Punishment and partner switching cause cooperative behaviour in a cleaning mutualism

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Abstract: What are the mechanisms that prevent partners from cheating in potentially cooperative interactions between unrelated individuals? The cleaner fish *Labroides dimidiatus* and a client reef fish both benefit from an interaction as long as the cleaner eats ectoparasites. However, cleaner fish prefer some client mucus, which constitutes cheating. Field observations suggested that clients control such cheating by using punishment (chasing the cleaner) or by switching partners (fleeing from the cleaner). Here, we tested experimentally whether such client behaviours result in cooperative cleaner fish. Cleaners were allowed to feed from Plexiglas plates containing prawn items and fish flake items. A lever attached to the plates allowed us to mimic the behaviours of clients. As cleaners showed a strong preference for prawn over flakes, we taught them that eating their preferred food would cause the plate to either chase them or to flee, while feeding on flakes had no negative consequences. We found a significant shift in cleaner fish foraging behaviour towards flake feeding after a few learning trials. As punishment and terminating an interaction caused cleaners to feed against their preferences in our experiment, we propose that the same client behaviours improve the service quality of cleaners under natural conditions.

Keywords: mutualism, cooperation, punishment, partner choice, biological market, *Labroides dimidiatus*

Introduction

The iterated prisoner's dilemma (IPD) game, with all its many theoretical extensions (Dugatkin 1997, Hammerstein 2003), does not appear to provide a general framework for the evolution and maintenance of cooperation between unrelated individuals. Few examples of intraspecific cooperation fulfil its assumptions (Hammerstein 2003), and no example of interspecific mutualism is thought to solve the IPD (Bergstrom et al. 2003). Instead, a variety of alternative control mechanisms have been proposed, based either on game theoretic modeling or on empirical results. Such alternative mechanisms include

pseudoreciprocity (Conner 1986), the threat of terminating an interaction (Johnstone & Bshary 2002), "passive partner choice" usually called "sanctions" (Kiers et al. 2003), active partner switching (Ferrière et al. 2001, Bshary & Schäffer 2002) and punishment (Clutton-Brock & Parker 1995). The mutualism between the cleaner wrasse *Labroides dimidiatus* and its client reef fish seems to be particularly rich in differing game structures and corresponding control mechanisms (Bshary & Noe 2003, Bshary & Bronstein 2004). While a variety of studies support the mutualistic nature of cleaning interactions in general (Grutter 1999, Grutter and Lester 2002, Cheney and Côté 2003), there is experimental evidence for an important conflict: cleaner fish prefer some mucus over ectoparasites (Grutter & Bshary 2003). Hence, clients face the challenge of how to make cleaners feed against their preference in order to receive a high service quality during interactions.

Predatory clients could counter any mucus feeding by the "threat of reciprocity" (cheating and eating the cleaner, Bshary & Bronstein 2004), and that threat may account for an almost unconditional cooperative behaviour of cleaners towards predators (Bshary 2001). The vast majority of clients, however, does not prey on cleaners (Bshary 2001) and therefore has no means to counter-exploit a cheating cleaner fish. Therefore, the game between cleaners and these non-predatory clients is asymmetric in that clients have no means to threaten a cleaner in response to its cheating (as IPD based strategies like Tit for Tat do). Field observations suggest that these clients use mainly two alternative control mechanisms: resident clients with access to only one cleaning station respond to cleaner fish cheating with aggressive chasing (punishment) of the cleaner (Bshary & Grutter 2002), while visiting clients with access to several cleaning stations respond with immediate flight and visit another cleaning station (partner switching) for their next inspection (Bshary & Schäffer 2002). Here, we test experimentally whether such apparent punishment or partner switching causes cleaner fish to feed against their preference, the essential requirement for a mutually beneficial outcome of interactions.

Methods
The study was carried out in March/April 2003 at the Lizard Island Research Station, Great Barrier Reef, Australia. We caught 24 cleaner fish from the reef surrounding the island. The fish were kept individually in aquaria ranging in size from 69x25x30 cm to 95x35x35 cm. Fish were fed daily with mashed prawn flesh or with a mixture of mashed prawn flesh and fish flakes (referred to as "flakes"), depending on the experimental treatment . The "flakes" mixture was prepared fresh every day with one third volume of flakes and two third volume of prawn. The food was spread on plastic (Plexiglas) plates (Grutter and Bshary 2004). Cleaners learned to feed from the plates within one to three days of exposure. The plates had a variety of uniform colours, grey, beige or white. Each cleaner was exposed to all different colours to become accustomed to the presentation of unfamiliar stimuli (to avoid potentially neophobic cleaners). The experiments began after the fish had been in captivity for at least 20 days.

The experiment consisted of three phases, namely the initial preference test, the subsequent learning phase, and the final foraging test.

1) The initial preference test: we offered the cleaners an unfamiliar plate with seven prawn items and seven flake items. The items were placed within a 5 x 3 grid, each grid cell being 1 x 1 cm in size. The location of the each item (prawn or flake) was determined by random sequences where 0 meant prawn and 1 meant flake. The central grid cell was always left empty (Fig. 1). After 3 trials that allowed cleaners to become familiar with the plates, we conducted the initial preference test. We offered the plate three times to each cleaner and scored the first 7 items eaten. This meant that we could possibly find a 100% preference for either prawn or flakes. We used the total of 21 food items eaten in the three trials to calculate the degree of preference of each cleaner for one food as expressed in % terms eaten of that food type.
2) The learning phase: we split the 24 cleaner fish into three experimental groups of 8 individuals each. In the first group ("control") cleaners were allowed to continue to eat as they chose. In the second group ("client flees") cleaners were trained such that eating the less preferred food items had no consequences while eating a preferred item led to the immediate removal of the plate. The plate was offered to the fish again after 60 s (with the remaining food items on it but the already eaten ones not replaced) until the cleaner ate a second preferred food item. That let to the removal of the plate until the next learning trial 120 min later. In the third group ("client chases") cleaners were trained that whenever a preferred item was eaten, the plate would chase them in the aquarium for about 1-2 seconds. The cleaner was then allowed to forage again until it had eaten its preferred food for a second time, which led to first chasing and then the plate being removed until the next education trial 120 min later. To improve the probability that cleaners might learn what the consequences were of eating either their preferred or less preferred food, we offered two preferred and twelve non-preferred items during the first 3 learning periods. This ensured that cleaners ate also from their less preferred food and therefore could learn that there were no negative consequences of doing so. After another 3 learning trials with the standard 7:7 distribution of food items, the learning phase was terminated.
3) Final foraging experiment: Each cleaner was allowed to interact once with a plate that did not respond to the cleaner's choice of food. Again, we scored the first 7 items eaten, allowing for the possibility of a 100% bias for either food.

The time schedule for each cleaner was the following: Day 1: three introductory trials plus the three preference tests. Day 2: four rounds of learning trials. Day 3: two rounds of learning trials followed by the experimental trials. All items were similar in size, weighing about 0.0002g each. Trials were scheduled so that cleaners received about 0.005-0.01g of food per day. We tested individuals of all three groups simultaneously. Thus, individuals of all three groups were tested with the same batch of flake food.

Wilcoxon matched pair tests were used to evaluate whether each of the three different teaching phases had a significant influence on cleaner fish foraging behaviour in the final experiment as compared to the initial preference tests.

Results

In the initial preference test, all 24 cleaners ate more prawn items than flake items. The least extreme preference observed was 15 prawn items to 6 flake items. Thus our cleaners showed a highly significant preference for prawn (Wilcoxon-Test, n=24, T=0, p < 0.0001). On average, 91% of the first 7 items eaten were prawn items. Therefore, all cleaners could be trained that feeding on flakes had no consequences but feeding on prawn led to either chasing or fleeing.

As the plates did not 'respond' to cleaner fish's foraging behaviour in the final experiment, individuals of all three groups had the opportunity to eat the seven prawn items first and then the seven flake items if still hungry. We found that individuals of the control group significantly increased their initial preference for prawn during the learning trials, ending with a 100% preference for prawn as opposed to a 92% preference for prawn during the initial preference tests (Wilcoxon-Test, n=8, two ties, resulting n=6, T=0, p=0.032, Fig. 2). In contrast, the other two experimental groups altered their foraging behaviour significantly in the opposite direction. Cleaners which had been exposed to plates being removed (fleeing) after they had eaten prawn ate significantly less prawn items during the final experiment than during the initial preference tests (Wilcoxon-Test, n=8, one tie, resulting n=7, T=0, p=0.016, Fig. 2). Similarly, cleaners, which had been exposed to plates chasing (punishing) them if they had eaten prawn ate significantly less prawn items during the final experiment than during the initial preference tests (Wilcoxon-Test, n=8, T=0, p=0.008, Fig. 2).

Discussion

Our experiment was based on the following chain of arguments. First, clients benefit from cleaning interactions if cleaners eat ectoparasites but pay a cost if cleaners feed on mucus. Second, cleaners prefer mucus over parasites in a choice experiment (Grutter & Bshary 2003). Third, clients therefore have to control cleaner fish behaviour in a way that makes cleaners feed against their preference. Fourth, field observations indicated that either client aggression (chasing) towards the cleaner or client fleeing (and visiting a different cleaner for their next inspection) may be such control mechanisms that promote cleaners to feed mostly against their preference (Grutter & Bshary 2003).

In our simple learning experiment, we offered the cleaners the same key stimuli that they also receive under natural conditions (Bshary & Grutter 2002). They were offered a choice between a preferred food type and a less preferred food type. Eating the less preferred food type had no negative consequences whereas eating the preferred food type let to either fleeing of the food source (as clients with access to several cleaning stations do) or to the food source chasing the cleaner (as resident clients with access to only one cleaning station do). These stimuli, provided with the help of a lever attached to a Plexiglas plate, resulted in significant changes in cleaner foraging behaviour after only six teaching sessions. Both fleeing and aggression resulted in increased consumption by the cleaners of the less preferred food items. As the control group increased their preference for the preferred prawn food during the learning trials, the previous result is not due to a sequence effect and a preference shift towards flakes. Thus, fleeing and aggression results in cleaners feeding against their preference. We therefore propose that the partner switching and the aggression used by clients under natural conditions serve

the same purpose, hence causing the cleaners to feed on ectoparasites against their preference for mucus. Our results thus provide the first experimental evidence that terminating an interaction (and subsequent partner switching) and punishment are partner control mechanisms used by animals to promote cooperative behaviour.

As we observed a significant shift towards prawn feeding after only six learning trials, cleaners therefore learned this task very rapidly. This may seem surprising at first as primates often fail to choose in a similar task: when asked to point at either a small amount or a large amount of food, most species point at the large amount even if they receive always the food they had not pointed to (and hence pointing at the smaller food item would yield the larger benefit, summarized by Genty et al. 2004). We propose that cleaners learned this task very rapidly in our experiment because they often may have to forage against their preferences in the wild. As cleaners have more than 2000 interactions per day (Grutter 1997) they receive feedback about the consequences of their actions over 2000 times a day. These are ideal conditions for instrumental conditioning to take place. Cleaners are therefore likely to have learned to vary their own behaviour according to client responses under natural conditions. In our experiment, they only had to apply their knowledge to a new combination of food items (flake and prawn) and to a new form of clients (Plexiglas plates). There is also experimental evidence that cleaners can recognize individual clients (Tebbich et al. 2002), which is a cognitive prerequisite to link their own behaviour to the response of particular clients so that punishment may yield future benefits to the client. Our study also suggests that the cognitive demands for partner control mechanisms like fleeing or punishment may not be so high that the costs constrain the evolution of these mechanisms, at least for vertebrates.

Acknowledgements

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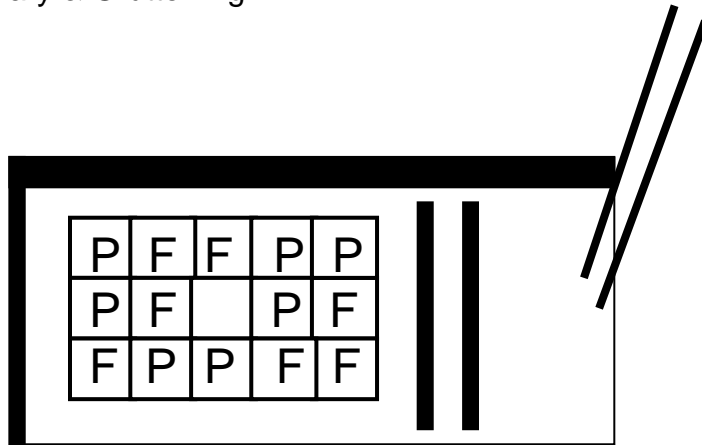
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Figure Legends

Fig. 1: Experimental Plexiglas plate. The stripes on the plates that are illustrated in black had two different colouration patterns, either pink and black or yellow and beige. The initial preference test was conducted with one plate type and the teaching trials and the final experiment with the other plate type. In each of the three treatment groups, four individuals were first tested on the pink and black plates, and the other four individuals were first tested on the beige and yellow plates. Fourteen food items were offered in each trial. Grid cells were filled from the upper left to the lower right, according to a random, but balanced, sequence. Once one food type had been selected 7 times, the remaining grid cells were filled with the other food type. Each random sequence was used only once in each of the three treatment groups; the sequence shown in the figure is just one possibility. P: Prawn item; F: Flake item. On the right of the plate is the lever (40 cm long) that allowed the experimenter to react to cleaner fish's foraging behaviour according to the treatment group (no reaction, fleeing, or chasing in response to prawn feeding).

Fig. 2: The percentage of prawn items eaten in the three treatment groups during the initial preference test (grey columns) and during the final experiment after the teaching period (white columns). Shown are the median and the interquartiles for N = 8 individual cleaners for each treatment.

Bshary & Grutter Fig. 1

P	F	F	P	P
P	F		P	F
F	P	P	F	F