The decision making on contest by social cognitive abilities in a cichlid fish

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社会的認知能力を用いた闘争の意思決定）

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Content

General Introduction

Many researchers have studied on “animal cognition” experimentally. The term of “Cognition” was variously defined in literature. A more general definition includes perception, attention, memory formation and executive functions related to information processing such as learning and problem solving (Brown et al. 2011). Most studies of animal cognition have been conducted in birds and mammals, particularly non-human primates.

There were few studies on cognition in fish, mainly because fish have largely been viewed as automatons throughout scientific history. Their behavior was thought to be controlled by unlearned processing, i.e. stereotyped responses to appropriate cue (sign stimuli). In other words, their behavior has long been viewed as stereotyped and not strongly influenced by context or experiences (Brown et al. 2011). For example, it is known well that three-spined sticklebacks (*Gasterosteus aculeatus*) respond aggressively against any fish with red belly even if the fish is model (Tinbergen 1948). However, researchers have now realized that fish exhibit a rich array of sophisticated behavior and that learning plays an important role in behavioral development of fish (Brown et al. 2011). In fact, since 1960s there has been a rapid increase in the number of papers published on learning in fish and those published since 1991 has risen dramatically. Although a number of studies about fish cognition increased, these are focus on simple learning or one information using. In other words, there are few researches in fish on cognitive abilities reported in mammals and birds.

The evolution of brain and cognition has been thought to be strongly related and described as a linear series of increasing complexity and advancement, from ‘lower’ to ‘higher’ groups in vertebrate. Thus, fish has been recognized as ‘most primitive’ or ‘least evolved’ until recent times (MacLean 1990).In fact, the forebrain of

fish was small and seemed not to development than mammals and birds. However, recent researches show that vertebrate brain has been far more conservative than previously thought (Striedter 2005, Brown et al. 2011). Therefore, although it is thought that fish have cognitive abilities reported in mammals and birds, there are no studies to test such cognition.

In many animals, there are contests over limited resources, e.g. mates and food (Huntingfold & Turner 1987). Although a winning individual can get such resources, “fighting” involves costs, e.g. expenditure on energy and time, and risk of injure (Reddon et al. 2011). Losing individuals cannot only obtain these resources, but also suffer such costs often more than winning individuals (Haller 1992). If a fight is clearly unwinnable, it is best to make a decision to avoid the fighting and subsequently the fighting costs. Thus, natural selection is likely to favor individuals that have such social cognitive ability, e.g. gathering and processing information about fighting abilities. However, it is thought that the main information used in contests in fish is size difference (Hsu et al. 2008). Contest with size-matched rivals is especially high fighting cost (Enquist et al. 1990). Thus, individuals facing with size-matched contestants should gather information other than size difference and use social cognitive abilities. However, little is known about the way to make a decision to avoid by social cognition on fighting abilities in fish (Brown et al. 2011).

In this thesis, I studied whether a highly social fish, *Julidochromis transcriptus*, has high cognitive ability with the aid of aquarium experiments. In Chapter I, individual recognition and duration of memory were studied of this fish. These abilities are thought as the base of cognitive ability, especially information processing. In fact, many species of mammals and birds can discriminate conspecific individuals quickly and accurately, and remember for a long time (Tibbetts & Dale 2007). Although it is also reported that fish can discriminate conspecific individuals

(Griffiths 2003), duration of memory in fish has not been researched. In order to study cognitive abilities in fish, I test the base at first. In Chapter II, I examined the multiple social cognitive abilities, i.e. direct information, winner/loser effects, social eavesdropping and transitive inference. Although some researchers studied these abilities in fish, each cognitive ability has been independently studied in different fish species and nobody treated all abilities in one species. Thus, we do not know the relations and interactions among these cognitive abilities. In Chapter III, I tested about order effects of gathering information in transitive inference. Some studies demonstrated about transitive inference in mammals, birds and fish (Grosenick et al. 2007, Vasconcelos 2008, White & Gowan 2013). Many studies of transitive inference use the task of inferring social dominance, where a subject animal A first directly interacts with B (e.g. A subordinate to B: A<B), and then indirectly observes the interaction of B and an unknown C (B<C), using both direct and indirect information to infer its own relationship with C (i.e. A<C). However, information order may influence information processing and motivation of gathering information, especially in complex scenarios, e.g. transitive inference (Bakker et al. 1989, Jonides et al. 2008, Brown et al. 2011). When subjects gather information such as the order of previous experiments, they may gather indirect information by observation because the contestants, at least one of contestant, are known. In a view from cognition, we have little understanding of the effects of presentation order in transitive inference. Through this thesis, I tested multiple cognitive abilities in one species and order effect of transitive inference for the first time in vertebrates.

Chapter I: Individual recognition and memory

Abstract

Animal contests are costly and tend to escalate when rivals have similar competitive abilities. Individuals that remember dominance relationships with rivals may avoid repeated agonistic interactions and hence avoid the costs of repeated escalation of contests. However, it can be difficult to experimentally disentangle the effects of memory from those of loser effects (losers behaving subordinately due to prior defeats). Here I test whether loser effects or individual memory mediate contest behaviour in the African cichlid, *Julidochromis transcriptus*. I find that on days three and five after initial contests, losers display subordinate behaviour to contest winners, but not to novel contestants. However, this effect disappears after seven days, at which time losers do not display subordinate behaviour to either rival. These results show that (1) this fish can recall a previously dominant contestant for up to five days and (2) as no subordinate displays were shown to the novel contestant, there are no evidences for loser effects in this species. Such short–term memory of past interactions may have broad significance in social species with repeated interactions.

Introduction

Contests to gain monopoly of a resource (e.g. mates, food, or territory) are widespread in animals, but may entail high costs in terms of time and energy expenditure and risk of injury, especially where contestants have similar fighting ability (Huntingford and Turner 1987, Enquist et al. 1990). Numerous strategies are employed to avoid unnecessary escalation of contests. Commonly, noninjurious behaviours are used to signal and assess the competitiveness of rivals without actual combat occurring (Briffa and Sneddon 2010). In addition to (or even in the absence of) signalling and communication, experiences of previous contests may influence aggression in subsequent encounters. Winner and loser effects can influence whether individuals escalate agonistic interactions or avoid further combat, and are widespread through animal taxa. Following a contest victory, individuals are often more likely to win subsequent contests against different individuals, whereas losing has the opposite effect (Dugatkin 1997; Hsu et al. 2006). Numerous physiological mechanisms may contribute to these effects, including socially induced changes in brain neuromodulators and hormonal responses to social interactions (Winberg and Nilsson 1993; Huber and Delago 1998; Oyegbile and Marler 2005). The adaptive value of winner–loser effects may arise through avoiding costs of contests including energy investment in fighting, the risks of injury, and the increased exposure to predation, or winner effects may prime individuals to win future contests so that the winner effect is a resource in itself (Hock and Huber 2008).

However, in social species where encounters with rivals are frequent and recurring, such physiological responses may lead to inappropriate responses in rapidly shifting social conditions. When multiple social partners are encountered in quick succession, physiological priming based on recent agonistic encounters may be too

coarse of a behavioural strategy if rivals of both lower and higher fighting ability are subsequently encountered. Moreover, in social groups, winner and loser effects are predicted to only evolve when there are considerable asymmetries in fitness benefits of contests between dominant and subordinate members (Mesterton–Gibbons 1999). Such extreme asymmetries are rare in social systems, where linear dominance structures are the norm (Wong et al. 2008). In these cases, discriminating individuals (i.e. individual recognition; Tibbetts and Dale 2007) and memory of past encounters with individuals may play a more important role in mediating behaviour in repeated contest scenarios (Hick et al. 2014). Because the maintenance of accurate memory is costly (Dukas 1999), the duration of memory should be tuned to the environment in which an individual finds itself. In fluctuating environments, the duration of memory is predicted to be short (Braithwaite 2005), because stored information will not long stay relevant. For example, in sticklebacks specific handling skills are retained for 10 days in a very variable environment, while in a stable environment memory lasts for 25 days (Mackney and Hughes 1995).

When prior experience of contest interactions influences subsequent behaviour, it is important to assess whether animals are using actual memory of individuals (Johnsson 1997) or are influenced by physiological changes (i.e. winner and loser effects; Hsu et al. 2006). However, it can be experimentally difficult to disentangle these effects during repeated contests because subordinate or dominant behaviour towards previously encountered rivals would have the same emergent behaviour whether caused by physiological changes or actual memory. While there are several studies that examine the duration of dominance relationships between individuals (golden hamster, Lai and Johnston 2002; jungle crow, Izawa and Watanabe 2008; Iberian wall lizard, Lopez and Martin 2001; paradise fish, Miklosi et al. 1992; rainbow trout, Johnsson 1997), and a wealth of separate studies examining winner and loser

effects (for review see Hsu et al. 2006), an interaction between memory and loser effects cannot be ruled out. To accurately assess the duration of memory of dominance, loser effects must be ruled out using contests against novel individuals. If apparent individual memory is simply a by–product of loser effects, the losing individual should behave subordinately against both novel and familiar contestants. Conversely, a difference in behaviour towards novel and familiar individuals will be seen if true memory underlies behavioural changes following encounters. Moreover, memory and physiological effects may persist for different periods of time, and simultaneous tests of the duration and interaction of individual memory and loser effects are therefore required.

I investigated the duration of memory of dominant individuals measuring the duration of response of male *Julidochromis transcriptus*, a group–living Lake Tanganyikan cichlid fish that breeds and shelters in rock crevices (Konings 1998; Awata et al. 2006). Although monogamy is the most common tactic exhibited by the fish, very large individuals of both sexes often mate with multiple partners at different nests (polygamous harem) and small individuals stay at breeder's nest as helpers assisting brood care (Taborsky and Limberger 1981). Male was chosen because non–related helpers are not females but often males that conflict with territorial males. Due to competition for limited habitat, contests between social partners, e.g. mates or group members, are frequent and repeated (Awata and Kohda 2004), and individuals may profit from mechanisms to avoid repeated contest escalation. The frequency of aggressive behaviour increased with decreasing the body size differences (Awata and Kohda 2004)). If *J. transcriptus* adopt individual memory, the duration is expected to be short since repeated interactions mean information about rivals must be frequently updated (Braithwaite 2005). To separate the contributions of winner/loser effects and individual memory on social behaviour following contests, I examined the duration of

subordinate behaviour following contest losses against novel and previously encountered rivals.

Materials and methods

The *Julidchromis transcriptus* used in this study were laboratory–reared descendants of wild–caught fish from Lake Tanganyika, Africa. Experiments were conducted with *J. transcriptus* males (total length; TL, 66.90–81.55mm), which were raised and kept in groups in my laboratory. Three days before the start of the experiment, fish were measured (TL) and each placed in 30cm × 17cm × 15cm glass tanks (house tank) with 2 cm coral substrate, filtration and aeration. The fish were visually isolated from one another: all sides of the tanks were covered with opaque sheets. This isolation lasted for 14 days prior to the experiment to avoid effects of previous contests (Hsu et al. 2006). The tanks were kept at 24–26 °C, and a 12:12h light:dark cycle. The fish were fed with artificial flake food (Tetramin) twice a day.

Experiment 1

Encounters between two paired males (unfamiliar each other) were staged in a glass tank (30cm × 17cm × 15cm) for 30 min. All 34 pairs were size–matched (TL difference was within 3 mm and preliminary experiment showed that the size difference was sufficiently small that I could not a priori predict the winner of the contest). Because contests between size–matched fish are generally longer than between fish of different sizes (Enquist et al. 1990), the contests needed 30 min to be resolved. The fish were put into the tank together at the same time in order not to provide any information about tank that may affect their behaviour. In each case

contest was ended with the one fish behaving aggressively (performing chases and bites towards the contestant, and being defined as winner) and another fish adopting submissive behaviours and retreating when another fish approached (being defined as loser). Following contests, each fish was returned to a house tank and isolated for either three days (12 pairs), five days (12 pairs) or seven days (10 pairs; see Fig. 1).

After each interval in isolation, the fish were placed into paired test tanks, which allowed visual exchange but prevented any physical interaction. Fish were allowed to settle in these tanks for ten minutes with an opaque sheet between tanks to prevent visual exchange. The opaque sheet was then removed and the interactions between the fish were recorded by video camera (HDR–CX370, Sony). After 10 minutes the opaque sheet was replaced and the winner was removed and replaced with a novel stimulus fish. After 10 min habituation, the sheet was removed and interactions between the focal fish and the stimulus fish were again video–recorded (see Fig. 1). Prior winners were always introduced first to avoid the possibility that interactions with novel fish would have subsequent effects (i.e. winner effects) on the behaviour of the losing fish towards a previous rival.

To evaluate aggressive behaviour, I measured “rushing time” (the time fish rush against glass barrier) and “time in near zone” (when the bottom of the tank was divided into three zones, i.e. each zone is 5.7 cm, the time fish stayed in a zone near glass barrier) using video–recordings of the first 30 seconds of interaction. If the focal fish tend to escalate aggression, the fish stay in near zone and rush against glass barrier more, and this value in “rushing time” and “time in near zone” may reflect aggressive motivation. This observation period was chosen because behaviour did not vary greatly over the ten minutes recording period and a few minutes were needed for some fish to aware of contestant. I defined subordinate behaviour as fish showing shorter rushing time and less time in near zone than their opponent. In my preliminary experiments,

losing fish showed shorter rushing time (Wilcoxon signed–ranks test: T=55.0, N=11 p<0.005) and less time in near zone (Wilcoxon test: T=1.0, N=11 p<0.005) than winners, but winner behaviour was not significantly different to novel fish behaviour (rushing time, Mann–Whitney U–test: U=106.5, $N1$=11, $N2$=10 p=0.90; time in near zone, U=124.5, $N1$=11, $N2$=10 p=0.35), therefore I cannot distinguish winners and novel fish with these indices.

Statistical analyses were conducted in R version 3.0.0 (R Development Core Team, 2013). Wilcoxon signed–rank tests were used to analyse whether losers behave submissively against winners but not against novel stimulus fish. Kruskal–Wallis tests and Steel–Dwass tests as post hoc tests were used to analyse whether loser effect exist, i.e. losers behave similar in three intervals (three, five and seven days).

Experiment 2

In Experiment 1, I cannot distinguish whether animals were using memory to recall previous rivals or based subsequent behaviour on some other cue associated with dominance, e.g. dominance badges or signals (Colgan 1983), darker body or eye colour as an indicator of social rank (O'Connor et al. 2000; Volpato et al. 2003) in fish, feather coloration in great tits (Järvi and Bakken 1984). Therefore, I expanded my experiments to exclude such effects.

Experimental design consisted of four size–matched fish encountering each other in two rounds of pairwise encounters (Fig. 2). In the first phase, paired fish encounter one another for 30 min in one of two glass tanks (tank 1 or tank 2) to determine winners (W1 in tank 1 or W2 in tank 2, respectively) and losers (L1 in tank 1 or L2 in tank 2, respectively). After these initial contests, the fish were swapped and placed into paired test tanks such that W1 was adjacent to L2 and W2 adjacent to L1 (i.e. the fish were unknown each other). An opaque divider was again placed

between tanks for 10 minutes to allow fish to settle, then removed and the interactions between the fish were recorded by video camera. I carried out six experimental sessions. As for experiment 1, I measured “rushing time” and “time in near zone” to evaluate aggressive behaviour.

Statistical analyses were conducted in R version 3.0.0 (R Development Core Team, 2013). Wilcoxon signed–rank tests were used to analyse whether dominance badge exist, i.e. both winners and losers behave similar. If dominance badge exist in this species, losers behave submissively despite winners are unknown to them.

Ethical note

All experiments were conducted in compliance with Regulations on Animal Experiments at Osaka City University and the Japan Ethological Society.

I chose to handle fish without anaesthetizing them because the effectiveness of anaesthetizing in eliminating/reducing the stress of handling is not clear (Thomas and Roberston 1991; Congleton 2006). Anaesthetics also seem to elicit prolonged cortisol elevation which influences contest behaviour (Wagner et al. 2003), and may cause death in fish (Cho and Heath 2000). When I measured fish, I netted and placed the fish on top of several sheets of tissue saturated with water and covered the fish with another layer of wet tissue. Directly after being measured, the fish were fed with flake food. All fish started to show regular feeding behaviour in five seconds.

In escalated contests, fish engaged mouth wrestling. All contests were video–taped and also carefully monitored by an observer. The observer would intervene and terminate contests if either fish appeared to suffer visible physical injury (e.g. scale loss, wounds, abnormal behaviour) or intensive biting. However, no interventions were required because most escalations were brief and no physical injures to fish were observed. All fish were returned to their home tank after contests, fed with flake food

and visibly inspected. No fish appeared to suffer physical damage from contests.

Results

Experiment 1

All pairs escalated contests and established dominant–subordinate relationships. There was no difference in body size between winner and loser fish (Wilcoxon test: T=583.0, N=34 p=0.95).

There were significant differences in the rushing time of losers against winners and novel fish after three days (Wilcoxon signed–rank test: T=15.0, N=12, p<0.005) and after five days (Wilcoxon test: T=5.0, N=12, p<0.005; Fig. 3a). However, these differences disappeared by seven days after initial contests (Wilcoxon test: T=17.0, N=10, p=0.98). There was an overall significant difference in rushing time against previous winner (Kruskal–Wallis test: $H2$=12.74, p<0.005). There was no difference in rushing time against novel fish for any interval (Kruskal–Wallis test: $H2$=3.21, p=0.20).

For time in the near zone, there were significant differences in loser behaviour towards winners and novel fish three days and five days after initial contests (three days: Wilcoxon, T=23.5, N=12, p<0.005, five days: T=7.0, N=12, p<0.05) but not after seven days (T=6.0, N=10, p=0.75; Fig. 3b). There was an overall significant difference in time in near zone against previous winner (Kruskal–Wallis test: $H2$=9.01, p<0.005). There was no difference in rushing time against novel fish for any interval (Kruskal–Wallis test: $H2$=3.21, p=0.20).There was an overall significant difference in time in near zone against novel fish (Kruskal–Wallis test: $H2$=6.30, p<0.05). However, I did not find a significant difference for any pairwise interval comparison (Steel–Dwass

test: three days vs five days, t=2.02, $N1$=12, $N2$=12, p=0.11; three days vs seven days, t=2.12, $N1$=12, $N2$=10,p= 0.09, five days vs seven days, t=0.08, $N1$=12, $N2$=10, p=0.99).

Experiment 2

All pairs escalated contests and established dominant–subordinate relationships. There was no difference in body size between winner and loser fish (Wilcoxon test: T=62.5, N=12, p=0.07).

There was no significant difference in either rushing time or time in near zone between winners and losers (rushing time, Wilcoxon: T=20.0, N=12, p=0.48, Fig. 4a; time in near zone, T=17.0, N=12, p=0.67, Fig. 4b).

Discussion

In this study, I measured the subordinate and agonistic behaviour of male *Julidochromis transcriptus* to rivals after a period of separation following initial contests. I found that individuals who had recently lost a contest behaved subordinately to familiar rivals in subsequent encounters. Taking this result in isolation, I might conclude that loser effects operate in *J. transcriptus* and cause behavioural changes in subsequent agonistic encounters. However, this subordinate behaviour was only observed when focal fish interacted with their winners. When focal fish were allowed to interact with novel rivals after having lost a previous bout (experiment 1), there was no evidence of subordinate behaviour, suggesting that it is not loser effects but rather memory of individual rivals that drives changes in behaviour following contests in *J. transcriptus*. When losing fish were presented with a novel winning fish (experiment

2), they did not display subordinate behaviour, suggesting that it was individual memory rather than some form of dominance badge affecting behaviour. These results highlight the care that needs to be taken to dissociate physiological changes and memory, because the emergent behaviour in the same in both cases but may have different underlying causes. In this study, I presented the same order for loser, i.e. at first previous winner then novel fish. However, if the winner and novel fish were presented in a randomized order, I might have much stronger evidence.

I also measured the duration of individual memory by monitoring the interactions of rivals on days three, five, and seven after initial contests. I found that subordinate behaviour towards dominant rivals disappeared after seven days, at which time focal fish reacted similarly to both novel and dominant prior rivals. These results suggest that duration of memory of dominant individual in *J. transcriptus* is between five and seven days. However, the possibility that fish still remember the dominant rivals after seven days but try to fight will not be completely refused, I think the possibility will be very small because of no difference in response against between novel and dominant prior rivals. I therefore conclude that *J. transcriptus* males have a memory span for social relationships of between five and seven days, the first such report of this phenomenon in vertebrates

Traditionally, forgetting was thought of as a failing of memory, but over the past two decades I moved towards the idea that the ability to change previously learned responses may be advantageous (Kraemer and Golding 1997). For species with complex social systems, individuals frequently interact with the same social partners, and mechanisms that facilitate avoidance of costly contest interactions may be adaptive. The recognition of individuals and memory of past encounters that I observe in *J. transcriptus* may allow animals to reduce the costs of repeated escalation of contests. In contrast, it is not clear how loser effects could contribute to reducing the costs of

frequent repeated contests with same opponents except through total avoidance on agonistic interactions with all individuals (Hsu et al. 2006; Hock and Huber 2008; but see Arnott and Elwood 2009; Gaecia et al. 2012). Changing social conditions can rapidly alter the selection acting on individuals (Cronwalis and Uller 2009), and fixed behavioural patterns may therefore become maladaptive if conditions change. Because *J. transcriptus* frequently has aggressive interactions with the same individuals, the information of particular individual updates. Therefore, memory durations of between five and seven days may allow social animals to avoid unnecessary confrontation in the short term but also update information about dominance hierarchies as they might change in the medium term. To verify this idea, comparisons of memory duration must be made with other species that vary in their social structure; in this regard, the Lake Tanganyikan species flock provides a perfect opportunity for such comparisons.

When social conditions are extremely variable, the benefits of memory may be few. Here *J. transcriptus* males recalled rivals for five days at least, but far longer memory durations have been shown in other fish species, suggesting that capacity for memory is greater than I observed. Paradise fish are able to remember and respond to predator stimuli for > 3 months (Miklosi et al. 1992), and three–spined stickleback handling skills for prey can be recalled for > 25 days (Mackney and Hughes 1995). Finally, in their natural environment, *J. transcriptus* have frequent interactions with many conspecific fish and need to recall dominance relationships with multiple individuals at the same time. When social conditions are complex and fluctuating, the costs of memory of multiple individuals may be prohibitive. For example, in guppies, mate choices in changing social environments may be based on rules of thumb rather than immediate memory of individual encounters (Jordan and Brooks 2012). Thus, to fully understand the role of memory in mediating contest behaviour in social animals like *J. transcriptus*, future work should consider both duration and scope of memory of

multiple individuals simultaneously (e.g. Griffiths and Magurran 1997a).

Figures

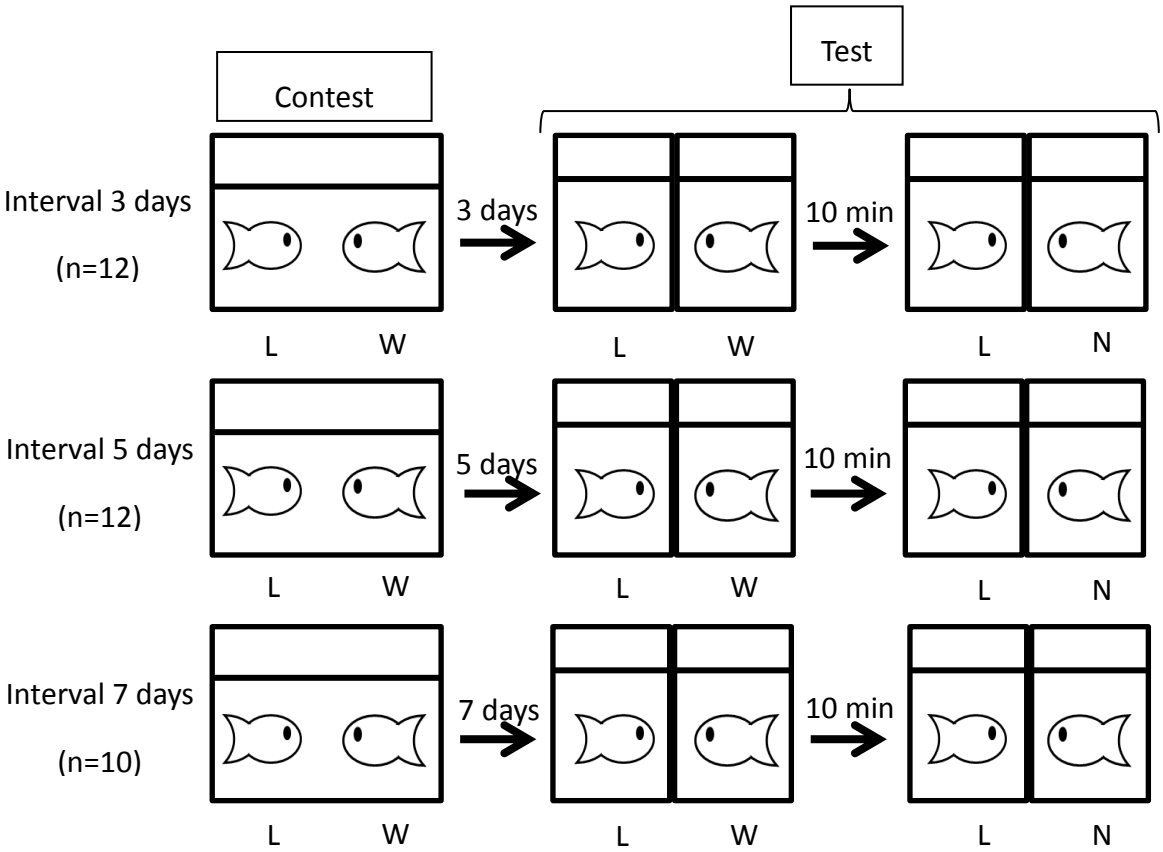

Figure 1. Procedure of Experiment 1.

After interval, focal fish (previous loser) was faced with previous winner. Then, focal fish was confronted with novel fish. W: previous winner, L: previous loser (focal fish), N: novel fish for loser. (See text for detail)

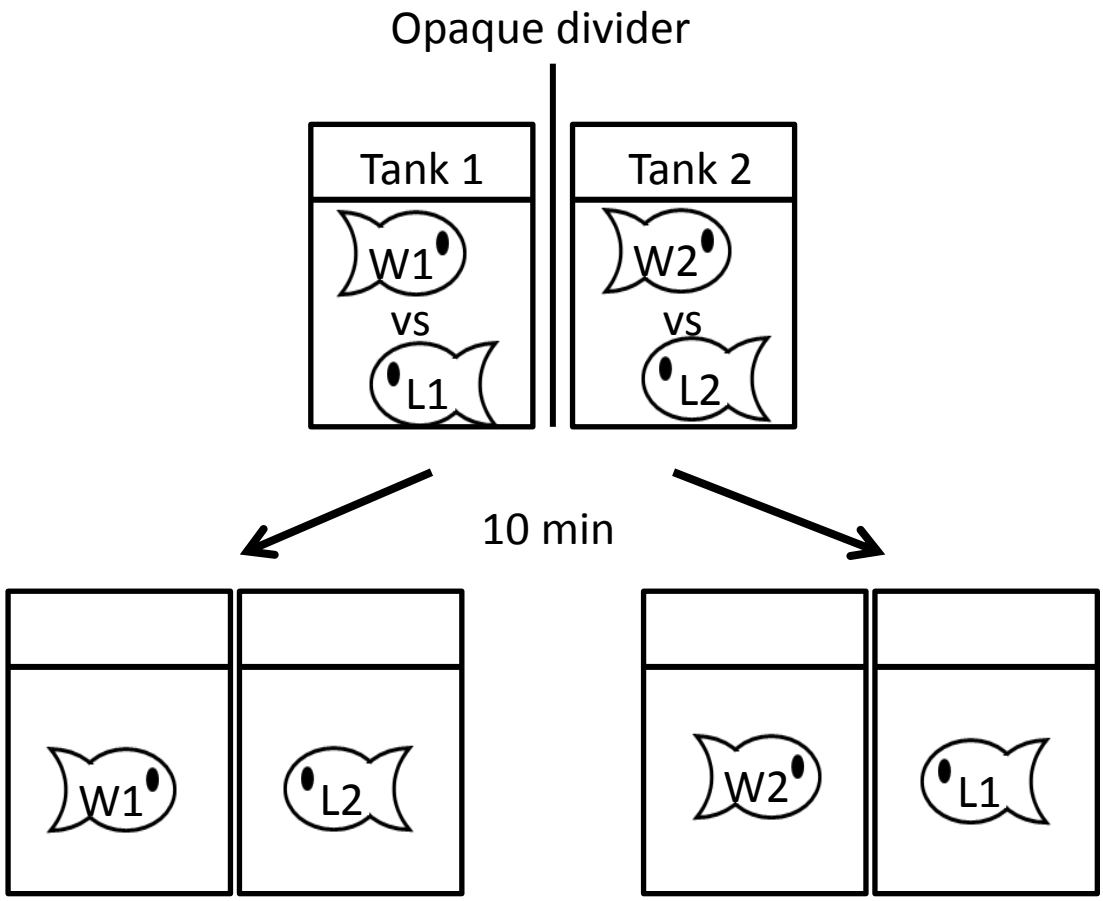

Figure 2. Procedure of Experiment 2.

In tank 1, winner was W1 and loser was L1. In tank 2, winner was W2 and loser was L2. After contests, fish was switched their opponent, i.e. W1 vs L2 and W2 vs L1, and their behaviours were video–recorded. (See text for detail)

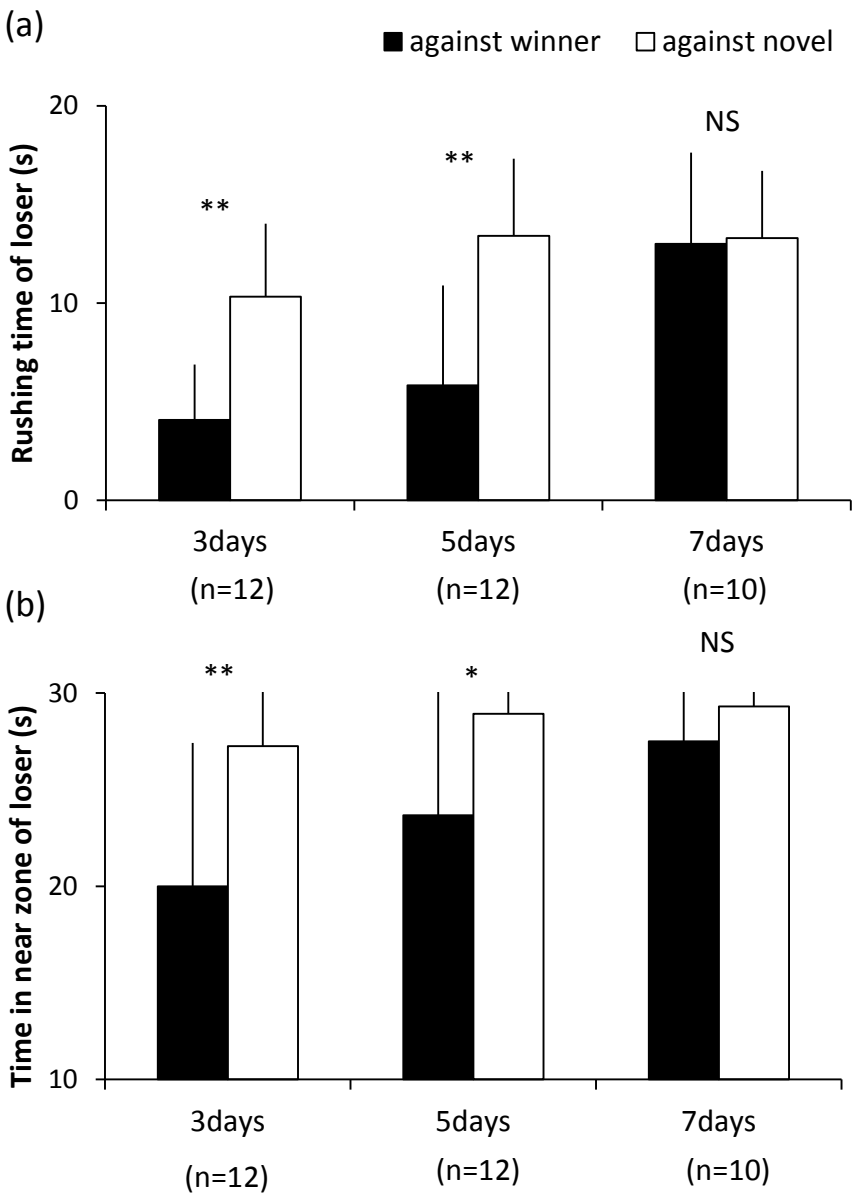

Figure 3. Results of experiment 1.

(a) rushing time (mean, SD) and **(b)** time in the near zone (mean, SD) of losers against previous winners (filled bars), and against novel rivals (open bars). *: $p<0.05$, **: $p<0.005$, NS: $p>0.05$

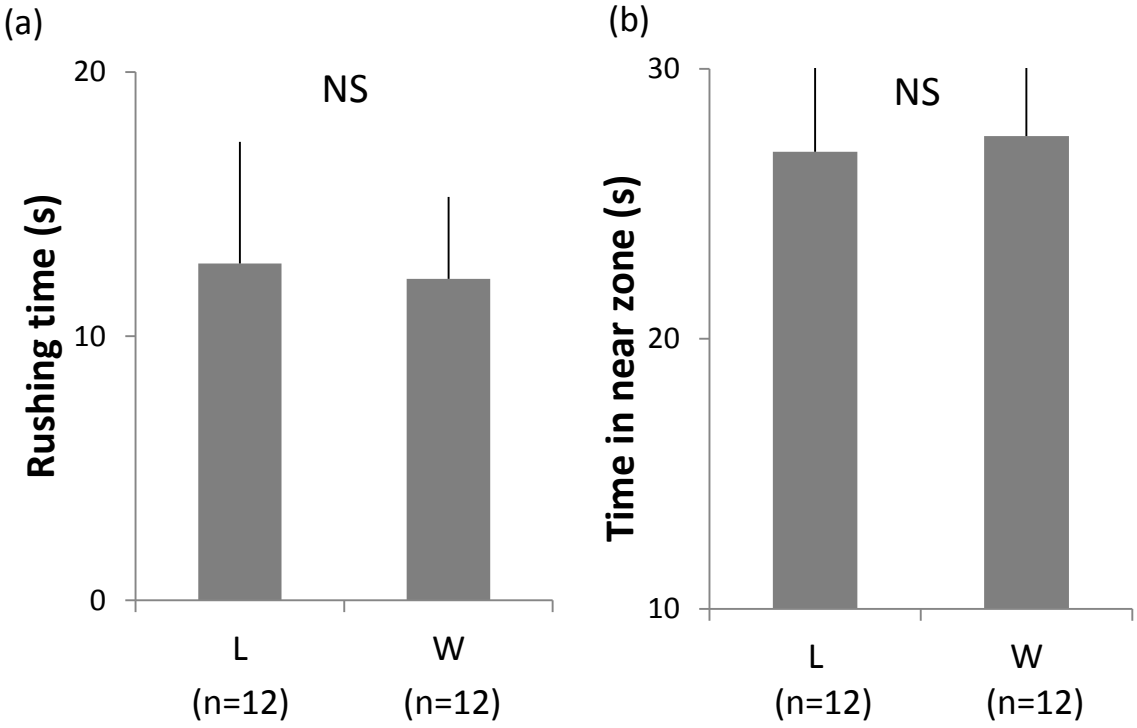

Figure 4. Results of experiment 2.

(a) rushing time (mean, SD) and **(b)** time in the near zone (mean, SD) of losers (L1 and L2, Fig. 2) and winners (W1 and W2, Fig. 2). NS: $p>0.05$

Chapter II. Testing cognitive abilities: transitive inference, winner/loser effects and social eavesdropping

Abstract

Theory suggests that living in large social groups with dynamic social interactions often favours the evolution of enhanced cognitive abilities. Studies of how animals assess their own contest ability commonly focus on a single cognitive task, and little is known about the diversity or co–occurrence of cognitive abilities in social species. I examined how a highly social cichlid fish *Julidochromis transcriptus* uses four major cognitive abilities in contest situations; direct experience, winner/loser effects, social eavesdropping and transitive inference (TI). I conducted experiments in which fish assessed the social status of rivals after either direct physical contests or observed contests. Individuals used direct information from a previous physical encounter to re–establish dominance without additional contact, but winner/loser effects were not observed. Social eavesdropping alone was ruled out, but I found that transitive reasoning was used to infer social dominance of other individuals of unknown status. My results suggest that in stable hierarchical social groups, estimations of contest ability, based on individual recognition pathways such as TI and direct experience, are more prevalent than social eavesdropping or winner/loser effects. I suggest that advanced cognitive abilities might be widespread among highly social fishes, but have previously gone undetected.

Introduction

Throughout animal kingdom, contests over limited resources (e.g. mate, territory and food) are widespread but may entail high costs in terms of time, energy and risk of injury (Huntingford & Turner 1987). Behaviour in iterated animal contests may be influenced by prior experiences (Hsu et al. 2006), potentially reducing costs or incidence of fighting in repeated encounters. Winning experiences, for example, tend to increase the probability of winning, e.g. winner effects, while losing experiences tend to decrease it, e.g. loser effects (Hsu et al. 2006; Benelli et al. 2015a, b). Furthermore, social eavesdropping, the ability to monitor the contests between unfamiliar individuals and use the information in subsequent aggressive interactions, may reduce fighting costs with unknown individuals (Oliveira et al. 1998).

In large social groups, especially those with dominance hierarchies (Jordan et al. 2010a, Jordan et al. 2010b) individuals frequently interact with familiar and unfamiliar group members, and also with unknown individuals from outside the group (e.g. Byrne & Whiten 1988; Awata & Kohda 2004; While & Gowan 2013). Increased cognitive abilities will be favoured if they allow individuals to indirectly infer their dominance relationship with unknown individuals and avoid costly aggressive interactions for dominance. An individual's place in the social order can be learned through direct interactions with others, including engaging with strangers. However, the costs of these interactions increase cumulatively with the size of the society since the likelihood of encountering stranger increases. By observing interactions between the stranger and known individuals, with whom a social relationship has already been established, an animal may predict their own relationship to unknown individuals. This component of cognitive ability is called transitive inference (TI; Hogue et al. 1996; Paz–y–Miño et al. 2004; Engh et al. 2005; Grosenick et al. 2007; MacLean et al. 2008;

Vasconcelos 2008 for review). A variety of studies have suggested that transitive inference may be used by higher vertebrates (e.g. Hogue et al. 1996; Peake et al. 2002; Peake & McGregor 2004; Engh et al. 2005), and TI studies are mainly documented in animals with high sociality, such as apes, monkeys, hyena, chickens and corvids (Gillan 1981; Bond et al. 2003, Paz–y–Miño et al. 2004; Engh et al. 2005), as well as a territorial and social fish (Grosenick et al. 2007, White & Gowan 2013).

This raises the question whether transitive inference only occurs in those species with high cognitive abilities in general. While transitive inference is typically observed in animals with highly organized societies, the pattern of co–occurrence of other cognitive abilities such as social eavesdropping and winner/loser effects is not well understood (Hsu et al. 2006, 2011). Despite this, analyses of the contributions of different components of cognition are rare, with studies most often focussing on a single component of cognitive ability (Hsu et al. 2011). In some cases, multiple cognitive factors have been examined in a single species, with conflicting results. Despite using TI with direct experience and information gathered by eavesdropping, hens in stable groups do not use social eavesdropping alone (Hogue et al. 1996). Further, in *Melanochromis auratus*, a group living cichlid with strict linear dominance and high sociality, winner/loser effects do not operate (Chase et al. 2003). In contrast, in Siamese fighting fish, both social eavesdropping (e.g. Oliveira et al. 1998; McGregor et al. 2001) and winner/loser effects (e.g. Wallen & Wojciechowski–Metzlar 1985) are observed, but there is no evidence that this species use transitive inference to infer social dominance.

While the general cognitive abilities of fish are not fully understood, there are reports of cognitive capacity in some fish that mirror those in higher vertebrates, e.g. social eavesdropping, individual recognition and winner/loser effects (Hsu et al. 2006, 2011; Alfieri & Dugatkin 2011; Brown & Laland 2011). The social intelligence

hypothesis predicts that highly increased cognitive ability, e.g. recognizing group members and transitive inference will be favoured in highly organized, large societies (Byrne & Whiten 1988; Bshary et al. 2002; Bond et al. 2003; Brown et al. 2011; Bshary 2011). Cooperatively breeding cichlids in Lake Tanganyika often have large groups associated with frequent social interactions with known and unknown individuals (e.g. Awata et al. 2005; Heg & Bachar 2006; Wong & Balshine 2010), which may favour the development of high cognitive abilities (e.g. Byrne & Whiten 1988, Bond et al. 2003, Alfieri & Dugatkin 2011). *Julidochromis transcriptus* and its congeners are cooperatively breeding cichlids with a highly organised social system (Awata & Kohda 2004; Awata et al. 2005, 2006, 2008, 2010; Heg & Bachar 2006; Kohda et al. 2009). Breeding groups consist of multiple unrelated males and females that frequently share paternity and cooperatively raise their brood (Awata et al. 2005). Breeding members frequently interact with each other, but strangers of varying social status often approach the territory or nests of the members (Awata & Kohda 2004). This fish is therefore an ideal species in which to examine social cognitive capacity, and is amenable to experimental manipulation of social experiences. Here I use male *J. transcriptus* to examine and disentangle the effects of transitive inference, social eavesdropping, winner/loser effects and direct fighting experience to determine the relationship between these cognitive abilities and how they interact to influence social interactions.

Materials and Methods

Study animal and housing condition

I obtained the subject fish *J. transcriptus* from commercial breeders. Experiments were conducted in my laboratory at Osaka City University. I used males (60–80 mm in total length) that had been kept with females in either 30 × 40 × 60 cm tanks of 20 individuals or 45 × 40 × 180 cm stock tanks with 60 individuals, both at 26 ^{o}C under 12:12 h light–dark cycles (Awata et al. 2006). Stock tanks contained multiple shelters of half–cut flower pots, stones and tiles put on coral gravel bottom. Water was aerated and filtered using sponge and external canister filters, and dissolved nitrogenous waste was never measured to be above acceptable levels. Commercial flake food (Tetramin) was provided twice a day. Prior to experiments, fish were successfully housed for more than one year, and frequently bred in captivity. Similar–sized males that had not encountered each other during this period were used in all experimental trials (average size difference: 1.07 ± 0.89 mm 0 – 3.6 mm; 1.66 ± 1.49 %, 0 – 6.52 %). Glass aquariums (30 × 18 × 20 cm^3) with gravel substrate were used for all experiments (Fig. 1). Behaviour was recorded with video cameras (HDR–CX370, Sony) in all experiment trials.

Experiment procedure

Many previous studies of TI used 3–term series tasks using individuals A, B and C (e.g. Bryant & Trabasso 1971; Hogue et al. 1996; Peake et al. 2004). In this study I also designed the 3–term series task using three individuals in test of TI, which are associated with several control experiments (Fig. 2). I conducted five independent experiments to determine the effects of direct interactions and observation of

interactions on subsequent aggressive and submissive behaviour. Experiments consisted of a pre–phase I, an optional pre–phase II (depending on the treatment group), and a test phase (Fig. 2). In preliminary experiments on direct physical contests, I observed that two size–matched fish put in a tank often continued to fight for at least five minutes after introduction, probably because of their similar body size and similar fighting ability. In these preliminary trials, dominance relationships took up to 15 minutes to become stable. Thus, I allowed 30 minutes to establish dominance after introducing two size–matched fish to arena tanks. I refer to individuals with established pairwise dominance as 'A' and 'B', and an individual that had not yet established pairwise dominance with individual A as a stranger 'C' and will use this terminology throughout the paper. During the test phase I recorded the following responses of each individual: (i) rushing time: time spent rapidly moving towards the glass barrier with open mouth in an aggressive fashion, and (ii) the time spent in each of three zones in test tank: the near, middle and far sections (each 6 cm width) from the border glass. Details of the experimental set–up and experimental sequences with predictions of each test are shown in Figs. 1 and 2. Sample sizes in respective experiments were ten or twelve focal individuals.

In experiment 1, I assessed how dominance during direct interactions in an 'arena tank' affected subsequent behaviour in a 'test tank' (Fig. 2*a*). In pre–phase I, two fish were placed in the arena tank for 30 min and their aggressive and submissive interactions were videotaped and monitored (10 pairs). The fish that performed aggressive acts toward and chased the other fish in a unidirectional manner or much more frequently than opponent during the last 15 minutes was regarded as winner and labelled 'A'. The losing fish was labelled 'B'. During the last half time, typically losers retreated from the winner if approached, and often showed submissive behaviour or could flee from the winner. The winner did not continue to attack the loser when the

latter performed subordinate displays, and therefore the losers were not persistently attacked or chased.

Fish A and B were then moved to each of the test tanks using a hand net, and visually isolated from each other using an opaque divider (Figs. 1, 2*a*). Ten minutes after introduction, the opaque sheet was removed allowing fish to visually interact, and then their behaviours were recorded for 10 minutes (test phase). I analysed total time (seconds) that fish attacked the glass divider with their mouth open and time in each of the three zones (near, middle and far; Fig. 1), using video recordings of the first 30 seconds on interaction. By analysing only the first 30 seconds, I excluded possibility that one's behaviour influences another's, i.e. collusion effects (Paz–y–Miño et al. 2004). I expected the winner A to attack the glass barrier more and to spend more time in the near zone than the loser B during the test phase.

Experiment 2 acted as a control for experiment 1. In pre–phase I, two fish 'C_1' and 'C_2' were put in two tanks separated with a 7 cm distance for 30 minutes (Fig. 2*b*), and they observed other fish but did not attack the glass barriers (10 pairs). These fish therefore had visual exposure, but did not have any experiences of direct contacts each other. These fish were then placed in the test tank and their behaviours were recorded as for the experiment 1 (Fig. 2*b*). I expected both fish to spend equal time in the near area during the test phase, because they are strangers to each other.

In experiment 3, I tested the hypothesis that winning or losing a fight increases the probability of winning or losing again (winner/loser effects, Whitehouse 1997; Oliveira et al. 2009). In pre–phase I, two arena tanks, each of which contained two fish, were used (Fig. 2*c*). As in experiment 1, I labelled the winners fish A_1 and A_2 and the losers fish B_1 and B_2 after 30–min observations. In the test phase, A_1 and B_2 or A_2 and B_1 were allowed to be visually interacted and their behaviours were recorded (12 pairs). If winner/loser effects were operating, I would expect the previous winners

A_1 and A_2 to exhibit more aggressive behaviours than the previous losers B_1 and B_2. In this experiment I used fish of size match, not the approach of "random–selection (Hsu et al. 2006; Benelli et al. 2015a), because this experiment 3 is also control for experiment 5 where focal fish face the same size fish.

Experiment 4 was conducted to test whether fish that were allowed to observe fighting contests of other fish altered their behaviour when they engaged those fish in subsequent fighting contests (eavesdropping hypothesis; Oliveira et al. 1998; Hsu et al. 2006, 2011). An observer fish C (in a separate observer tank) was allowed to observe interactions between two fish in an arena tank for 30 minutes (pre–phase I in Fig. 2*d*). I then transferred the observer fish C into one compartment of a test tank and the winner fish A into another compartment (10 pairs). Their behaviours were recorded for 10 minutes (test phase in Fig. 2*d*). If these animals use social eavesdropping to infer social dominance, the observer fish C would respond less aggressively when interacting with the winner A (Fig. 2*d*).

In experiment 5, I tested the hypothesis that *J. transcriptus* can use bi–directional transitive inference for inferring social dominance of strangers, using three individuals (12 triads). In the pre–phase I, two fish were placed in an arena tank, and watched by an observer fish C who was physically separated from the arena tank (Fig. 2*e*). The observer fish C was allowed to watch the interactions between the two fish for 30 minutes. The winner of the dyadic interaction was labelled A, and the loser B. The physically interacting fish A and B in arena tank ignored the fish C that observed the formers. In the pre–phase II, the winner A and the previous observer C was transferred into a new arena tank, while the loser B was moved to a new observer tank to be allowed to observe interactions between A and C from the separated tank (Fig. 2*e*). As in the pre–phase I, I observed the two fish in the arena tank for 30 minutes and determined dominance relationships. If fish C was dominant over fish A, the winner

fish C and observer fish B were transferred to a test tank, and their behaviour was recorded for 10 minutes (Fig. 2*e*). If fish C was subordinate to fish A in the arena tank, I discarded the trial from the analyses. If fish C and B can use bidirectional transitive inference, it is predicted that both should correctly infer that C > B, despite that both fish had never experienced direct interactions between them. In this case, C and B would behave in an aggressive and submissive way during the test phase, respectively, which would be identical to the behavioural patterns of the winner A and the loser B in the test phase of experiment 1.

Ethical notes

This research adheres to the ASAB/ABS guidelines for the Use of Animals in Research (ASBS/ABS 2014). All experiments were conducted in compliance with the Regulations on Animal Experiments in Osaka City University and the Japan Ethological Society. No permits were needed from Japanese government for experiments involving *J. transcriptus*.

I opted to handle fish without anaesthetizing them because the effectiveness of anaesthetizing in eliminating/reducing the stress of handling is not clear (Congleton 2006). When I measured fish, I netted and placed the fish on top of several sheets of tissue saturated with water and covered the fish with another layer of wet tissue.

In escalated direct physical contests in arena tank, fish engaged in bouts of mouth biting where the two fish grasped each other's jaws and pushed each other (Sopinka et al. 2009). All contests for 30 minutes were videotaped and monitored by an observer. The observers had a rule to intervene and terminate contests if either of the fish appeared to suffer visible physical injury (e.g. scale loss, wounds, bleeding or abnormal swimming behaviour). However, no interventions were required because escalations in fight involving mouth biting were not observed to cause physical injuries

to the fish. After contests were resolved, losers were often able to avoid attacks from the winners by sticking on the side or the corner of the aquarium although no refuge was put in the tank. In preliminary experiments, I observed that in the test tank containing a refuge (half of a small flower pot), some individuals stayed in refuges considerable time, which largely affected their reaction times and positions, and thus I did not put a refuge in test tanks. No refuge was put in arena tanks in order to make the condition of arena tank the same as test tanks.

None of the fish in arena tanks appeared to suffer physical damage from the contests. These losers were highly aggressive to unknown fish in subsequent test–phase experiments 10 minutes later (e.g. exp. 2 and 3), showing that they were not damaged. After test phase experiments, fish were introduced to new stock containers, in which they took foods well and were all in good condition for more than 1 month, and were used in other experiments (Hotta et al. 2014).

Statistical methods

Statistical analyses were performed using R. 2.13.2 (R Development Core Team 2011). Wilcoxon signed–rank tests and Mann–Whitney *U*–tests were used to compare rushing time within matched pairs and between fish in different experiments, respectively, during the test phase. Beta binominal generalized linear models (GLZ) were used for analyses of the proportion of time in the three zones during the test phase. Likelihood ratio tests were applied to test the significant effects of explanatory variables (fish group, zone and its interaction).

Results

(a) *Effect of direct experience*

In experiment 1, the winner A more frequently attacked the loser B through the glass barrier (Wilcoxon signed–ranks test, T = 0.0, P = 0.004) and spent more time in the near zone than the loser B (beta binomial GLZ, fish group x zone: χ_2^2 = 12.95, P = 0.002, Fig. 3*a*). In contrast, in experiment 2, rushing time and time in zones were not different between fish C_1 and C_2, which had not had direct contact experiences (T = 20.0, P = 0.82 in rushing time, fish group x zone: χ_2^2 = 0.24, P = 0.89; fish group: χ_1^2 = 0.004, P = 0.95 in time in zones, Fig. 3*b*). Comparing the two experiments, responses of the winner A in experiment 1 and the strangers in experiment 2 were not different (Mann–Whitney U–test, z = –0.29 P = 0.77 in rushing time, fish group x zone: χ_2^2 = 5.31, P = 0.07, fish group: χ_1^2 = 0.04, P = 0.85 in time in zone, N = 20, data of fish C_1 and fish C_2 in experiment 2 were pooled). However, responses of the loser fish B in experiment 1 and the strangers in experiment 2 were significantly different (z = –4.24, P < 0.0001 in rushing time, fish group x zone: χ_2^2 = 5.96, P = 0.05 in time in zone, N = 20).

(b) *Winner/loser effects*

In experiment 3, I tested whether fish used winner/loser experience to infer dominance relationship. Contrary to the winner/loser effects expectation, neither rushing time nor the time in the three zones of the winners (A_1 and A_2) differed from those of the losers (B_1 and B_2; Wilcoxon signed–ranks test, T = 22.0, P = 0.57 in rushing time; beta binomial GLZ, fish group x zone: χ_2^2 = 1.58, P = 0.45, fish group: χ_1^2 = 0.07, P = 0.79 in time in zones, Fig. 3*c*).

(c) *Social eavesdropping*

In experiment 4, I tested whether fish used social eavesdropping to infer dominance. Contrary to the social eavesdropping expectation, both rushing time and the time in the three zones of the fish C, which had previously observed a contest between fish A and fish B, did not differ from those of the opponent fish A, who defeated B (Wilcoxon signed–ranks test, $T = 24.0$, $P = 0.77$ in rushing time; beta binomial GLZ, fish group x zone: $\chi_2^2 = 5.83$, $P > 0.05$, fish group: $\chi^2 = 0.50$, $P = 0.48$ in time in zones, Fig. 3*d*) Comparing across experiments, rushing time and duration in each zone were not different between observer C of this experiment and observer C_1 and C_2 in experiment 2 (Mann–Whitney *U*–test, $z = 0.59$ $P = 0.69$ in rushing time, fish group x zone: $\chi_2^2 = 0.75$, $P = 0.69$, fish group: $\chi_1^2 = 0.05$, $P = 0.82$ in time in zone, $N = 20$ for both experiments, data of fish C against A in experiment 4 and fish C_1 and fish C_2 in experiment 2 were combined).

(d) *Transitive inference*

In experiment 5, I tested whether fish can infer dominance by a combination of observation and their own experience. As expected, fish B, having lost a fight against fish A after observing fish C defeating fish A, less frequently attacked fish C through the glass barrier (Wilcoxon signed–ranks test, $T = 0.0$, $P = 0.0002$) and spent less time in the near zone than the winning fish C (beta binomial GLZ, fish group x zone: $\chi_2^2 = 7.53$, $P = 0.02$, Fig. 3*e*). This result confirms that fish B correctly inferred that it was subordinate to fish C after watching fish C defeated fish A, that had defeated fish B. Comparing across experiments, I found that rushing time significantly differed between fish B in experiment 5 and the loser fish B in experiment 1 ($U = 23.5$, $P = 0.01$), but the time in three zones was not different (fish group x zone: $\chi_2^2 = 2.62$, $P =$

0.27, fish group: $\chi_1^2 = 0.08$, $P = 0.78$). Further, rushing time did not differ between the fish C in experiment 5 and the winner fish A in experiment 1 (Mann–Whitney *U*–test, $U = 46.5$, $P = 0.37$), but the time in each zone just differed (beta binomial GLZ, fish group x zone: $\chi_2^2 = 5.98$, $P = 0.05$). Overall in experiment 5, fish B performed similarly to the losers B in experiment 1, and fish C similarly to the winners A in experiment 1. However, the aggressiveness of fish C in experiment 5 was similar to that of stranger C_1 and C_2 in experiment 2 that had no information about the dominance relationship ($z = 0.76$, $P = 0.44$ in rushing time, fish group x zone: $\chi_2^2 = 0.51$, $P = 0.78$; fish group: $\chi_1^2 = 0.01$, $P = 0.91$ in time in zones).

Discussion

My results support the hypothesis that the cichlid *Julidochromis transcriptus* can infer social status of unknown individuals using transitive inference. Using only observations of a social interaction between a stranger and a known individual, focal animals in my experiments changed their behaviour in a manner consistent with having inferred their social status relative to that of a stranger.

My first experiment showed that fish used direct information from a previous physical encounter to re–establish dominance without additional physical contact, which confirms that my protocol for the test phase is adequate. The second experiment showed that physical encounters are necessary to establish pairwise dominance. My third experiment clearly showed that winner/loser effects do not operate in this species. In my fourth experiment, the operation of social eavesdropping alone was ruled out, as I observed no differences in the responses of the observing fish against winner of an observed contest (Oliveira et al. 1998; Hsu et al. 2006, 2011). In experiment 5, the

focal male (B), which had no direct contact with the winning male (C) behaved submissively against C in test phase, suggesting transitive inference by the focal male. Alternatively, this submissive response might be caused by loser effects or social eavesdropping. However, experiment 3 demonstrated that loser effects do not cause a submissive response, and experiment 4 demonstrated that this fish does not use social eavesdropping alone. Any effects of physical characteristics on competition outcomes can also be ruled out because fish were of equal size and sex, and there are no other phenotypic indicators of dominance in this species (e.g. 'status badges'; Moller 1987; Beani & Turillazzi 1999). Thus, my fifth experiment shows that male *J. transcriptus* infer the relative social status of an unknown stranger without physical interaction, using transitive inference (Hsu et al. 2006, 2011, Whitehouse 1997, Oliveira et al. 2009).

Integration of cognitive ability from multiple sources

Social eavesdropping and winner/loser effects appear not to operate in *J. transcriptus*, potentially because transitive inference is a more effective method for determining social relationships. While observation of social interactions is required to obtain social information about strangers during transitive inference, my results suggest that eavesdropping must be accompanied by direct contact with one of the competitors to determine social relationships in *J. transcriptus*. In highly social animals, individuals repeatedly interact allowing more accurate inference of social dominance of strangers than would be possible using social eavesdropping or winner/loser effects. In contrast, winner/loser effects and social eavesdropping may be effective in less social species, where inferring dominance of strangers using TI would be difficult as the same members rarely encounter each other multiple times (Hsu et al. 2006, 2011). This suggests that in highly social animals, transitive inference, rather than social

eavesdropping or winner/loser effects, may be used to infer social structure. I suggest transitive inference may be mutually exclusive from social eavesdropping or winner/loser effects in the majority of species: the former being prevalent in highly social species and the latter in relatively less social species. This prediction is consistent with previous studies of highly social species, e.g. chickens (Hogue et al. 1996), territorial cichlids (Grosenick et al. 2007), river trout (White & Gowan 2013) and a highly social cichlid *M. auratus* (Chase et al. 2003), and also with less social species, e.g. the fighting fish *B. splendens* (Oliveira et al. 1998; Mcgregor et al. 2001; Witte & Nobel 2011), paradise fish (Francis 1983) and green swordtail (Beaugrand & Goulet 2000). A powerful test of this hypothesis may be achieved using related animal species with different levels of sociality (e.g. Bond et al. 2003; MacLean et al. 2008).

Transitive inference

My results exhibit two noteworthy points in the effects of transitive inference (TI) of this fish: first, the strength of the effect of TI, and second, the ratio of individuals that can effectively use TI. The behaviour of the losing fish in experiment 5, which did not have direct experience with its rival, was identical to those of losing fish in experiment 1, which did have direct experience with its rival. This similarity suggests that fish might infer their relative social dominance using transitive inference as effectively as through direct physical encounters, which will be a novel finding in studies of vertebrate TI. Comparing behaviour I also found that the level of aggression towards subordinates by fish that had correctly inferred their social dominance was equal to that of strangers who had no contest experience. Thus, while TI was correctly used to infer that an individual was inferior to its rival, I cannot be certain that individuals correctly inferred that they were dominant to rivals in experiment 5. This is also the

case of other studies on TI in e.g. hens (Hogue et al. 1996) and pinyon jays (Paz–y–Miño, et al. 2004).

In my final experiment, almost all individuals (11/ 12 individuals) used TI and modified their behaviour appropriately. This rate of TI use is comparable to other vertebrates, e.g. hens (15/15 individuals, Hogue et al. 1996), great tits (10/10, Peake et al. 2002) and pigeon jays (6/6, Paz–y–Miño 2004), and suggests that *J. transcriptus* uses TI in the complex social groups that exist in nature (Awata et al. 2005). It is worth exploring whether naïve individuals or less socially experienced fish are equally able to use transitive inference. Social experiences are known to influence subsequent behaviour in other fish species (Jordan & Brooks 2012), as well as highly social spiders and many other species (Jordan et al. 2014), and modifying social experiences in future studies will be shed light on the effects of experience and ontogeny on cognitive ability (e.g. Budaev et al. 1999; Frost et al. 2007; Brown & Laland 2011).

While studies of fish cognitive abilities are rarely compared with those on birds and mammals, recent work suggests that social fish have considerable cognitive ability (e.g. Bshary et al. 2002; Peake & McGregor 2004; Bshary & Grutter 2006; Bshary et al. 2006; Hsu et al. 2006, 2011; Bshary 2011). For example, individual recognition has been documented in many social fishes (e.g. Hert 1985; Griffiths & Magurran 1997a, b; Balshine–Earn & Lotem 1998; Bshary 2011; Brown & Laland 2011; Ochi et al. 2012). To predict the social status of strangers using transitive inference, it is necessary to recognise the individuals and recall their social status (Grosenick et al. 2007; Hsu et al. 2006, 2011). Although studies of fish memory are even more scarce than those on recognition, there is evidence that fish can remember social information for a considerable time, for example when making mate choice decisions (e.g. Milinski et al. 1990; Dugatkin & Godin 1993; Griffiths & Magurran 1997a, b; Dugatkin 2000; Tebbich et al. 2002), and integrate it with other information

in future social contexts (Dugatkin 2000; Dugatkin & Earley 2004; Frost et al. 2007; Bshary et al. 2006; Bshary 2011; Witte & Nobel 2011; Jordan & Brooks 2012, Hotta et al. 2015a). These studies suggest that advanced cognitive abilities such as transitive inference may occur across fish taxa. A fascinating future research direction will be to compare the use of TI between fish and mammal and bird species (Byrne & Whiten 1988, 1992; Bond et al. 2003, MacLean et al. 2008) to establish if either group uses TI more accurately to infer ambiguous social relationships, or if the effects of TI on behaviour differ. Finally, my study emphasises the importance of future studies evaluating the operation of social eavesdropping, winner/loser effects and transitive inference at the same time, which can be achieved using the experimental design I employ here.

Figures

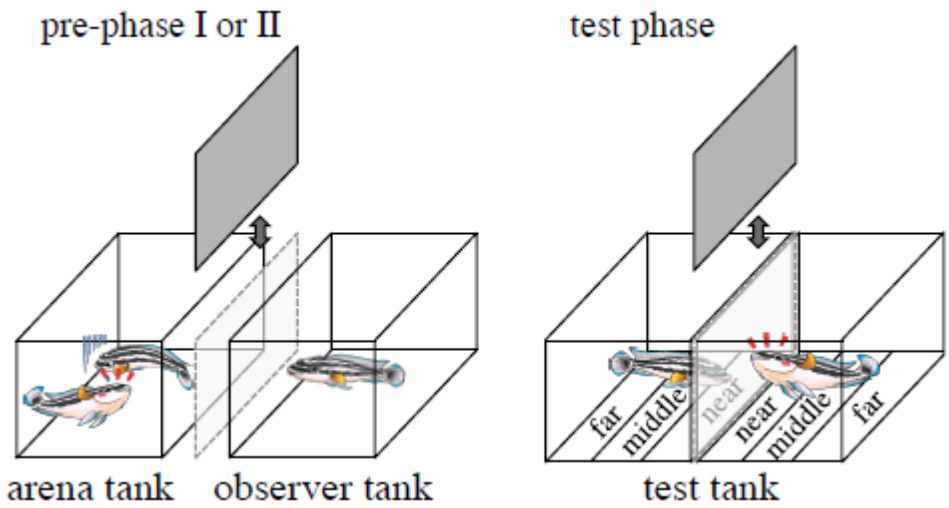

Fig. 1 Pictures of an arena tank, an observer tank (used in the pre–phase I and II) and test tanks (used in the test phase).

These tanks all measured 30 × 18 × 20 cm^3 (W × D × H). The arena tank and the observer tank were separated with 7 cm distance. Movable opaque sheets are between the arena tank and the observer tank, and between the two adjacent test–tanks. The bottom of the test tanks was divided into three zones: near, middle and far zones, each having a width of 6 cm.

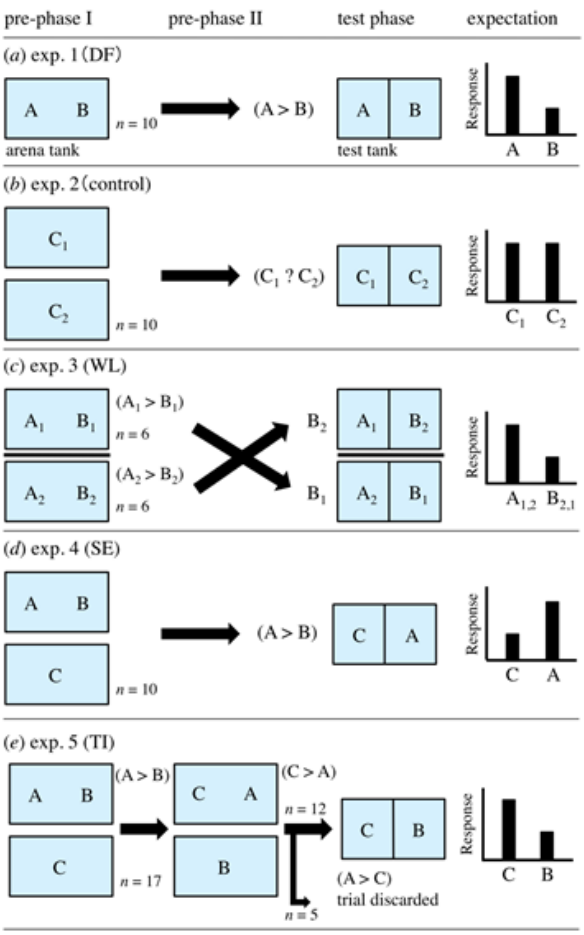

Figure 2. Design of the experiments.

All five experiments contained pre–phase I (and II if needed) and test phase. The expected responses (rushing time or time spent near the opponent, see text) are shown at the right when the predictions from the different hypotheses tested are supported. (*a*) experiment 1. A and B had a direct encounter during the pre–phase and re–establish dominance during the test phase. (*b*) experiment 2. C_1 and C_2 had only visual contact in the pre–phase and establish dominance during the test phase. (*c*) experiment 3 (testing winner/loser effects). A1 and A2 dominated B1 and B2 in arena tank, respectively. If A_1 and A_2 dominate against B_2 and B_1 against grass barrier in test phase, respectively, the loser–winner effect will operate. (*d*) experiment 4 (testing eavesdropping hypothesis). Fish C observed interactions A > B. If the fish C exhibited submissive behaviours against A, but if it exhibited aggressive behaviours against B, the prediction from eavesdropping hypothesis will be supported. (*e*) experiment 5 (testing transitive inference hypothesis). Fish C observed interactions A > B, thereafter B observed interactions C > A. According to the transitive–inference hypothesis, fish C is expected to dominate over fish B against grass barrier.

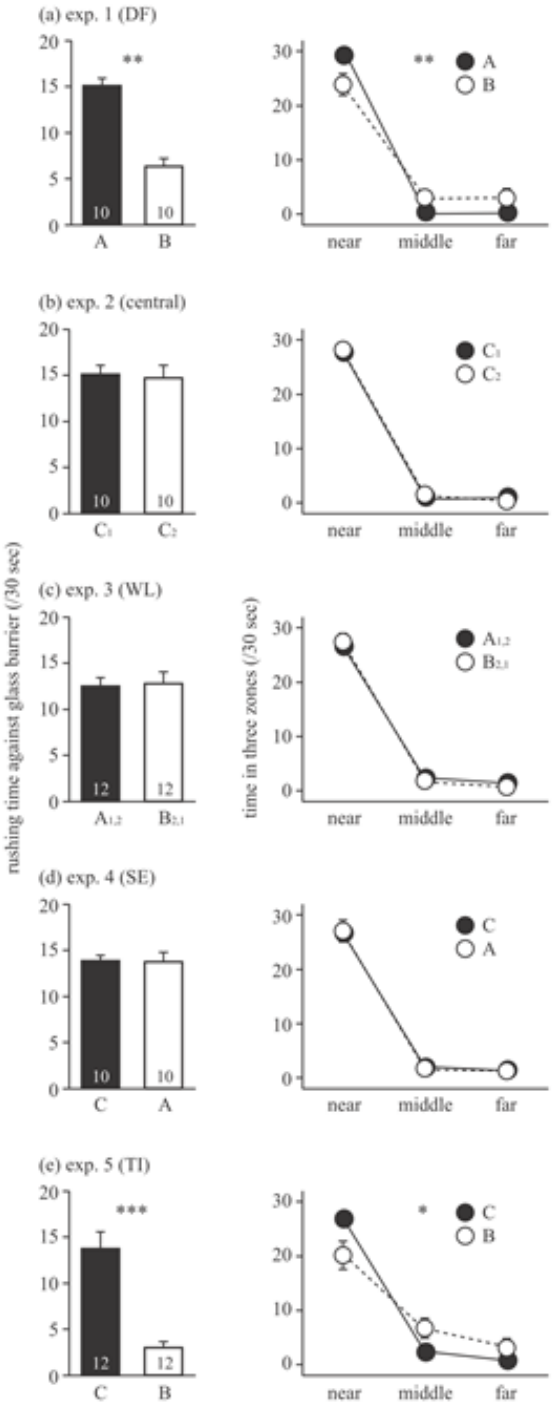

Figure 3. Results of five experiments testing different hypotheses of cognitive abilities.

Right and left panels show the rushing time against grass barrier per 30 seconds and the time in three (near, middle and far) zones per 30 seconds, respectively. (*a*) experiment 1 testing whether direct interactions affect the next fight between the same individuals. (*b*) experiment 2 with two strangers (*c*) experiment 3 testing winner/loser effect. (*d*) experiment 4 testing eavesdropping effect. (*e*) experiment 5 testing transitive inference. See details of fish combinations and expectations from the different hypothesis in figure 2. Error bars indicate s.e.m. Numerals in bars show sample sizes. Asterisks denote the significant differences in rushing time between fish groups by Wilcoxon signed–rank tests (left panels) and the significant interactions between fish group and zone on the proportion of time stayed in each zone by beta binomial GLZs (right panels): * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Chapter III: Order effects in transitive inference

Abstract

Transitive inference (TI) is the ability to infer social relationships between individuals (e.g. if A<B & B<C, then A<C), and has been documented in a variety of vertebrates. Many studies of TI use the task of inferring social dominance, where a subject animal A first directly interacts with B (e.g. A subordinate to B: A<B), and then indirectly observes the interaction of B and an unknown C (B<C), using both direct and indirect information to infer its own relationship with C (i.e. A<C). However, order effects are known to influence learning, especially in complex scenarios, and I have little understanding of the effects of presentation order in transitive inference. Here I show that the cichlid *Julidochromis transcriptus* can use TI to correctly assess social relationships when information is presented in the order opposite to that most commonly employed in studies of TI. I find that focal individuals (A) can transitively infer their relationships with an unknown individual (C) when initially given indirect experience (i.e. eavesdropping that B<C) and then given direct experience (A<B). I conclude that *J. transcriptus* can infer social relationships when experiencing first indirect and then direct social information. I suggest that in this and many other species, transitive inference may occur in either presentation order, and future studies of TI should account for order effects of social information.

Introduction

Transitive inference (TI) is the ability to infer unknown relationships between objects by using multiple sources of information (Vasconcelos 2008). For example, knowing that A<B and B<C, the subject may infer that A<C. Although TI has long been considered a developmental milestone in human social and linguistic ability (Piaget 1970), after the methodological development for animals (e.g. Bryant and Trabasso 1971), a number of studies revealed that a variety of vertebrate social animals can also transitively infer social relationships (e.g., chimpanzee: Gillian, 1981; monkey: D'Amato and Columbo 1988, 1990; lemur: MacLean et al. 2008, Tromp et al. 2014; rat: Davis 1992; Birds: von Fersen et al. 1991; Bond et al. 2008; Mikolash et al. 2013; Weiß et al. 2010; Fish: Groesnick et al. 2007).

Many TI studies document that highly social animals can infer the contest ability of unknown individuals based on information from direct experience and from social eavesdropping (e.g. Paz–y–Miño et al. 2004; MacLean et al. 2008; White and Gowan 2013; Tromp et al. 2014) or operant conditioning (e.g. Gillian, 1981; von Fersen et al. 1991; Davis 1992). Theoretical models predict that increased cognitive ability offers an adaptive advantage and evolves more easily in animals living in complex social groups (Byrne and Whiten 1989). By inferring an unknown individual's relative dominance rank, animals may avoid costs of direct fights, including time and energy expenditure and risk of injury or predation. Thus, TI likely plays an important role in social rank estimation and the establishment or maintenance of dominance hierarchies (Cheney and Seyfarth 1986; Hogue et al. 1996). In large and stable social groups, individuals frequently interact with many other group members within the communication network (McGregor and Peake 2000). High cognitive abilities such as TI will be favoured in these social groups where frequent interactions

may otherwise lead to increased aggressive interactions (Kaiser 2014).

Previous studies of TI demonstrate that subjects that directly fight with the known animal first, and are then allowed to observe aggressive interactions between known and unknown conspecifics, can infer dominance relationships of the unknown animal. Surprisingly, in most experimental studies of TI (Grosenick et al. 2007 being an exception for using only indirect information), the subject animal obtains information from direct fights with animal B first (A<B), and then gets indirect information from eavesdropping on the contest between B and an unknown C (B<C). Using this protocol, individual A has been shown to correctly infer the relative contest ability of animal C (i.e. A<C) in multiple species (e.g. Hogue et al. 1996; Paz–y–Miño et al. 2004; Weiß et al. 2010; Mikolash et al. 2013). But in natural social conditions, it is more likely that an incoming individual (A) will try to join a social group that has an already established dominance hierarchy (Jordan et al 2010a, b). Indeed, in many species individuals visit groups many times before finally attempting to join them. This gives a potential joiner (A) the opportunity to observe the interactions of unknown members of the group before directly interacting with them (Vasconcelos 2008).

More generally, in the field of learning and behaviour, the effect of presentation order and serial learning are well known to influence retention of information (Domjan 2010). It cannot be assumed for instance that a series of stimuli will be remembered in the same way presented in a different order (Domjan 2010). In fact, researches of TI using operant conditioning tasks considered information presentation order (Steirn et al. 1995). In the context of social learning, indirect observations (i.e. that two unknown individuals have a certain dominance relationship, B<C) may constitute a less salient source of information, and so be more difficult to recall when presented with a direct interaction (A<B). Remarkably however, there appear to have been no studies of TI in which social information is presented in this

alternative order (observation first and direct interaction second). I therefore do not know whether transitive inference is possible under these potentially more common social conditions.

Julidochromis transcriptus is a species indigenous to Lake Tanganyika, Africa (Konings 1996) and is a cooperatively breeding cichlid with a highly organized social system (Awata and Kohda 2004; Awata et al. 2005; Heg and Bachar 2006; Kohda et al. 2009), which may favour higher cognition. This fish can recall memories of social events after 5 days (Hotta et al. 2014). In the previous experiment (see Chapter II, Hotta et al. 2015b), I used three unknown individuals (A, B and C). At first, A lost a competitive interaction with B (i.e. A<B) and then A observed C defeating B (B<C). As expected, in the first encounter, A behaved subordinately to C (A<C), suggesting that A infers social dominance transitively. I also showed that eavesdropping does not directly affect the reaction of the observer A to the interacting fish B or C in the absence of direct interaction (e.g. Oliveira et al. 1998), nor did I observer evidence of winner/loser effects following direct interactions (e.g. Hsu et al. 2006; Hotta et al 2014). Taken together, these results demonstrate that *J. transcriptus* has the ability to perform TI to infer the dominant rank of unknown individuals when direct information precedes indirect information.

The purpose of this study is to test whether this fish can perform TI in the complementary presentation: indirect (eavesdropping) followed by direct (contest). Because my previous study showed no evidence that fish use social information alone to estimate the competitive ability of unknown individuals (Hotta et al. 2014), it is possible that TI is impossible when presented with social information prior to direct contests. It is therefore essential to establish whether fish can infer their relative dominance rank when the information order of social eavesdropping and direct experience is switched.

Material and Methods

Subjects and Housing

Experiments were conducted in my laboratory at Osaka City University. The cichlid *J. transcriptus* used in this study were laboratory–reared descendants of wild–caught fish from Lake Tanganyika, Africa. I used males (60.6–76.0 mm in total length, TL) that had been kept with females in stock tanks, either 30×40×60 cm glass tanks of 20 individuals or 45×40×180 cm glass tanks of 60 individuals. These stock tanks contained multiple shelters of flower pots, stones and tiles put on coral gravel bottom and water was aerated and filtered. The tanks were kept at 24–26℃ at a 12:12–h light/dark cycle. Fish were fed with artificial flake food (Tetramin) twice a day.

Three days before the start of the experiments, fish were measured TL and each placed in 30×17×15 cm glass tanks (house tank) with 2 cm coral substrate, filtration and aeration. The fish were visually isolated from one another: all sides of the tank were covered with opaque sheets. This isolation lasted for 14 days prior to the experiment to avoid any effects of previous contests or social experience (Hsu et al. 2006, Hotta et al. 2014).

Experimental procedure

I designed 3–term series tasks (Bryant and Trabasso 1971) that have previously been used in studies of social transitive inference (Hogue et al. 1996, Paz–y–Miño et al. 2004, White and Gowan 2013). I made 31 triads containing individuals were size–matched (TL difference was within 3 mm, and preliminary experiments showed that the size difference was sufficiently small that I could not a priori predict the winner of the contest). This experiment consisted of three phases, a pre–phase I, a pre–phase II

and a test phase.

In the pre–phase I, two fish were placed in an arena tank, and watched by an observer fish A that was physically separated from the arena tank for 30 min. My preliminary experiment showed that dominance relationships took up to 15 min to stabilize, i.e. one fish behaving aggressively (performing chases and bites) and another fish adopting submissive behaviours and retreating when another fish approaches. Thus I allowed 30 min to establish dominance after introducing two size–matched fish to arena tanks. The tank housing the observer fish A was separated by 7 cm space from the tank housing the physically interacting fish (B and C), and I did not observe any behavioural interactions among observing and interacting fish. After 30 min, I measured the dominance relationship between the interacting fish, referring to the winner of the dyadic interaction as “C”, and the loser as “B” (Fig.1).

In the pre–phase II, the loser fish B and the previous observer fish A were transferred into a new arena tank, and the winner fish C was moved to a new observer tank to be allowed to observe interactions between A and B at a distance of 7 cm. As in the pre–phase I, I observed the physical interaction during 30 min and determined dominance relationships. In this study, the effect of information order was evaluated by comparison with results from experiments using traditional social information presentation order (direct followed by indirect). The experience of winning and being observed did not influence contest behaviour in *J. transcriptus* (Hotta et al 2014). If fish B was dominant over fish A, the loser fish A and the observer fish C were transferred to a paired test tank (Fig.1, n=18). Conversely, when the fish A was dominant over the fish B, the experiment was terminated and the fish were returned to own house tank (n=13). At first, an opaque sheet was placed between the test tank and fish were acclimated for 10 min. After the acclimation period, the opaque sheet was removed and behaviour was recorded for 10 min by video camera (HDR–CX370,

Sony). This procedure allowed visual, but not physical, interaction.

During the first 30 seconds of exposure in the test phase, I measured the following responses of each individual to evaluate aggressive behaviour: “rushing time” (when the focal fish rapidly swims towards the glass barrier) and “time in near zone” (when the bottom of the tank was divided into three zones, i.e. each zone is 5.7 cm, the time fish stayed in a zone near glass barrier). Subordinate behaviour was defined as one fish showing shorter rushing time and less time in near zone than their opponent. These responses and the observation time were similar to previous studies (Hotta et al. 2014).

To create a directly comparable experimental design to previous studies of transitive inference, I allowed fish C to observe the contest between A and B, because traditional TI studies allow an audience to observe all contests. However, this has the effect of presenting social contest information to fish C in the traditional order, creating the potential for ‘normal’ transitive inference on the part of C. Using normal TI, fish C may therefore infer that it is dominant over A, and be more aggressive towards A in the final trials. This increased aggression may elicit a subordinate response in A that is unrelated to transitive inference. To test for this possibility, I compared the level of aggression shown by C to that shown in contests between two unfamiliar fish that were placed in test–phase contests but had no previous social interactions. The null expectation is that fish C will show levels of aggression that are not significantly different to those of unfamiliar fish, and consequently that any change in behaviour in A is due to transitive inference rather than a reaction to increased aggression by C. Note that this test does not eliminate the possibility that C is performing TI, but only that this does not result in increased aggression by C. Alternatively, if fish C shows higher than normal aggression, I cannot conclude that any change in the behaviour of A is a consequence of TI.

All experiments were conducted in compliance with Regulations on Animal Experiments at Osaka City University and the Japanese Ethological Society. During physical contests in pre–phase I and pre–phase II, when fish had the opportunity to engage in open aggression, e.g. mouth wrestling, all contests were video–taped and monitored by an observer. If either fish appeared to suffer visible physical injury, the observer would have terminated the contests. However, I did not observe any fish suffering physical injury.

Statistical analyses

Statistical analyses were conducted in R version 3.0.0. Wilcoxon signed–rank tests were used to compare rushing time and time in near zone between fish A and C during the test phase to determine whether fish A acted subordinately in the absence of direct experience with C (i.e. used transitive inference to infer that C is dominant over A). Additionally, Mann–Whitney U tests were used to compare the traditional and reversed social information presentation order, in order to determine whether the order of social information presentation influences TI.

Results

In the test phase, rushing time by fish A against fish C was significantly shorter than fish C against fish A (Wilcoxon signed–rank test: T=290, N=18, p<0.005; Fig. 2a). Fish A stayed in the near zone for a significantly shorter total time than fish C (Wilcoxon test: T=243, p<0.01; Fig. 2b). Thus, fish A behaved subordinately to fish C. In addition, the level of aggression shown by fish C was not different from that shown by unfamiliar fish that had no previous social interactions (Mann‑Whitney test: U=162, N1=18, N2=14, in rushing time, p=0.18; U=164, N1=18, N2=14, in time in near zone, p=0.15).

Further, rushing time and time in the near zone of fish A in the present study (with reversed social information presentation order) was not significantly different from rushing time and time in the near zone of fish A in my previous study (with traditional social information presentation order; Mann–Whitney test: U=173, N1=18, N2=15, in rushing time, p=0.17; U=128, N1=18, N2=15, in time in near zone, p=0.80).

Discussion

In this study, I tested whether the male cichlids *Julidochromis transcriptus* use TI to infer the relative contest ability of an unknown individual by observing a physical contest between two unknown individuals first, and then directly interacting with the previous loser. This order of social experience has never before been tested in the context of transitive inference, with previous studies either beginning with direct exposure (contests) and following with indirect exposure (eavesdropping), or using only indirect social information (Grosenick et al. 2007). However, in learning studies, the order of presentation is known to have strong effects on learning and memory

(Domjan 2010). Moreover, indirect experiences followed by direct experiences are potentially more ecologically relevant and more common in group joining animals than direct followed by indirect experiences (Bergmuller et al. 2005; Jordan et al. 2010a, b). My results showed that subject fish (A) were able to transitively infer their social relationship with an unknown fish (C) when first observing C interact with B, and then directly interacting with B. Focal fish behaved subordinately to C despite never having interacted directly with this previously unknown fish. There are two explanations for why fish A would behave subordinately to fish C. Firstly, the subordinate behaviour of fish A may be a direct response to increased aggression from fish C, which has used 'traditional' transitive inference to determine it is dominant over fish A. However, I found that the aggressive behaviour of fish C was not different from that of unfamiliar fish, and therefore does not explain the observed change in the behaviour of A. It should be emphasized that this test does not confirm that fish C is not using TI in the traditional manner, only that it does not show any observable behavioural correlate of TI if it is being used. An alternative explanation for this pattern of behaviour is that fish A is displaying a loser effect (Hsu et al. 2006) due to a recent contest loss (against B), and that fish C displays some form of winner effect (i.e. dominance badge, Colgan 1983), or that social eavesdropping on the interaction between B and C is sufficient to change the behaviour of A. However, my previous studies find no evidence that *J. transcriptus* exhibits dominance badges, loser effects, or social eavesdropping (Hotta et al. 2014).

The comparison between traditional and reversed social information presentation order shows that there are no differences in the social responses of fish A against winner C, suggesting that both orders of presentation are equally effective in inducing transitive inference. Furthermore, almost all individuals displayed TI under both order presentations (11/12 fish behave submissively against A, i.e. they can do TI

for A>C in the previous study, and 17/18 in the present study; χ^2–test, χ^2=0.015, p=0.91). Combined, my current and previous results demonstrate that a single source of social information is not sufficient to induce submissive behaviour to an unknown individual, and that the combination of direct and indirect social experience is sufficient for TI, independent of presentation order.

A previous study on this species suggests that *J. transcriptus* do not use eavesdropping alone to determine dominance relationships (Hotta et al. 2014). However, I cannot judge whether observing fish are able to determine the social relationships by eavesdropping, but do not use this knowledge in their subsequent direct interactions, or if they are simply unable to assess dominance relationships through observation alone. In the present study, this fish eavesdrops on the interactions of two unknown fish first, and if it then loses in a direct contest with the observed loser, it transitively infers its relationship with the observed winner. This strongly suggests that these fish are able to assess and remember social relationships based on observation alone, but do not use this information in the absence of a direct interaction. This is in keeping with observations of other social animals that use TI but not social eavesdropping alone (e.g. hen: Hogue et al. 1996; pinyon jay: Paz–y–Miño et al. 2004), and I suggest these animals are also able to recognize and memorize the outcome of observed contests, but do not modify their behaviour based on indirect experiences alone. It is possible that observed contests provide less reliable social information, and animals making judgements on indirect information alone may suffer increased costs of inappropriate social contests.

When attempting to join social groups of unknown individuals, individuals will most likely indirectly observe the interactions of some or most members before directly interacting with them. Prospecting behaviour, in which an animal visits groups numerous times before eventually joining them (Bergmuller et al. 2005), would allow

for numerous indirect observations of social interactions, allowing rapid assessment of dominance relationships using transitive inference when joining groups. Prior to this study, however, it was not known if animals could perform TI when presented indirect followed by direct social information. In my experiment, I show that fish are able to perform TI when presented with information in the order expected when joining new social groups. Further TI studies should be conducted across taxa focusing in the order of presentation of social information.

In conclusion, I show that *J. transcriptus* can transitively infer their social relationship with unknown individuals, and that the order of social information presentation (direct and indirect) does not influence the ability to perform TI. My experimental procedures provide a straightforward method to test TI and other social abilities in fish (direct fighting, eavesdropping, winner/loser effects).

Figures

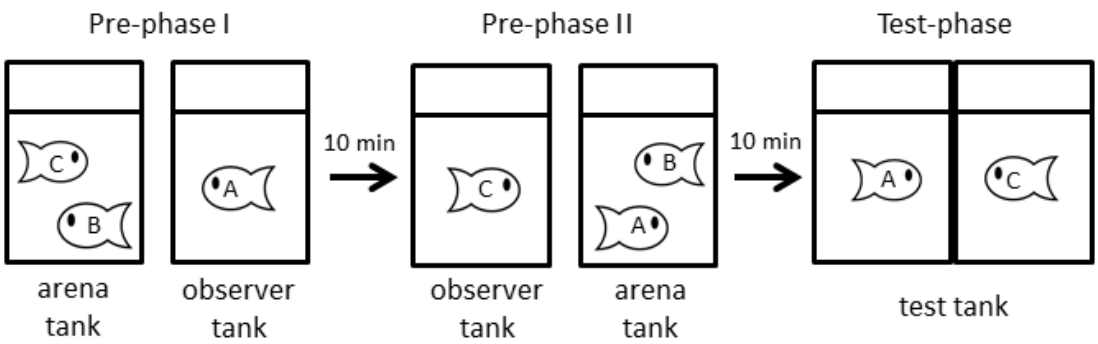

Figure 1. Procedure of experiments.

In pre–phase I, A observes that C defeats B (B<C). Then A is defeated by B (A<B), and C observes that contest in pre–phase II. Finally, in test–phase, A is faced to confront with C and their behaviors are video–recorded. (See text for detail)

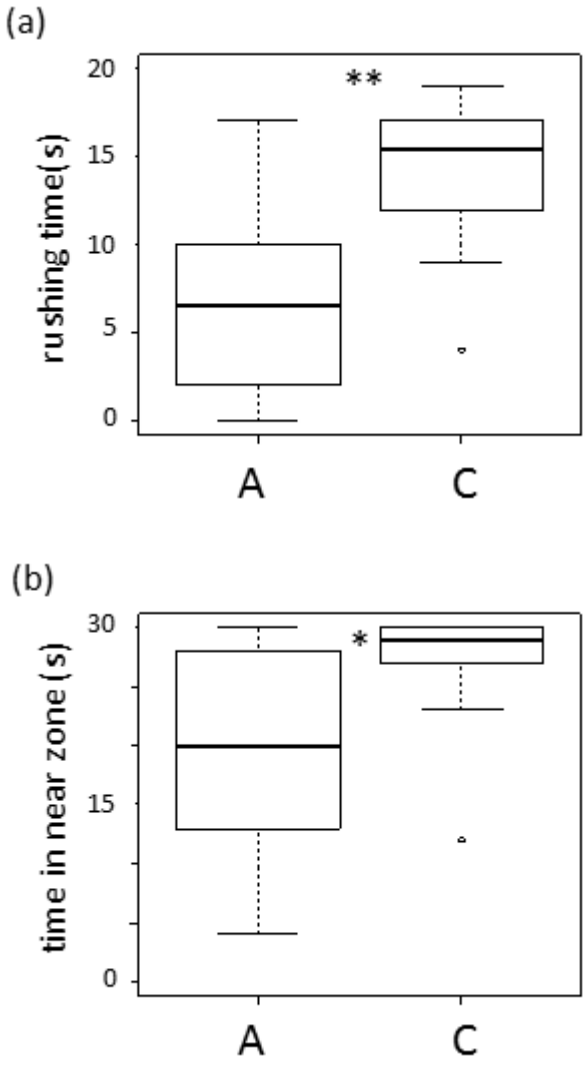

Figure 2. Results of experiment.

(a) rushing time and (b) time in near zone of A and C in test phase. *p<0.01, **p<0.005.

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Hotta, T., Takeyama, T., Jordan, L.A., Kohda, M. (2014) Duration of memory of dominance relationships in a group living cichlid. Naturwissenschaften 101: 745–751. (Chapter I)

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