Cannibalistic tadpoles that pose the greatest threat to kin are most likely to discriminate kin

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Inclusive fitness theory predicts that altruism should often be directed towards reproductive relatives, but it is unclear whether individuals that are most likely to help or harm relatives are also most likely to identify kin in the first place. Here I show that species and sibships of spadefoot toad tadpoles (*Spea bombifrons* and *S. multiplicata*) that were most likely to produce an environmentally induced cannibalistic morph were also most likely to avoid eating kin. Moreover, tadpoles avoided eating kin when they expressed the cannibal phenotype, but not when these same individuals reverted to the non-cannibalistic morph. Thus, individual tadpoles facultatively adjust their level of discrimination according to how likely they are to harm kin. In general, sensory systems and/or decision rules enabling recognition may be especially likely to evolve among those individuals that are most often faced with the problem of discrimination.

Keywords: cannibalism; cannibalistic polyphenism; conditionality; context-dependent behaviour; kin recognition; kin selection

1. INTRODUCTION

Inclusive fitness theory (Hamilton 1964) furnishes a general explanation for the evolution of altruism by positing that individuals can propagate their genes not only by reproducing themselves but also by helping relatives to reproduce. Although many organisms recognize and aid kin as expected (reviewed in Fletcher & Michener 1987; Hepper 1991; Sherman *et al.* 1997; Queller & Strassmann 1998), a critical but untested prediction of this theory is that individuals who are most often in a position to help or harm relatives should also be most likely to discriminate kin (Reeve 1989; Sherman *et al.* 1997).

Cannibalistic species are ideal for testing whether individuals that are most likely to harm relatives are also most likely to discriminate kin. A cannibal that can recognize and avoid destroying kin should often be selectively favoured over individuals lacking this ability. Indeed, many cannibalistic species have well-developed kin-recognition abilities (Walls & Roudebush 1991; Pfennig & Collins 1993; Pfennig *et al.* 1993, 1994; Pfennig 1997; but see Walls & Blaustein 1995). Moreover, cannibalism rates vary across species, populations, and individuals (Fox 1975; Polis 1981; Elgar & Crespi 1992), enabling one to determine whether cannibalistic tendencies covary with kin-recognition ability.

I studied the possible coevolution of kin-recognition abilities and cannibalistic tendencies in polymorphic spadefoot toad tadpoles (genus *Spea*). These tadpoles often occur as two alternative morphotypes: omnivores that feed mostly on detritus, and carnivores that have enlarged cranial muscles to facilitate ingestion of large prey, including conspecifics (Bragg 1965; Pomeroy 1981; Pfennig 1992a). Carnivores are induced facultatively when individuals eat freshwater shrimps or other tadpoles (Pomeroy

1981; Pfennig 1990); when carnivores cease to eat these prey, however, they often revert to the omnivore morphology (Pfennig 1992a,b). The underlying mechanism is that tadpoles acquire from these prey thyroid hormone, which induces and maintains the carnivore phenotype (Pfennig 1992b).

Carnivores are much more cannibalistic than omnivores (Pfennig & Frankino 1997) and the two morphs differ in their treatment of kin: omnivores school preferentially with siblings, whereas carnivores generally avoid siblings (Pfennig *et al.* 1993). When carnivores are confronted with siblings, they tend to avoid eating them (Pfennig *et al.* 1993). These well-developed kin-recognition abilities enabled me to test whether kin discrimination, as assayed by avoidance of kin cannibalism, was most pronounced among tadpoles that were most prone to cannibalism.

I specifically sought to address three questions. First, is kin recognition more pronounced in species that are more likely to produce carnivores? Second, within species, do different sibships vary in ability to recognize kin and, if so, are individuals from families that are most likely to develop into carnivores also most likely to avoid eating kin (as occurs, for example, in facultatively cannibalistic tiger salamander larvae (Pfennig *et al.* 1994))? Third, do tadpoles preferentially avoid eating kin when they express the carnivore phenotype, but not when these same individuals revert to the omnivore phenotype?

2. METHODS

(a) *Experimental animals*

I studied *S. bombifrons* from 12 sibships whose parents were captured from two ponds and *S. multiplicata* from 23 sibships whose parents were captured from a single pond near Portal,

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Arizona, USA (31°48′ N, 109°05′ W). After hatching, four or five groups of eight full siblings from each sibship were chosen randomly, and each group was placed into a separate tank (28 cm × 18 cm × 10 cm) filled with 6 l of dechlorinated water. All tadpoles were reared with seven siblings; this is the upper range of tadpole densities in natural ponds (Pfennig *et al.* 1991). Tadpoles were reared under identical conditions and fed during rearing and throughout the experiments live anostracan fairy shrimps *ad libitum* daily. At two weeks after hatching I scored tadpoles as being omnivore or carnivore morphotypes by characterizing the shape of the head and mouthparts (see, for example, Pfennig 1992*a,b*). I determined whether sibships differed in their propensity to produce carnivores by comparing the proportion of tanks containing each sibship that produced a carnivore (Pfennig & Frankino 1997). The experiments involved a total of 392 *S. bombifrons* tadpoles ((11 sibships × 4 replicate tanks per sibship × 8 tadpoles per tank) + (1 sibship × 5 replicate tanks per sibship × 8 tadpoles per tank)) and 920 *S. multiplicata* tadpoles (23 sibships × 5 replicate tanks per sibship × 8 tadpoles per tank).

(b) *Experimental procedures*

I tested the kin-discrimination abilities of tadpoles 2–5 weeks after they had hatched (Gosner (1960) developmental stages 34–36) by introducing two equally sized 'stimulus' animals (both omnivore-morph larvae) and one 'test' animal (a carnivore or an omnivore morph; omnivores occasionally prey on smaller tadpoles) to an opaque plastic cup (12 cm tall × 9 cm diameter) filled with 250 ml of dechlorinated tap water. One stimulus animal was an unfamiliar full sibling of the test animal (i.e. they had been reared in separate tanks) and the other was an unfamiliar non-sibling. Stimulus tadpoles were from the lower end of the natural size distribution and were about one third of the snout–vent length (SVL) of test animals. All test animals were similarly satiated at the start of the test (i.e. test animals were fed shrimps *ad libitum* immediately before testing). To keep track of kinship identities, I stained half of the stimulus animals red and half blue by placing tadpoles for 12 h in a 0.000 25% aqueous solution of either neutral red or methylene blue (Waldman 1981). To control for the effects of these marks on test animals' prey preferences, in half of the cups the test animal's sibling was stained red, and in half they were stained blue. These marks did not affect larval mortality. An observer checked each cup at least once every hour between 08.00 and 22.00 and recorded when cannibalism occurred and the colour of the surviving stimulus animal. All kin-discrimination trials were allowed to run until cannibalism occurred. In eight trials, both stimulus animals were eaten simultaneously; these were excluded from analysis. I used a χ^2-test to compare the proportion of siblings consumed with the proportion expected (0.5) if cannibalism were random with respect to kinship. I tested the kin-discrimination abilities of 28 *S. bombifrons* carnivores, 46 *S. multiplicata* carnivores and 101 *S. multiplicata* omnivores. Stimulus (prey) animals were used only once; test animals were also used only once, except as noted below.

I also investigated whether prey preferences of individual tadpoles differed depending on whether they were expressing the carnivore or the omnivore phenotype at the time of the test. I created both morphs within the same individuals by altering the diet of 46 focal *S. multiplicata* carnivores immediately after they had eaten a stimulus animal. I switched the diets of these animals from fairy shrimps to rabbit chow, which simulates the detritus that omnivores feed on in nature. Carnivores whose diet
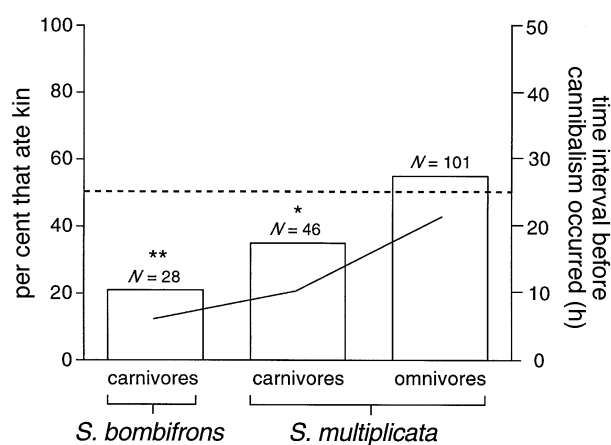

Figure 1. Relation between the percentage of tadpoles that ate kin (bars) and mean time-interval before cannibalism occurred (curve) for two different species and morphotypes. Asterisks indicate that the percentage of tadpoles that ate kin was significantly (*$p < 0.05$; **$p < 0.005$) less than 50% (heavy, dashed line), the value expected if cannibalism were random with respect to kinship.

is thus modified often revert to the default omnivore morphology (Pomeroy 1981; Pfennig 1992*a,b*). Within a week, half (23) of the carnivores had reassumed the omnivore morphology. I then tested for a second time the kin-discrimination abilities of these reverted animals as well as of carnivores that did not revert (the latter served as controls).

3. RESULTS

Different species and sibships varied significantly in cannibalistic tendencies: 29 out of 49 tanks (59%) containing *S. bombifrons* produced a carnivore, whereas only 36 out of 115 tanks (31%) containing *S. multiplicata* produced a carnivore ($\chi^2 = 11.162$, d.f. = 1, $p = 0.0008$). Similarly satiated *S. bombifrons* carnivores, *S. multiplicata* carnivores and *S. multiplicata* omnivores differed significantly in their propensity to engage in cannibalism, as assayed by the mean interval of time before cannibalism occurred per sibship in each group (Kruskal–Wallis non-parametric ANOVA: $H = 13.19$, d.f. = 2, $p = 0.0014$; figure 1). The mean interval of time before cannibalism occurred correlated highly positively with the percentage of individuals that ate kin across these three groups ($r^2 = 0.93$; figure 1).

Different *S. multiplicata* sibships varied in their propensity to produce carnivores and discriminate kin. The probability that an omnivore from a particular sibship would eat a relative was significantly inversely correlated with the probability that a member of its sibship would express the carnivore morphology ($r = -0.62$, $n = 23$ sibships, $p < 0.002$; figure 2).

Kin-discrimination abilities of individual *S. multiplicata* carnivores differed before and after they had reverted to the omnivore morphology. Before reversion, only 6 out of 23 focal carnivores (26%) ate kin, whereas 17 out of 23 ate non-kin ($\chi^2 = 5.261$, d.f. = 1, $p = 0.022$). One week later, after these same individuals had reverted to omnivores, they now behaved as normal omnivores and no longer discriminated kin: 12 out of 23 (52%) reverted individuals ate kin, whereas 11 out of 23 ate non-kin ($\chi^2 = 0.435$,
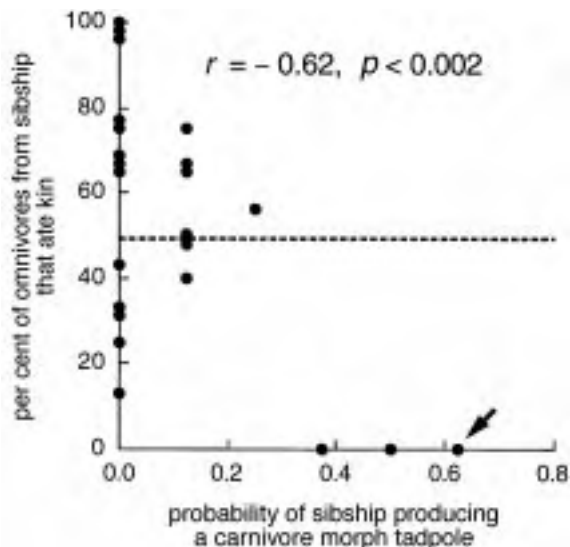
Figure 2. Relation between the proportion of omnivores from each of 23 sibships that ate kin and the proportion of carnivore-morph tadpoles produced by that sibship. The heavy dashed line is the value expected if cannibalism were random with respect to kinship (i.e. 50%). An outlier analysis revealed that only the data point indicated by the arrow was an outlier; even with this point removed from the analysis, the correlation is still significant ($r=-0.52$, $p=0.013$).
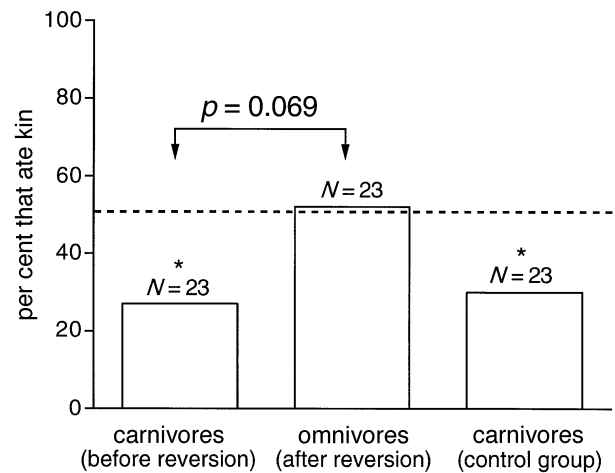

Figure 3. Kin-recognition abilities of 23 *S. multiplicata* tadpoles when these individuals were expressing the highly cannibalistic carnivore phenotype, of these same 23 individuals after they had reverted to the less cannibalistic omnivore phenotype, and of 23 control carnivores that did not revert, but which were the same age as reverted carnivores. Asterisks indicate that the percentage of tadpoles that ate kin was significantly ($p<0.05$) less than 50% (heavy, dashed line), the value expected if cannibalism were random with respect to kinship.

d.f. $=1$, $p=0.835$). Thus, individuals ate significantly fewer kin than expected before reversion ($p=0.022$) but not after reversion ($p=0.835$). This finding suggests that the prey preferences of individual tadpoles differed, depending on which morphotype they were expressing at the time of the test. Indeed, the level of kin discrimination before and after reversion just missed being significantly different when a conservative analysis was used (McNemar's non-parametric test for repeated measures (Lehner 1996): $\chi^2=3.286$, d.f. $=1$, $p=0.069$; figure 3).

It might be contended that reverted individuals ceased to discriminate kin, not because these individuals were less cannibalistic, but because these individuals were hungrier (see, for example, Pfennig *et al*. 1993). However, focal animals were hungrier before reversion than after reversion: cannibalism actually occurred sooner before reversion (mean $\pm$ s.d. $=13\pm20$ h) than after reversion (mean $\pm$ s.d. $=24\pm15$ h; Wilcoxon matched-pairs signed-ranks test: $z=-2.007$, $p=0.045$). The change in discrimination was also independent of age: control carnivores that were the same age as the reverted carnivores discriminated kin (figure 3).

4. DISCUSSION

I hypothesized that kin recognition should be most pronounced among individuals that are most likely to harm kin. As predicted, variation in propensity to engage in cannibalism correlated positively with variation in kin-recognition abilities (figures 1–3; see also Pfennig *et al*. 1994).

At the proximate level, individuals appear to express kin discrimination facultatively only when they are likely to harm kin (figure 3). Similar examples of context-dependent kin discrimination have been reported from diverse taxa (reviewed in Waldman 1988; Sherman *et al*.

1997). Such plasticity in the expression of kin recognition may arise developmentally in *S. multiplicata* if only carnivores possess the sensory and neural structures to discriminate kin. Alternatively, carnivores and omnivores may be equally capable of assessing kinship, but they may obey different 'decision rules' (Reeve 1989). Results from previous experiments point to the latter explanation: carnivores cease to discriminate kin when they become more food-limited (Pfennig *et al*. 1993) and only a shift in decision rules could account for such a rapid change in prey preferences.

At the evolutionary level, kin discrimination will be selectively favoured when Hamilton's rule is satisfied (Hamilton 1964), i.e. whenever $rb-c>0$, where r is the coefficient of relatedness between cannibal and its potential prey, c, is the cost of the act in terms of future offspring production that the cannibal loses by not eating the prey, and b is the benefit of the discrimination in terms of extra offspring that non-cannibalized prey gain. Plasticity in the expression of kin discrimination may be selectively favoured in *Spea* tadpoles, for at least two adaptive reasons. First, the differential kin threat hypothesis states that the different frequencies of kin cannibalism between morphs may selectively favour the expression of kin discrimination among individuals that are more frequently in a position to harm kin. Under this hypothesis, both tadpole morphs pay a similar cost of discriminating kin (i.e. $c_{carnivore}=c_{omnivore}$), but the cumulative indirect benefits of discrimination exceed this cost for carnivores (i.e. for carnivores, cumulative $rb>c$).

To see why carnivores may have more to gain from kin discrimination than omnivores, suppose that a proportion, p, of individuals in a pond is a focal tadpole's kin. For a non-discriminating carnivore that cannibalizes n tadpoles, the expected decrement in fitness would be proportional to np, and for a non-discriminating omnivore

that cannibalizes m tadpoles, the expected decrement would be proportional to mp. Because carnivores are much more cannibalistic than omnivores (figure 1; Pfennig & Frankino 1997), $np \gg mp$. Thus, non-discriminating carnivores stand to suffer greater potential cumulative losses to their inclusive fitness than do non-discriminating omnivores (i.e. cumulative $b_{carnivore} >$ cumulative $b_{omnivore}$). In contrast to carnivores, selection may not favour the expression of kin discrimination in omnivores, because the direct costs of discrimination (such as diminished growth or survival associated with foregoing a meal) may exceed the omnivore's relatively small cumulative losses in indirect fitness associated with not discriminating (i.e., because cumulative b is small for omnivores, $c > rb$).

A second explanation for why individuals discriminate kin when they express the carnivore morphology, but not when they express the omnivore morphology, is the differential rejection cost hypothesis. According to this hypothesis, kin discrimination (and the passing up of an especially nutritious meal (Crump 1990)) is more costly personally to an omnivore than to a carnivore (i.e. $c_{omnivore} > c_{carnivore}$). In particular, because omnivores tend to develop much more slowly than carnivores (Pfennig 1992a), the resources in a conspecific's body may be more valuable to an omnivore than to a carnivore (i.e. because c is large for omnivores, $c > rb$).

The above two hypotheses make different predictions regarding the kin discrimination of large and small individuals of each morphotype. In particular, the differential kin-threat hypothesis predicts that the larger a carnivore is, the more discriminating it should be. However, this hypothesis predicts that there should be no difference in discrimination abilities between large and small omnivores. The rationale behind these predictions is that large carnivores are more cannibalistic and thus pose a greater potential threat to kin than do small carnivores, but that large omnivores are not more likely to eat conspecifics than are small omnivores (as evidence, there was a highly significant inverse correlation between a carnivore's size and the time it took to cannibalize its prey ($r = -0.69$, $n = 27$, $p < 0.0001$), but there was no such relationship for omnivores ($r = -0.23$, $n = 30$, $p = 0.216$)). By contrast, the differential rejection-cost hypothesis predicts that for both morphs, larger individuals should be more discriminating than smaller individuals, because the resources in a conspecific's body should be more valuable to a small tadpole than to a large tadpole. As predicted by the differential kin-threat hypothesis, but not by the differential rejection-cost hypothesis, *S. multiplicata* carnivores that ate non-kin were significantly larger (mean SVL $\pm$ s.d. $= 13.43 \pm 1.86$ mm) than same-aged carnivores that ate kin (mean SVL $\pm$ s.d. $= 11.45 \pm 2.73$ mm; two-tailed t-test: $t = -2.24$, d.f. $= 25$, $p = 0.034$), whereas omnivores that ate non-kin (mean SVL $\pm$ s.d. $= 15.78 \pm 0.97$ mm) did not differ from same-aged omnivores that ate kin (mean SVL $\pm$ s.d. $= 15.73 \pm 1.74$ mm; two-tailed t-test: $t = -0.08$, d.f. $= 30$, $p = 0.934$).

In sum, kin discrimination was most pronounced among species, morphotypes, and sibships that were most likely to engage in cannibalism, i.e. those that posed the greatest potential threat to kin. Thus, as predicted by optimal discrimination theory (Reeve 1989; Sherman *et al.* 1997), individuals that are most likely to harm their relatives are most likely to identify their kin. More generally, the results of this study suggest that recognition systems (i.e. sensory systems and decision rules that enable the discrimination of, for example, kin or mates) may be especially likely to evolve among those individuals (e.g. cannibals or females) that are most often faced with the problem of discrimination.

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