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Context-dependent kin discrimination in larvae of the marbled salamander, *Ambystoma opacum*

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Abstract. The context-dependent nature of kin discrimination was examined in larvae of the marbled salamander, *Ambystoma opacum*. In a graphical analysis, relationships between aggression and social and ecological factors were explored. Increasing relatedness between individuals was predicted to reduce aggressive encounters, except when correlates of fitness (e.g. body size) differed between individuals and/or when environmental conditions increased the profitability of aggression. These predictions were tested in a multifactorial experiment in which genetic relatedness, food quantity, relative body size and the potential for physical encounters were manipulated. The effects of each factor and their interactions on larval aggression and correlates of fitness (body size and timing of metamorphosis) were examined. Frequency of aggression depended upon an interaction between kinship, food quantity and relative larval size. Thus, in accordance with our graphical model, kinship-based aggression varied in a context-dependent fashion. Low food level reduced body size and increased the time required to reach metamorphosis. When larvae were not separated by a barrier, growth was inhibited in the smaller, but not the larger, individual of a pair. Moreover, the larval period was significantly shortened in chambers where barriers were absent. Neither kinship nor initial relative size, either alone or in combination with the other factors, significantly affected larval performance. These results show that the frequency of larval aggression in *A. opacum* depends upon prevailing social and environmental conditions; however, the influence of aggression on larval growth and metamorphosis remains unclear. Previous inferences regarding context-dependent kin discrimination in amphibians have been based on independent, interspecific studies or concurrent, intraspecific comparisons between individuals of alternative morphologies. The present results provide direct evidence that simultaneous variation in multiple socio-ecological factors may alter aggression in a single amphibian species in which kin discrimination is known to occur.

The ability to discriminate between kin and non-kin (often called kin recognition) is especially germane to kin selection theory and the concept of inclusive fitness (Hamilton 1964a, b). Kin discrimination can enhance aid-giving behaviours or preferential treatment of kin and preclude its misdirection toward unrelated individuals (discussed in Holmes & Sherman 1983). Moreover, kin discrimination can influence key ecological processes that may ultimately affect population and community structure; for example, it may enable individuals to direct competition and intraspecific predation (cannibalism) away from kin (Blaustein & O'Hara 1982).

Hamilton (1964a, b) predicted that, all else being equal, an animal's behaviour towards another individual depends upon the relative costs and benefits of expressing the behaviour, as well as the genetic relatedness of the two individuals. Since Hamilton's prediction, numerous studies have revealed a diverse array of taxa that appear to distinguish kin from non-kin (e.g. Fletcher & Michener 1987; Blaustein et al. 1988; Hepper 1991). Kin selection is often invoked as the primary force driving the evolution of kin

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discrimination, even though other explanations may be just as plausible (in Grafen 1990; Barnard 1991; Blaustein et al. 1991). For example, behaviour that is biased towards kin may be the result of individuals identifying and interacting with members of their own species ('species recognition': O'Hara & Blaustein 1982; Grafen 1990). Aggregations of kin may form without discrimination between individuals if relatives share a preference for a particular habitat (e.g. Blaustein et al. 1987; Pfennig 1990). Moreover, attempts to elucidate the adaptive basis of kin discrimination have been complicated by the variable nature of this behaviour; i.e. whether discrimination occurs often depends on prevailing ecological and social conditions (Blaustein et al. 1987; Waldman 1988; Reeve 1989; Beecher 1991).

We explored the context-dependent nature of kin discrimination and its functional consequence. First, we present a graphical examination of context-dependent kin discrimination, using aggression as a behavioural assay. Second, we use this model, and the factors contained within, for an empirical examination of context-dependent kin discrimination and its possible fitness consequences in the aquatic larvae of the marbled salamander, *Ambystoma opacum* (Ambystomatidae). Like other ambystomatid salamanders, *A. opacum* has a complex life cycle (Wilbur 1980) in which there is an aquatic larval stage, followed by terrestrial juvenile and adult stages. Female *A. opacum* differ, though, from the predominantly 'pond-breeding' members of this family: in autumn, females deposit their eggs underneath objects covering the dry basins of ephemeral woodland ponds. Following egg deposition, females remain with their developing clutch until winter rains flood the nests, which stimulates the larvae to hatch (Noble & Brady 1933). Larvae metamorphose in May–June as the temporary ponds they inhabit begin to dry.

We selected *A. opacum* for our study because its larvae, like those of some other amphibians, have been useful subjects for examining aggression and cannibalism among kin (Walls & Roudebush 1991; Pfennig & Collins 1993; Pfennig et al. 1993, 1994). Aggression is easily quantified and its preferential direction toward non-kin is predicted: aggressive biting and attempts at cannibalism have been observed in larval *A. opacum* (Smith 1990), and kin discrimination reduces larval aggression in this species (Walls & Roudebush 1991). For *A. opacum*, variation in body size and, thus, the potential for cannibalism may result from asynchronous hatching (Jackson et al. 1989; Petranka 1990; Scott 1990) and variation in initial egg size (Kaplan 1980a, b). If these size-related disparities in growth rate continue throughout the larval period (Kaplan 1980a), the potential for size-dependent cannibalism may be greatly increased.

A GRAPHICAL EXAMINATION

As a form of interference competition, aggressive behaviour may benefit an individual's direct fitness by inhibiting a competitor's foraging. Moreover, aggression that escalates to generate fatal injuries eliminates competitors and may provide nutrition for the aggressor if cannibalism follows (Crump 1992). Aggression may also be costly because of the energy expended in the pursuit and capture of prey, the risk of retaliation, the costs of maintaining a cannibalistic morphology, and the potential risk of acquiring diseases by consuming infected conspecifics (Crump 1992; Elgar & Crespi 1992; Pfennig et al. 1991; Walls & Semlitsch 1991).

In terms of inclusive fitness, we assume that aggression becomes more costly as it escalates. In contrast, the benefits of aggression presumably cease to accrue beyond some point because fitness cannot increase infinitely. Thus, to construct simple cost–benefit curves, we envision the costs of aggression to increase linearly and its benefits to increase asymptotically. Fig. 1a depicts a cost function (*C*) and four different benefit functions. Our model includes an environment with variable food resources. Competition for food is a significant factor in many systems (e.g. Schoener 1982, 1983; Connell 1983) and food availability may influence interactions among relatives (Porter et al. 1980). The two highest benefit curves (*LD* and *LN*) correspond to a low food environment, and the lower benefit curves (*HD* and *HN*) correspond to a high food environment. We assume that the benefits of aggression are greater at low than at high food levels; i.e. a satiated individual in a high food environment will gain less in an aggressive encounter compared to a starved individual in a low food environment.

The difference between *LD* and *LN* and the difference between *HD* and *HN* are attributable to

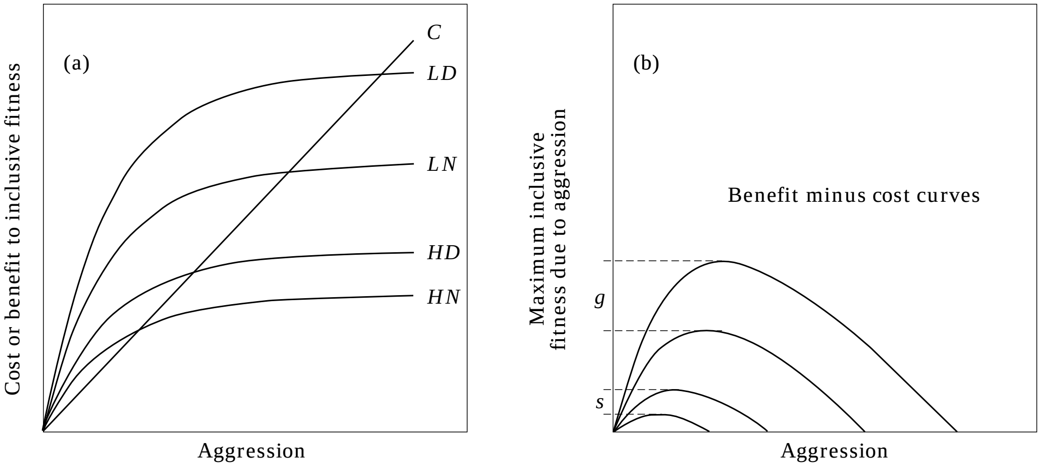

Figure 1. Cost–benefit curves for aggressive behaviour in different contexts (a) and the result of subtracting costs from each benefit curve (b). Costs (*C*) are assumed to be linear, but benefit curves depend upon the food resource level (*L* = low, *H* = high) and whether individuals discriminate kin (*D* = discriminating, *N* = non-discriminating). The cost of not discriminating kin from non-kin in the low food environment (*g*) is greater than it is in the high food environment (*s*).

the costs of aggression to indirect fitness. Aggression may incur many costs to indirect fitness (Fig. 2). For example, as a source of mortality for relatives, aggressive cannibalism lowers an individual's indirect fitness. Injuries (e.g. aggressive biting) may alter metamorphosis, decrease body size, or render victims more vulnerable to subsequent predation attempts, thus reducing indirect fitness via a decreased probability of survival (e.g. Kumar et al. 1983; Morin 1985; Semlitsch & Reichling 1989; Semlitsch 1990; Figiel & Semlitsch 1991; Parichy & Kaplan 1992). Survivors of aggressive attacks may experience lowered fitness if the injury is fatal or if disease-causing pathogens invade bite wounds (Walls & Jaeger 1987; Petranka 1989). Non-lethal aggression may influence long-term survival and reproduction as well, however (Harris 1989) because of factors that reduce larval growth (Petranka 1989; Smith 1990). Even for amphibians that survive to metamorphosis, aggression experienced in the larval stage may contribute to a reduced metamorphic size, which in turn may delay the age at which they first reproduce (Semlitsch et al. 1988; Scott 1994) as well as influence their competitive ability in the terrestrial environment.

Individuals may reduce or eliminate costs to indirect fitness by preferentially directing aggressive behaviours away from kin. In polymorphic populations, some individuals may discriminate kin from non-kin and display aggression towards non-kin, but other individuals may be non-discriminating (Blaustein et al. 1987). The latter group will accrue more costs for a given number of social encounters (the net benefits gained by aggression will be reduced). Thus, the difference between the *LD* and *LN* curves (Fig. 1a) depicts two groups of individuals in the low food environment: one discriminates kin from non-kin, and the other is non-discriminating. The same relationship exists for the difference between the *HD* and *HN* curves, only the two groups are in a high food environment. The relationship between the *LD* and the *LN* functions is identical to the relationship between the *HD* and the *HN* functions. The only difference between these two pairs of functions is the relative gain in inclusive fitness for a given amount of aggression due to differences in food levels.

By subtracting the cost function (*C*) from each of the benefit functions, we obtain a set of difference functions (Fig. 1b). If individuals behave optimally, the apex of each function is the maximum gain in fitness, due to aggression, that an individual can obtain in each of the four groups. The scale of the ordinate is arbitrary and represents only the portion of total fitness due to aggression. The individuals in the low food

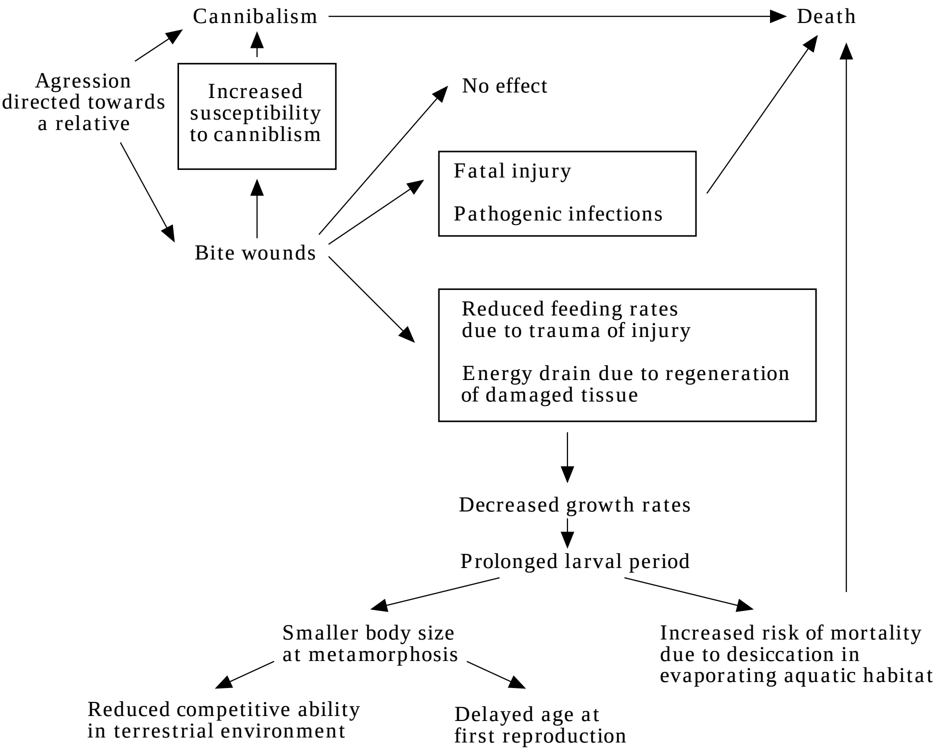

Figure 2. Costs of aggression to indirect fitness. Aggressive behaviour received from a relative can have a number of outcomes that may reduce the fitness of the victim and thus reduce the indirect fitness of the aggressor.

environment do not have higher fitness than the individuals in the high food environment; rather, they gain relatively more for each act of aggression. The difference between individuals in the low food environment (the *LD* and *LN* maxima, *g*) is much greater than the difference between individuals in the high food environment (the *HD* and *HN* maxima, *s*), although the two pairs of functions are identical. This disparity implies that the relative costs of not discriminating kin from non-kin is higher in the low food environment. Moreover, the costs of not discriminating may be so low in the high food environment that selection favouring discriminatory behaviour may be trivial. Thus, kin discrimination (expressed as differential aggression) may occur in one food environment but not in another.

Our graphical model (Fig. 1) may be used to consider additional factors, resulting in a landscape of aggressive behaviours, the surface of which depends upon genetic relatedness and food level. When interacting individuals are of the same reproductive value (e.g. of similar body size and, thus, similar survival and reproductive expectancies), aggression should decrease with increasing genetic relatedness and food level, resulting in the landscape shown in Fig. 3a. If one individual of a pair has significantly lower reproductive value than the other, however, then the landscape shown in Fig. 3b may result. In this case, the entire plane is elevated above that in Fig. 3a and the relationship between food, relatedness and aggression is changed. The relationship between relatedness and aggression is notably different between Fig. 3a and 3b, because the indirect costs of being aggressive to a relative with low reproductive value are very small. In many cases, the relative may die before becoming sexually mature. Thus, the value of a close relative to the aggressor's inclusive fitness may be no higher than that of non-kin if the relative's reproductive value, compared to that of the aggressor, is sufficiently low (Eickwort 1973; Milinski 1978; Charlesworth & Charnov 1980). The set of outcomes depicted

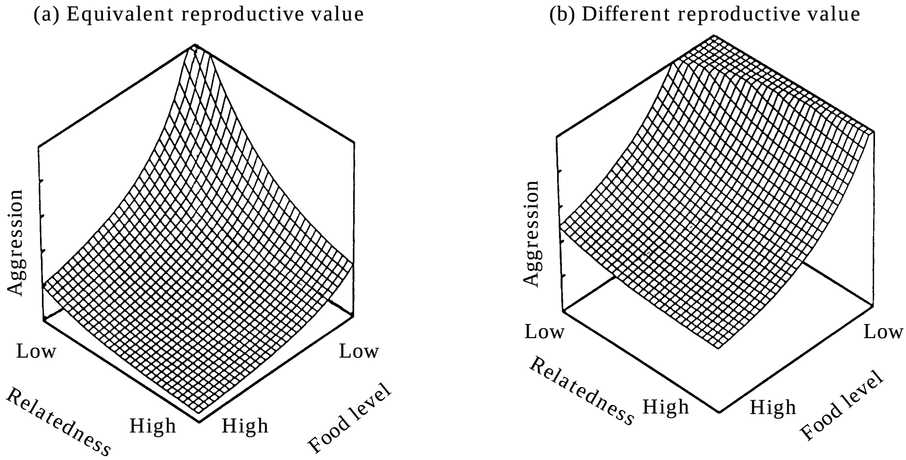

Figure 3. Aggressive behaviour landscapes when interacting individuals have the same reproductive value (a) and when reproductive values differ (b). The three-dimensional surface was generated following the procedures outlined in Wilkinson (1986).

in Figs 1 and 3 depend upon our initial assumptions and the relationships between the cost and benefit functions. Variations in these initial relationships may produce different, even contrary, results, which underscores our message that kin discrimination may be highly context-dependent.

AN EMPIRICAL EXAMINATION

We examined whether kin discrimination varies in different contexts in the marbled salamander.

Methods

Collection and rearing of test animals

From 7 to 9 November 1992, we collected three clutches of embryos from brooding females found beneath objects covering the dry basin of an isolated, temporary woodland pond near Chicot State Park (Evangeline Parish, Louisiana, U.S.A.). Individuals from this population have been examined in previous kinship studies (Walls 1991; Walls & Roudebush 1991; Walls & Blaustein 1995). Females may occasionally nest communally, thus mixing larvae from different parents (Noble & Brady 1933; Petranka 1990). We therefore collected clutches that were associated with a single brooding female and that were spatially isolated from other clutches. We maintained each sib-group individually on moistened paper in covered dishes (9.2 cm diameter, 4.6 cm deep), from the time that embryos were collected until hatching. To enhance variation in relative body sizes (see below), we manipulated the dates on which larvae hatched: half of each sibling group was hatched two weeks prior to its remaining half. Once larvae within each clutch reached the hatching stage (stage 45: Harrison 1969), we hatched half of the larvae by submerging them in dechlorinated tap water and then manually tearing their egg membranes with dissecting probes. The larvae in the remaining half of each clutch remained in their egg membranes for two additional weeks before being hatched in an identical manner. This two-week postponement of hatching had no detectable adverse effects. Indeed, embryos that have reached the hatching stage can remain quiescent within their egg capsules for months, yet will hatch readily once they are inundated with water (Petranka et al. 1982). Moreover, delayed hatching due to postponed filling of ponds has been reported in natural populations of this species (Jackson et al. 1989; Scott 1990).

Prior to the experiment, we reared the first larvae hatched in 38-litre glass aquaria. We divided these aquaria into width-wise halves with fibreglass mesh screen partitions (mesh size = 2 mm) that we placed perpendicular to the container's length. Each aquarium contained

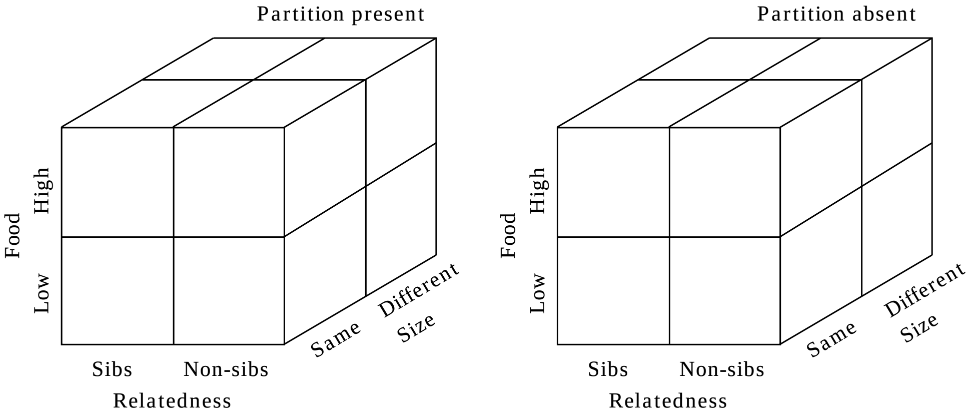

Figure 4. Experimental design employed to examine whether simultaneous manipulation of four factors influences context-dependent kin discrimination in larval *A. opacum*.

19 litres of dechlorinated tap water and housed 10–15 larvae from one clutch on one side of the partition and 10–15 larvae from another clutch on the opposite side. This procedure is sufficient to familiarize test larvae with the cues of individuals of varying genetic relatedness (and, thus, to avoid biasing their behaviour toward either siblings or non-siblings: Walls & Roudebush 1991). We used multiple aquaria such that the three possible pairs of sib-groups (e.g. J and K, J and L, K and L) were reared together in replicated groups. Before the experiment, we fed the larvae brine shrimp nauplii, *Artemia salina,* ad libitum, once per day. At hatching of the remaining larvae in each clutch, individuals within each treatment were paired haphazardly, then pairs were immediately placed in 28.5 × 17.7 × 12.0-cm opaque plastic containers holding 3.0 litres of dechlorinated tap water. We maintained larvae on a 12:12 h light:dark photoperiod at 15°C and replenished chambers with fresh dechlorinated tap water once per week.

Experimental design

We simultaneously measured the effects of genetic relatedness, initial relative body size, food level, the presence of a partition and their interactions on four response variables: (1) aggression, (2) body size of metamorphosing individuals, (3) the timing of metamorphosis and (4) body size of non-metamorphosing larvae. These factors were examined in a 2 × 2 × 2 × 2 factorial design (Fig. 4), with each of the resulting 16 treatments replicated in seven randomized blocks (total = 112 experimental chambers). Each block was a position on two adjacent laboratory benches, with two blocks for each of two of the sib-groups and three blocks for the third. We randomized the designation of sib-groups to each block, as well as the arrangement of the experimental treatments within each block.

Experimental pairs of larvae were either siblings or non-siblings that were of similar or dissimilar body size (i.e. hatched at the same time or 2 weeks apart). At hatching, a sample ($N = 30$) of the delayed group of larvae averaged (± SD) 14.6 ± 1.39 mm in total length (tip of snout to tip of tail). At the same time, a sample of the earlier-hatched larvae measured 20.5 ± 1.66 mm ($N = 29$). On average, larvae hatched 2 weeks apart differed in size by 5.9 mm when the experiment began, and this difference was significant (Student's *t*-test: $t = 14.75$, $df = 57$, $P < 0.001$). Food levels consisted of a low- and a high-feeding regime that varied, as follows, with time (i.e. as larvae grew). From weeks 1 to 4 of the experiment, we provided each larval pair with either a 4-ml (low food regime) or an 8-ml (high food regime) aliquot of brine shrimp nauplii once per day. We did not quantify the number of nauplii per aliquot, but, we assumed that each pair received a homogeneous sample because the prey suspension was thoroughly stirred prior to extracting each aliquot. From weeks 5 to 8, we fed larvae live black worms (Annelida: *Lumbriculus*); each larva received either one-half (14 mm long, low food regime) or one whole black worm (28 mm long, high food regime). From weeks 9 to 14 (the end of the experiment), we fed each larva either one (low food regime) or two (high food regime) live black worms. These values were chosen based on previous observations of the amount of food necessary for growth in larval *A. opacum* (S. Walls, personal observation). All larvae were fed once per day throughout the experiment.

Last, we manipulated the presence or absence of a partition, constructed from perforated, opaque Plexiglas (hole diameter = 2 mm). Partitions divided chambers width-wise into two halves and allowed us to separate each larva of a pair (either siblings or non-siblings). We used partitions to eliminate the potential negative effects of physical encounters and/or 'scramble' competition on larval growth and survival; i.e. aggressive interference (including visual displays) and monopolization of the food source were eliminated by the opaque barrier.

Experimental protocol and response variables

The experiment began on 22 December 1992, when we hatched the remaining larvae in each clutch and paired them with a conspecific according to our experimental design. Every day we noted whether cannibalism had been attempted and/or achieved; i.e. whether a larva was missing or killed. No mortality due to other factors was detected in our experiment.

Metamorphosis of at least one member of each pair occurred between 24 February (day 64) and 28 March (day 96) 1993. At this point, we calculated the time (days) required to reach metamorphosis. We additionally measured the wet mass (to the nearest 0.001 g) of both the metamorph and the remaining larva of each pair, after patting each individual dry to remove excess moisture. At the end of the experiment, all surviving individuals were returned to their pond of origin.

Between 22 and 27 February 1993 (2 months after the experiment began), we observed pairs of larvae to assess the possible effects of genetic relatedness, initial relative body sizes and feeding history on aggressive interactions. To eliminate the possible confounding influence of dominance established during previous physical interactions, we observed only pairs reared in partitioned chambers ($N = 56$), with each chamber observed only once. Before observations began, we removed both larvae of a pair from their respective sides of their rearing chamber and isolated each in an opaque habituation cup (7.3 cm diameter, 6.3 cm high, and filled with 150 ml of dechlorinated water) that was positioned in an unpartitioned chamber containing 3.0 litres of dechlorinated water. After a 30-min acclimation period, we allowed the larvae to swim out into the test chamber.

For the next 30 min, we recorded the frequency of four aggressive behaviour patterns, operationally defined as follows (Walls & Semlitsch 1991).

(1) *Move towards*: one larva approaches another; continued movement in that direction would result in physical contact between the two individuals.
(2) *Look towards*: one larva rotates its head in the direction of its opponent while remaining otherwise motionless; an extension of the individual's line of sight would contact the body of its opponent.
(3) *Bite*: one individual grasps its opponent with an open mouth.
(4) *Lunge*: an unsuccessful bite attempt, initiated as rapid and abrupt movement by one larva toward its opponent.

Bite and Lunge are unambiguous acts of aggression. Walls & Semlitsch (1991) demonstrated that larval *Ambystoma* Moved and Looked towards their opponents immediately before and after delivering a Bite or Lunge. Moreover, these larvae Moved and Looked towards their opponents significantly more often than did larvae that did not show Bite and Lunge (Walls & Semlitsch 1991). Thus, these movement and visual displays are associated with overt aggression in these larval salamanders.

We conducted our observations at air temperatures of 15–16°C and at night using a 15 W incandescent lamp, because larval *Ambystoma* are known to be more active at this time (Branch & Altig 1981). Observations were blind with respect to experimental treatment (including genetic relatedness of larvae), and we randomized the order in which replicates of the experimental treatments were observed. Larvae were returned to their respective halves of their rearing chambers at the end of each trial.

Statistical analyses

Preliminary analyses revealed that the four aggressive behaviour patterns were highly correlated (all bivariate correlations were ≥ 0.70).

Table I. ANOVA of the number of aggressive behaviours in all treatments

Source	*df*	*MS*	*F*	*P*
Kinship	1	912.07	0.55	0.46
Food	1	3877.79	2.32	0.13
Kinship × food	1	1263.50	0.76	0.39
Initial size	1	292.57	0.18	0.68
Kinship × initial size	1	3090.29	1.85	0.18
Food × initial size	1	1522.57	0.91	0.34
Kinship × food × initial size	1	7873.14	4.72	0.04
Error	48	1668.21		

To avoid potential problems associated with multicollinearity (Tabachnick & Fidell 1989, pp. 87–88), we created one composite response variable by combining the frequencies of the four behaviours for each larval pair. After examining the data for departures from normality (no transformations were necessary), we used a Type III SS univariate analysis of variance (ANOVA) to evaluate the effects of kinship, initial size, feeding history and their interactions on total larval aggression.

There was no evidence of multicollinearity in the larval performance data (all correlations ≤ 0.20), nor were there any departures from normality (no transformations were necessary). Thus, we used ANOVA to test for spatial block effects and multivariate analysis of variance (MANOVA) to test simultaneously for treatment effects and their interactions on (1) the size of the smaller individual of each pair, (2) the size of the larger individual of each pair and (3) the number of days to reach metamorphosis. The smaller individual of a pair was frequently the first to initiate metamorphosis. Because of the potential costs to reproduction of metamorphosing at a smaller size (Semlitsch et al. 1988; Scott 1994; Fig. 2), we assumed that larger individuals may experience higher fitness than smaller metamorphs. Thus, we analysed the relative body sizes of the larger and smaller individual in a pair, rather than the more traditional response variables of metamorph size versus the size of the remaining larva.

The MANOVA is a conservative test of a treatment effect; i.e. it accounts for inflation of the overall Type I error rate which may result from correlated, multiple response variables (Tabachnick & Fidell 1989). If the MANOVA revealed a significant overall effect of our experimental treatments, we then performed Type III SS ANOVAs on each of the three response variables to determine their potential contributions to this effect. Univariate ANOVAs for each variable (with Bonferroni adjustments of significance levels for the number of variables examined) may serve as a tool in interpreting the results of MANOVA; i.e. they aid in the assessment of which variable(s) may have contributed to a significant multivariate response (Tabachnick & Fidell 1989). Our overall statements concerning statistical significance rely upon the MANOVA, however. All analyses were conducted using Systat 5.1 (Wilkinson 1986).

Results

Aggressive behaviour

None of the main factors or their pair-wise interactions had a significant effect on the total number of aggressive behaviours observed (Table I). Aggression was significantly affected, however, by an interaction between kinship, food level and initial size difference. The three factors combined to create a complex behavioural pattern (Fig 5). In general, aggression was more frequent in the low food environment, unless the opponent was a sibling of the same initial size.

Larval performance

In the absence of a partition between individuals, cannibalism occurred in 14 of 56 chambers. We performed a multifactorial chi-squared test (Zar 1984) to assess whether the experimental factors influenced the number of individuals cannibalized. The null hypothesis of no significant difference was not rejected ($\chi^2 = 13.323$, $df = 7$, $0.05 < P < 0.1$). Although not statistically significant, 11 of the 14 cannibalism events occurred in

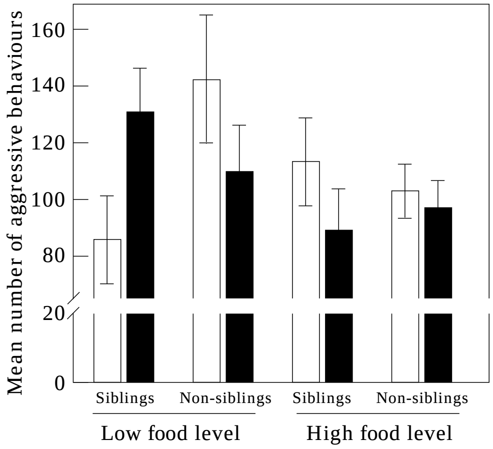

Figure 5. Mean (±SE) number of aggressive behaviours observed in pairs of larvae that were initially of the same (□) or of different (■) body sizes.

the low-food environments, and 10 of the 14 events occurred in non-sibling pairs. These acts of cannibalism produced unequal sample sizes between our experimental treatments.

ANOVA revealed no significant spatial block effect or interaction between blocks and treatments. We therefore pooled the block and error terms for the remaining tests (Zar 1984) and treated blocks as replicates. MANOVA indicated that food level and the presence of a partition had significant effects on larval performance (MANOVA, Table II). Subsequent ANOVAs indicated that food level significantly affected all three response variables (Table II, Fig. 6). The mean mass of the larger and the smaller individual of each pair was significantly lower in the low food treatment than in the high food treatment (Fig. 6a, b) and larvae took significantly longer to metamorphose in the low food treatment (Fig. 6c). The presence of a partition significantly increased the mass of the smaller individual and the number of days to reach metamorphosis, but had no effect on the mass of the larger individual (ANOVAs, Table II, Fig. 7).

Larval performance was not significantly affected by initial size variation, kinship or any of the two- or three-way interactions between the factors (MANOVA, Table II). There was a significant interaction between all four factors, however (MANOVA, Table II). This interaction is probably due to an effect on the mass of the smaller individual of each pair (ANOVAs, Table II, Fig. 8). The mean mass of the smaller individual was lowest for sibling pairs with an initial size difference, reared on low food levels and with a partition absent, but was highest for non-sibling pairs with an initial size difference, fed high food levels and with a partition present. None of the response variables showed a significant interaction effect between all four factors when the Bonferroni adjustment was taken into consideration, however (ANOVAs, Table II). Furthermore, because of cannibalism, our low sample size for some treatments weakened any inferences regarding the importance of this interaction effect.

DISCUSSION

This study demonstrates that the expression of kin discrimination in *A. opacum*, as assayed by aggression, is dependent upon the social and environmental context in which it is expressed. Food level, initial size variation and kinship all interact to produce a complex pattern of aggressive behaviour that may ultimately affect fitness. Behavioural effects on correlates of fitness were most evident in chambers without partitions. The smaller individuals of each pair were, on average, significantly smaller in chambers without partitions where behavioural interactions and/or scramble (exploitative) competition were possible. Furthermore, larvae took less time to metamorphose when partitions were absent. Low food level also reduced larval body size and increased the time required to reach metamorphosis.

Context-dependent Kin Discrimination

Although the cues and perceptive ability necessary for recognition of kin may be present, the actions taken by an individual define kin discrimination. These actions may vary, for the same individual, with the context in which discrimination occurs (e.g. Reeve 1989; Beecher 1991). Thus, kinship is not the only factor important for kin discrimination.

Context-dependent kin discrimination has been reported in tadpoles of *S. bombifrons* (Pfennig et al. 1993). Tadpoles preferentially associated with kin or non-kin, depending on whether they had cannibalistic tendencies. Cannibals (carnivores) preferred to associate with non-kin and avoided cannibalizing relatives, although kin cannibalism

Table II. Results of MANOVA and ANOVA for overall effects of food level, presence of a partition, initial size difference, kinship and all possible interactions on three response variables (correlates of fitness)

Source	Wilks' λ	MANOVA			ANOVA: Mass of smaller individual				ANOVA: Mass of larger individual				ANOVA: Number of days to metamorphosis			
		F	*df*	*P*	*df*	*MS*	*F*	*P**	*df*	*MS*	*F*	*P**	*df*	*MS*	*F*	*P**
Food	0.04	610.52	3, 80	<0.001	1	0.18	18.02	<0.001	1	0.29	30.49	<0.001	1	3506.09	237.90	<0.001
Partition	0.48	28.48	3, 80	<0.001	1	0.71	70.57	<0.001	1	0.004	0.39	0.53	1	512.09	34.75	<0.001
Food × partition	0.92	2.29	3, 80	0.08	1	0.01	1.12	0.29	1	0.001	0.14	0.70	1	2.24	0.15	0.70
Initial size	0.97	0.76	3, 80	0.52	1	0.01	0.63	0.43	1	0.01	0.91	0.34	1	32.96	2.24	0.14
Food × initial size	0.93	2.10	3, 80	0.11	1	<0.001	0.01	0.94	1	0.05	5.02	0.03	1	8.35	0.57	0.45
Partition × initial size	0.92	2.18	3, 80	0.10	1	0.03	2.71	0.10	1	0.04	4.44	0.04	1	45.53	3.09	0.08
Food × partition × initial size	0.99	0.23	3, 80	0.88	1	0.001	0.15	0.70	1	0.002	0.26	0.61	1	1.47	0.10	0.75
Kinship	0.98	0.40	3, 80	0.75	1	0.01	0.61	0.44	1	<0.001	0.004	0.95	1	0.002	<0.001	0.99
Food × kinship	0.98	0.49	3, 80	0.69	1	0.01	0.67	0.42	1	0.005	0.57	0.45	1	0.005	<0.001	0.99
Partition × kinship	0.98	0.43	3, 80	0.73	1	0.01	0.95	0.33	1	0.004	0.40	0.53	1	7.44	0.50	0.48
Initial size × kinship	0.96	1.09	3, 80	0.36	1	0.02	1.45	0.23	1	0.02	2.43	0.12	1	41.83	2.84	0.10
Food × partition × kinship	0.97	0.74	3, 80	0.53	1	0.01	0.50	0.48	1	0.01	0.95	0.33	1	4.32	0.29	0.59
Food × initial size × kinship	0.94	1.59	3, 80	0.20	1	0.004	0.39	0.54	1	0.04	3.89	0.05	1	10.26	0.70	0.41
Partition × initial size × kinship	0.97	0.92	3, 80	0.43	1	<0.001	0.03	0.87	1	0.01	1.21	0.28	1	19.85	1.35	0.25
Food × partition × initial size × kinship	0.88	3.55	3, 80	0.02	1	0.04	4.22	0.04	1	0.02	2.24	0.14	1	17.44	1.18	0.28
Error	—	—	—	—	82	0.01	—	—	82	0.01	—	—	82	14.74		

*Significance level for univariate tests is 0.016 (Bonferroni-adjusted for three response variables). Response variables are mass of the smaller individual of each pair of larvae, mass of the larger individual of each pair and the number of days to metamorphosis.

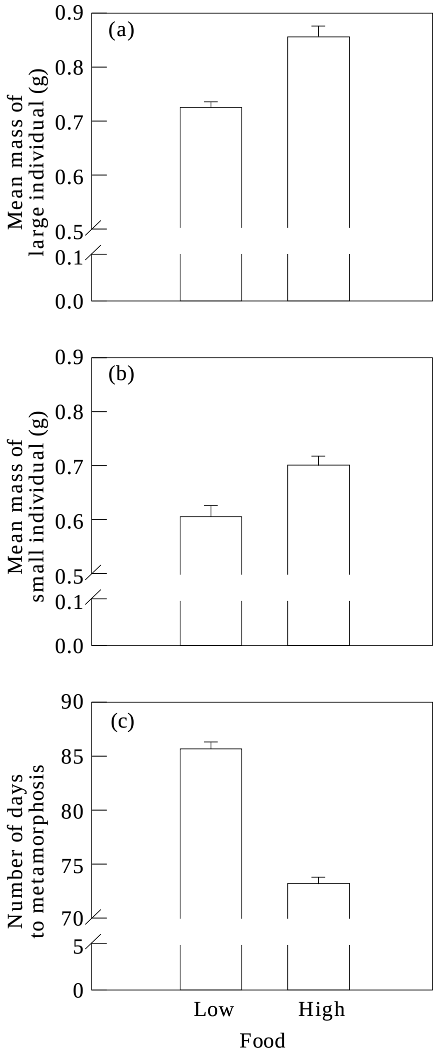

Figure 6. The effect of food resource level on the mass of (a) the largest and (b) the smallest individual of each pair, and (c) number of days to metamorphosis.

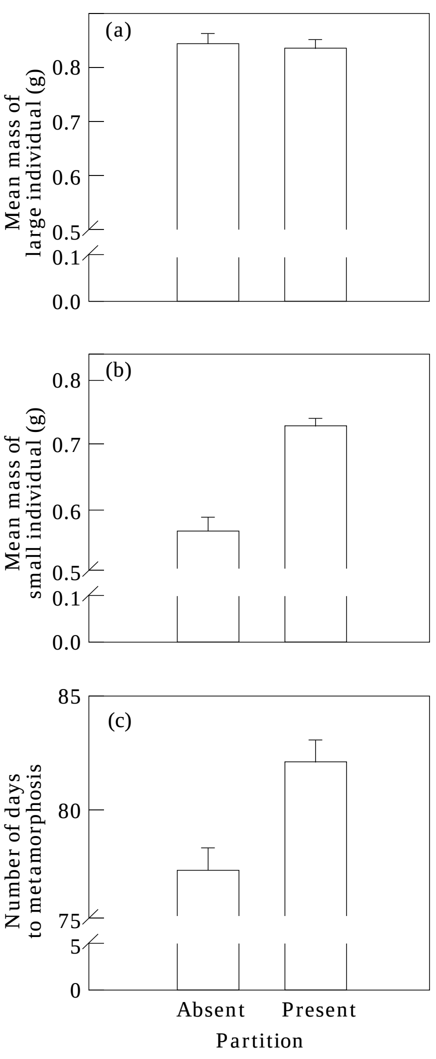

Figure 7. The effect of the presence or absence of a partition between salamanders on the mean mass of (a) the largest and (b) the smallest individual of each pair, and on (c) the number of days to metamorphosis.

occurred more frequently at low food levels. Thus, Pfennig et al. (1993) demonstrated that kin discrimination may vary with alternative morphologies and hunger levels. By simultaneously manipulating multiple ecological factors, however, we extend current knowledge of the context-dependent nature of kin discrimination in larval amphibians. Moreover, our factorial design permitted the detection of an interaction (indirect) effect of kinship on aggressive behaviour: the effect of relatedness on aggression depended upon food level and initial size of interacting individuals. The use of such designs to test for indirect effects in social behaviour are rare (cf. Sherratt & Church 1994), even though behavioural phenotypes are well known to be ‘condition-sensitive’ (e.g. West-Eberhard 1987, 1989; Pfennig & Reeve 1989).

In addition to our present observations, context-dependent discrimination in *A. opacum* has been observed in several other studies. Walls

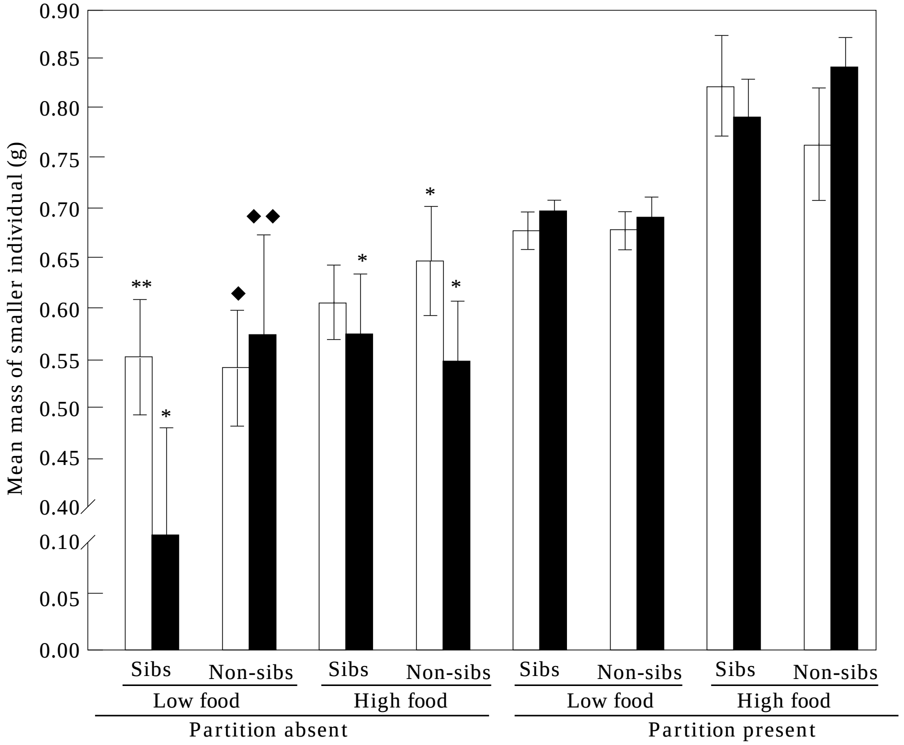

Figure 8. The mean mass of the smallest individual of pairs that were initially of the same (□) or of different (■) body sizes. Due to cannibalism, *$N=6$; **$N=5$; ◆$N=4$; ◆◆$N=2$; all other data cells $N=7$.

& Roudebush (1991) found that same-sized larvae of *A. opacum* were generally less aggressive towards siblings than non-siblings when tested under identical conditions. When larval *A. opacum* were housed with much smaller conspecifics, however, larvae cannibalized siblings significantly more often than non-siblings (Walls & Blaustein 1995). Thus, relative body size appears to be one context in which kin discrimination may vary in this species. In many animals, including amphibians, kin discrimination appears to depend strongly upon ontogeny (Pfennig et al. 1983; Blaustein et al. 1984, 1993; Breed et al. 1988; Waldman 1989; Walls 1991). Indeed, the primary basis for discrimination in *A. opacum* shifts from genetic relatedness in the larval stage to that of familiarity in metamorphs (Walls 1991). This switch in behaviour may reflect a change in selective pressures between the aquatic and terrestrial environments (Walls 1991).

Changes in kin discrimination have also been associated with changes in environmental and social contexts. Sibling aggression in birds may be mediated by food levels or food type (O'Connor 1978; Mock 1985; Mock et al. 1987; Drummond & Garcia Chavelas 1989; Parker et al. 1989). Maternal care in colonies of the prairie dog, *Cynomys ludovicianus*, shifts from infanticide to communal nursing once litters become mixed a few weeks after parturition (Hoogland 1985; Hoogland et al. 1989). Female acorn woodpeckers, *Melanerpes formicivorus*, may cannibalize a sister's eggs or incubate them in a communal nest, depending upon the intensity of intraspecific competition (Stanback & Koenig 1992). Similarly, sibling cannibalism is density-dependent in the catfish, *Clarias gariepinus* (Hecht & Appelbaum 1988).

Fitness Consequences

Larval aggression, as a form of interference competition, may have important consequences for components of fitness, such as body size and the timing of metamorphosis. Furthermore, plasticity in kin discrimination may be selectively favoured if such behaviour increases an individual's fitness in some contexts but not in others. Thus, aggression that is kin-biased may ultimately affect components of fitness in a manner that reflects the context. Our results of larval performance revealed an interaction between all four main factors, which corresponds with the interaction effect (between three variables) observed in our behavioural analysis. We regard the former result with caution, however, because of the low sample size in some treatments. Furthermore, although the MANOVA indicated a significant interaction effect, none of the ANOVAs were statistically significant. Thus it is unclear whether correlates of fitness in *A. opacum* may be influenced by kin discrimination.

In contrast to another experimental investigation with larval *A. opacum* (Smith 1990), we found no effect of variation in initial size on larval performance. Similar to Smith (1990), however, we found that food level had a significant effect on all of our response variables. Therefore, resource competition appeared to occur in at least the low food treatments. Moreover, in chambers without partitions, exploitative and/or interference competition significantly reduced the size of the smaller individual of each pair. This result is confounded with the timing of metamorphosis, however, because larvae in chambers without partitions metamorphosed sooner than did larvae in partitioned chambers. Thus, the smaller size may be partly due to early metamorphosis.

Such early metamorphosis at a small body size suggests a possible switch in strategies. Timing of amphibian metamorphosis may have important fitness consequences (Wilbur 1980; Werner 1986). Early metamorphosis in *A. opacum* may minimize interference behaviour in the aquatic environment. Early metamorphosis may not allow full exploitation of the aquatic environment, however, resulting in lower mass at metamorphosis and ultimately a reduction in reproductive output (Berven & Gill 1983; Smith 1987; Semlitsch et al. 1988; Scott 1994). Thus, *A. opacum* may spend more time in the larval stage in the absence of interference competition.

Our study demonstrates that kinship influences aggressive interactions, although its significance to measures of fitness remains unclear. Furthermore, the effect of kinship on aggression depends upon the social and environmental context, demonstrating that test conditions may influence the detection of kin discrimination abilities. Animals that discriminate kin may not do so in all contexts, but animals not known to discriminate may do so if test conditions are altered. Manipulating test conditions may produce more examples of context-dependent kin discrimination and, in turn, may illuminate the functional basis of kin biased behaviour.

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