brackets are sample sizes for all the colonies (number of workers, males or queens).

ZI: average diameter from zone-of-inhibition assay.

and challenged sides, respectively. Hence, over the duration of the experiment, the number of sexuals hatching per day (i.e. the rate at which sexuals were produced) in the challenged side was a significant 2.24-fold that of the control side (i.e. the odds ratio for the treatment effect; Wald statistic = 30.70, d.f. = 1, $P < 0.0001$) (the treatment main effect). As to the interaction effects, the two curves did not have the same slopes over time (interaction 'time × treatment': odds ratio = 0.98, Wald statistic = 24.61, d.f. = 1, $P < 0.0001$). The estimates of the model allowed plotting the 'fecundity curves' (see Methods) of the two halves (Fig. 1b). Compared with control sides and commensurate with the statistical test, fecundity in challenged sides was clearly accelerated early in the life cycle before a marked decline followed, from day 30 to 60 since the start of the treatment. Then fecundity increased slightly at the end of the colony life cycle (Fig. 1b). These patterns were not observed for colonies placed in favourable condition (Fig. 1b) where the statistical model yielded $a = 0.341$ (-0.341) and $b = -0.005$ ($+0.005$) for the control and challenged sides, respectively. Under favourable condition, the fecundity curves were similar for both challenged and control sides (Fig. 1b; 'Treatment': odds ratio = 0.84, Wald statistic = 2.02, d.f. = 1, $P = 0.155$) and for their slopes over time ('time × treatment': odds ratio = 1, Wald statistic = 1.37, d.f. = 1, $P = 0.240$).

DISCUSSION

In this study, we found a large and significant condition-dependent effect on the reproductive success and the life history of entire colonies of the bumblebee, *B. terrestris*, when individual workers were immune challenged. For example, challenging individual workers leads to a fitness cost for the entire social group (Moret & Schmid-Hempel 2001), because the colony produces fewer sexual offspring, particularly, under harsh environmental conditions (Table 1). Indeed, shifting from favourable to harsh temperature conditions, the cost of the immune defence affected first the production of queens (Table 1; temperature 24 °C) that are the biggest individuals and might be the most costly sexual offspring to produce. Then in harsh conditions, the colonies hardly managed to produce queens in each side of the nest and the cost of the immune challenge affected the production of males (Table 1; temperature 18 °C) that are the second largest individuals in bumblebee colonies. The difference between environments suggests that compensatory food intake or the absence of demanding concurrent needs (e.g. thermoregulation), such as is the case under favourable conditions, could typically mask the costs of using the immune system not only for individuals (Moret & Schmid-Hempel 2000) but also for the entire colony. Interestingly, neither the number of workers nor their body condition (fat body) was affected by the immune challenge (Table 1). While the exact physiological reasons for this are unclear, it is possible that favourable conditions could have allowed challenged workers to compensate for the cost of immune defence. In contrast, in harsh conditions, where compensation is constrained, workers could have reached the minimum threshold of fat content below which individual survival is jeopardized (Moret & Schmid-Hempel 2000; Rolff *et al.* 2000). Extrapolating from this result, it is then possible that under even harsher temperature conditions than this set in our experiment, the cost of the immune challenge could affect the number of workers produced.

In addition to fitness costs for the entire colony, an immune challenge of individual workers also lead to facultative changes in colony life history, again, only under harsh conditions (i.e. low ambient temperature) (Fig. 1). In particular, colonies with immune-challenged workers while producing fewer sexuals altogether increased their rate of production of sexuals (i.e. the treatment main effect). The significant treatment–time interaction effect showed that, at the same time, the average sexual was produced earlier in challenged colonies; hence, a shift in reproductive timing had occurred as is also visible from the fecundity functions in Fig. 1b.

An alternative, more parsimonious, interpretation of the difference between control and challenged sides is that the shift is simply because of lower overall production of sexuals. However, under these circumstances, the fecundity functions of control and challenged sides of colonies estimated by the statistical model underlying Cox regression (Fig. 1b) should have a similar shape, as the estimate of the model already controls for the difference in the overall number of produced sexuals. Instead, the fecundity function of challenged sides in the harsh environment demonstrates an early acceleration of the reproductive effort compared with control sides (corresponding to the treatment–time interaction effect).

In Hymenopteran insects, complementary sex determination (haplo-diploidy) (Cook & Crozier 1995) gives the mother queen full control over the sex of the offspring. In *B. terrestris*, at some point in the colony cycle, the mother queen switches to lay unfertilized eggs that develop into males; she rarely, if ever, reverts to laying diploid eggs (Duchateau & Velthuis 1988). The occurrence of this 'switch point' then triggers a suite of events in the colony – the reproductive phase of the colony has started. As no more diploid eggs become available, the workers must raise some of the still present diploid larvae into daughter queens. Whether such larvae develop into workers or daughter queens depends, on the one hand, on the feeding regime (Cnaani *et al.* 1997; Peerebom 2000) and, on the other, on the queen stopping to impose a worker development of the

young larvae through a non-volatile pheromone that is spread in the colony by her workers (Röseler 1970; Ribeiro *et al.* 1999; Cnaani *et al.* 2000). Unfortunately, what determines the timing of the switch point is still poorly understood. The general state of the colony and how well it grows clearly plays a role (Müller & Schmid-Hempel 1993), and many events happen very early in the colony life cycle thus making it possible to adjust quickly to a given environment (Müller *et al.* 1992). These mechanisms make it at least obvious why colony reproductive performance is lower under harsh conditions or when workers are challenged. But how could the switch point (the major triggering event) become accelerated under immune challenge? In fact, our finding contrasts in remarkable ways with the results of previous studies, which showed that food limitation alone, under otherwise favourable environmental conditions (i.e. warm temperatures), does not change the reproductive timing of colonies (Schmid-Hempel & Schmid-Hempel 1998), nor did variation in worker mortality as shown by experiments in the sister species *B. lucorum* (Müller & Schmid-Hempel 1992). It thus appears that food limitation or worker mortality as compared with immune challenge have drastically different consequences at the onset of reproductive events in the colony. How this is mediated remains very unclear at present.

The facultative shift in reproductive timing in colonies of *B. terrestris* is similar to the phenomenon of fecundity compensation reported for snails that are infected or challenged by trematodes (Minchella & Loverde 1981; Minchella 1985). Note, however, that in the studies of Minchella & Loverde (1981) it is not known whether the immune system was indeed activated or whether the response was triggered by another signal that indicated the presence of the parasites. Our experimental design ensured that the immune system was activated without the presence of a parasite, as shown by the antibacterial tests. Note that the mechanism underneath this observed change of the colony reproductive effort is not yet known and was beyond the scope of this study. However, recent studies in crickets suggest that the immune system might interact with general physiological processes leading to fecundity compensation (Adamo 1999). With the results of previous work in mind (Moret & Schmid-Hempel 2000), we could hypothesize that the physiological stress to which immune-challenged bumblebees are exposed may influence their individual behaviour, although this would depend on the level of environmental stress that individuals experience. Through the well-known phenomenon of 'amplification' of small differences in social insects (Bonabeau *et al.* 1997), this newly adopted behaviour by several individual workers could result in the observed life-history response at the level of the colony.

Our study demonstrates the existence of some kind of a social response by the colony to potential threats directed towards the individual workers. To our knowledge, this is the first demonstration of such a collective life-history adjustment/modification/change in a social animal in response to immune system activation. We therefore conclude that such plasticity in life-history traits in response to parasitism is not restricted to solitary organisms but has been maintained through the evolutionary transitions towards sociality (Maynard Smith & Szathmary 1995).

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