CANNIBALISM AND KIN DISCRIMINATION IN TADPOLES OF THE AMAZONIAN POISON FROG, DENDROBATES VENTRIMACULATUS, IN THE FIELD

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Two experiments were conducted to investigate the influence of kinship on aggression and cannibalism in the Amazonian poison frog, Dendrobates ventrimaculatus, in eastern Ecuador. Firstly, we placed pairs of kin and pairs of non-kin tadpoles in plastic cups, allowed them to interact over a food item and videotaped their behaviour. The videotapes were analysed for aggressive and associative behaviour. Secondly, we placed pairs of tadpoles in manipulated natural pools in the field, and left them together for one month. The results of the videotaped behavioural experiments did not indicate strongly preferential treatment of kin, although biting was rare in the kin treatments but common in some non-kin treatments. The field experiments indicated that both kin and non-kin tadpoles are likely to be cannibalized if they coexist with larger tadpoles in Heliconia pools for a substantial period of time. Ultimately, the study was inconclusive with respect to the occurrence of kin discrimination. However, the study provides important information relevant to the study of kin discrimination by dendrobatid tadpoles in the field.

Key words: cannibalism, kin recognition, Dendrobates, behaviour

INTRODUCTION

Kinship is of fundamental importance in understanding the evolution of behaviour (Hamilton, 1964). The study of kin recognition, or how and why animals do or do not recognize and discriminate among kin and non-kin, has received considerable attention (Alexander, 1979; Waldman, 1988; Sherman, Reeve & Pfennig, 1997). Kin recognition among anuran larvae comprises a large part of the literature on kin recognition (Waldman, 1991). Despite frequent demonstrations that anuran larvae can and do recognize kin, the function of such recognition has remained obscure in most cases.

Recent research (Pfennig, Reeve & Sherman, 1993) on tadpoles of the spadefoot toad has demonstrated a clear functional context for kin recognition and discrimination. In this species, there are two tadpole morphs, one of which is highly cannibalistic. Tadpoles were predicted to be more likely to cannibalize non-kin than kin because cannibalizing kin reduces the indirect component of the cannibal's inclusive fitness (Pfennig et al., 1993). This prediction was confirmed: cannibalistic tadpoles prefer to cannibalize non-kin, although cannibals will eat kin and non-kin indiscriminately when the cannibal is hungry, i.e. when its own survival is at risk.

Tadpoles of several species of poison frogs (genus Dendrobates) are highly cannibalistic (Wells, 1981; Weygoldt, 1987; Summers, 1990). Hence, this genus is an excellent candidate for investigations of kin recognition in a functional context. In this paper we present the results of laboratory and field experiments on cannibalism and kinship in the Amazonian poison frog, Dendrobates ventrimaculatus, from Amazonian Ecuador. The objectives of this study were to: (1) analyse behavioural interactions between related and unrelated pairs of tadpoles to determine if tadpoles discriminate behaviourally on the basis of kinship; (2) investigate the consequences of coexistence in the same pool for related and unrelated pairs of tadpoles.

Dendrobates ventrimaculatus lives in Ecuador, Peru and Brazil. The mating and parental system of D. ventrimaculatus from Pompeya in Sucumbios Province in Amazonian Ecuador has been described elsewhere (Summers & Amos, 1997). Briefly, recent field research suggests that this population has male care. Tadpoles are deposited in the pools by males, who carry them on their back from the pool over which they were oviposited (some are simply placed in the pool over which they were oviposited). The tadpoles grow and develop in the pool until metamorphosis, which can require several months in closely related species (Caldwell & Araujo, 1998). Typically, only one tadpole is placed in a pool, but two or more tadpoles are sometimes placed together in the same pool, and a maximum of seven have been found in a single pool (Summers & Amos, 1997; Summers, 1999, unpublished data). Genetic analysis suggests that both related and unrelated tadpoles are placed together (Summers & Amos, 1997). Hence, tadpoles may encounter other tadpoles in the same pool, and these tadpoles may be kin or non-kin.

METHODS

This investigation was carried out in the Quechua village of Limoncocha, and in nearby rainforest near Pompeya, a small Capuchin Mission on the Napo River, in Sucumbios Province, Ecuador, from 23 May to 4 August, 1997. We obtained tadpoles by collecting clutches of eggs in the field and raising them in plastic cups until they hatched. After hatching, the members of the clutch
were raised in separate plastic cups (one per cup) until placed in an experiment.

We fed at least one member of each clutch *ad libitum* with detritus, mosquito larvae, algae, and (unrelated) *D. ventrimaculatus* eggs. The other members were fed detritus only. After a period of approximately one week to ten days, a substantial difference in mass developed between tadpoles fed *ad libitum* and tadpoles given a restricted diet (mean ± SE: 46±3 mg (large), n=18; 15±1 mg (small), n=18; paired *t*-test: *t* = 9.695, *P*<0.0001). We also raised unrelated tadpoles in the same way. Tadpoles classified as unrelated were taken from clutches found in plants approximately 10 m or more apart, which is outside of the home range of males and females in this species (Summers & Amos, 1997).

After a substantial difference in mass developed, a large tadpole was placed together with a smaller tadpole in one of two treatments: with kin (presumed full siblings from the same clutch) or with non-kin (unrelated tadpoles from different clutches from different plants). Each related pair of tadpoles was matched with an unrelated pair of tadpoles, and the two treatments (kin and non-kin) were carried out on the same day. Fourteen sets of matched pairs (28 pairs) were used in the experiments. The pairs were matched so that the mass and size differences between the large and small tadpole were as similar as possible between the two treatments (kin and non-kin). There was no significant difference between the two treatments in either the initial mass of the small kin and non-kin tadpoles (paired *t*-test, *n*=14, *t*=0.306, *P*=0.7646) or the mass difference between the large and small tadpoles in the kin and non-kin treatments (paired *t*-test, *n*=13, *t*=1.775, *P*=0.101).

These matched pairs of tadpoles constituted the matched trials used in the paired tests presented in the results. We placed the two tadpoles of each pair together in a cup with approximately 50 ml of water (equivalent to a small to medium natural pool), and allowed them to acclimate to each other for five to eight hours. A food item (an unrelated egg or embryo) was then placed in the cup and the tadpoles were videotaped for from one half hour to one hour, depending on the availability of electricity. All matched trials were videotaped for the same period of time (so the total amount of observation time was the same for trials with related and unrelated tadpoles), and the data on tadpole interactions were analysed as events per second (e.g. bites per second), to adjust for time length differences between trials.

The behaviour of the tadpoles was scored later from the videotapes by a researcher who did not know the purpose of the experiment, nor which experimental pools contained kin or non-kin. Major categories of behaviour scored were: biting (large tadpole bites the small tadpole), feeding (large or small tadpole feeds on the food item), time spent in contact without aggression (the two tadpoles remain quiescent while in contact or within 1mm of each other), and chasing. Chasing was defined as the large tadpole moving toward the small tadpole, followed by the retreat of the small tadpole. It was not possible to place one large tadpole with one related and one unrelated tadpole simultaneously (e.g. Pfennig et al., 1993), because tadpoles were not sufficiently distinctive in colour or pattern to be individually identifiable, and attempts at marking were not successful.

We carried out further experiments with the kin and non-kin treatments in the field for those experiments that were started more than a month before the end of the study. The day after tadpoles were used in the first experiment (the videotaped behaviour experiment), we placed the tadpoles in *Heliconia* pools in the forest. The pools used form in the leaf and stem axils of *Heliconia* plants, and are the sites most commonly used for breeding by *D. ventrimaculatus* in this area (Summers, 1999).

Placing two tadpoles together replicates the most common type of multiple pool occupancy found in pool surveys: more than two tadpoles in a pool is relatively infrequent (Summers & Amos, 1997). Typically, one tadpole is larger than the other (K. Summers, unpublished observations). We matched the pools for volume for each pair of kin and non-kin. The pools were emptied, any eggs or tadpoles that were found in the pool were removed, and plastic flanges were affixed to the stem of the *Heliconia* plant (with waterproof plastic tape) to prevent adults in the area from using the pools for breeding. The water from each of the matched pools was mixed to equalize the amount of nutrients in the two pools, and the two tadpoles (one large and one small) from each treatment were placed in one of the two pools. We also set up control pools in the same manner, containing only a single small tadpole. These were used as controls for the natural levels of mortality of small tadpoles, without the presence of a large tadpole in the same pool.

The replicate pools were left for one month. The kin and non-kin treatments were fed four eggs over the course of this period, approximating the average availability of eggs in pools that occurs naturally (Summers & Amos, 1997). The control treatments were fed a single egg at the start of the experiment. After one month, the pools were drained and taken apart, and the number of surviving tadpoles was recorded by a researcher who did not know which treatments were which. We are confident that large tadpole remained alive in each pool because the pools were examined frequently (three times a week) and the large tadpole was usually seen in each pool during those inspections. If the large tadpole had died, it would have taken at least a week for the smaller tadpole to reach that size, and the absence of the large tadpole would have been detected during that time period.

This research and associated protocols were approved by INEFAN (the Ministry of Natural Resources of Ecuador); Permit No. 24-IC, and by the Animal Care and Use Committee of East Carolina University; Permit No. D145. Statistical analyses were carried out with
StatView (Abacus Concepts, 1992). Data were examined for normality and homogeneity of variances, and transformed as appropriate.

RESULTS

Analysis of the videotaped behaviour revealed a tendency for tadpoles to be more aggressive towards non-kin than towards kin. The number of bites per second did not differ between the treatments (Fig. 1, Wilcoxon signed rank test, $Z=1.095, n=14, P=0.273$), but there was a significant difference between treatments in the variance of this behaviour ($F$-test, $F_{1,14}=0.001, n=28, P<0.0001$). This means that biting between kin was rare, but there was a high variance in the frequency of biting by non-kin. The number of chases per second did not differ between the treatments (Fig. 2, paired $t$-test, $t_{13}=0.535, n=14, P=0.602$). Time spent in contact with each other also did not differ between the treatments (Fig. 3, paired $t$-test, $t_{13}=0.594, n=14, P=0.563$). The amount of feeding (number of bites per second) did not differ between the treatments, either for large tadpoles (paired $t$-test, $t_{13}=1.386, n=14, P=0.1890$), or small tadpoles (paired $t$-test, $t_{13}=1.515, n=14, P=0.154$).

For the field experiments on cannibalism, there were no significant differences among the experimental treatments or the control pools in pool volume (one-way ANOVA, $F_{2,20}=0.067, n=23, P=0.935$). There were no significant differences among the experimental treatments or the control pools in the starting weights of the small tadpoles (one-way ANOVA, $F_{2,22}=0.056, n=25, P=0.945$). Also, there was no significant difference between the kin and non-kin treatments in the mass differential between large and small tadpoles at the start of the field experiments ($t$-test, $t_{16}=0.936, n=18, P=0.363$). There was no difference between the treatments in the mortality of small tadpoles (there was 100% mortality for small tadpoles in both treatments), but there was a significant difference between the mortality of small tadpoles in the two experimental treatments (pooled results) and that of the controls (Fig. 4, Fisher’s exact test, $P=0.0017$). There was no significant difference between the treatments (kin versus non-kin; single tadpole controls were excluded because they started at a different stage) in the growth rates of the surviving (large) tadpoles ($t$-test, $t_{12}=1.341, n=14, P=0.205$).

DISCUSSION

The behavioural observations of interactions between kin and non-kin did not demonstrate any dramatic differences between the two treatments. Tadpoles did not chase or bite non-kin significantly more frequently than kin, nor did they spend significantly more time in
passive (non-aggressive) contact with kin than with non-kin. There was no significant difference between kin and non-kin in the amount of feeding done by either large or small tadpoles, so there was no evidence that non-kin tended to monopolize food more, or attempted to prevent the smaller tadpole from eating more vigorously. More complex tests combining different variables (e.g. the number of bites per time spent in contact) also failed to yield significant differences between the kin and non-kin treatments.

However, there was a significant difference between the kin and non-kin treatments in the variance of biting behaviour: biting among kin was consistently rare, whereas the amount of biting between non-kin was variable. This high variance means that the power of the statistical tests to detect preferential treatment was low. Power analysis (Zar, 1996) indicates that to achieve a probability of 75% of detecting a significant difference between the kin and non-kin groups (assuming the mean difference of 0.002 bites per second or 7.2 bites per hour found in this study) would require a sample size of at least 83 trials. Hence, it would be premature to conclude that preferential treatment of kin is absent in this species.

The inconclusive nature of the results of this experiment make it worthwhile to consider potential pitfalls in the methodology that could be corrected by future researchers. We believe that the behaviours we recorded, particularly chasing and biting, are likely to be correlated with tadpole mortality, as has been suggested by other researchers (e.g. Caldwell and Araujo, 1998). However, one possible problem is that the videotaped trials were not long enough. In the field, it may take days or weeks for a small tadpole to succumb to the attacks of a larger tadpole. Hence, the number of interactions observed over the short duration of the videotaped experiments is likely to be relatively small, making detection of significant differences between the kin and non-kin treatments difficult.

The results of the field experiments suggest that, even if there is some tendency to be less aggressive toward kin than toward non-kin, this tendency may usually be insufficient to prevent cannibalism of small tadpoles by larger kin in the field. All of the small tadpoles with larger tadpoles were cannibalized, regardless of kinship status. The mortality of small tadpoles in the experimental treatments (kin and non-kin) was significantly higher than that of single small tadpoles in the control treatment, implying that the increased mortality was due to the presence of the larger tadpole. The effect is unlikely to be due simply to starvation as a result of the presence of another tadpole, as the experimental tadpoles were given four times as much food as the single tadpole controls. The effect was also unlikely to be due to water fouling, as single small tadpoles given more than four eggs in feeding experiments did not show high mortality rates (Summers, 1999), and apparently healthy tadpoles were frequently found in pools containing more than four eggs in various stages of decomposition in pool surveys (K. Summers, unpublished observations).

The results presented here suggest that preferential treatment of kin in tadpoles of this species may be less well developed than in the spadefoot toad tadpoles studied by Pfennig et al. (1993). Even if this is the case, it does not necessarily mean that these tadpoles do not recognize their kin. Tadpoles may recognize their kin, and yet not discriminate between kin and non-kin behaviourally (Waldman, 1991). This is particularly likely if the costs of altruism (i.e. refraining from cannibalism) are high (Pfennig, 1998).

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