Kinship affects morphogenesis in cannibalistic salamanders

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INCLUSIVE fitness theory predicts that organisms can often increase their fitness by helping relatives. Indeed, many animals modify their behaviour towards kin in a fashion consistent with theory1-4. Morphogenesis may also be sensitive to kinship environment, especially in species that facultatively produce distinct morphs that differ in their ability to harm relatives, such as those that produce alternative cannibalistic and non-cannibalistic phenotypes5-9. We tested this hypothesis by examining whether consanguinity affected the probability that structurally distinctive cannibal morphs10,11 would develop in larval Arizona tiger salamanders (Ambystoma tigrinum nebulosum). We report here that when tiger salamander larvae are reared in mixed-brood groups they are significantly more likely to develop the cannibal morphology and at an earlier age than siblings reared in pure-sibling groups. In general, morphogenesis may be responsive to kinship in any species that facultatively develops structures that can be used against conspecifics as weaponry.

Tiger salamander larvae occur in nature as two alternative morphotypes10,11: a 'typical' morph that feeds mostly on invertebrate prey, and a larger, physically distinctive 'cannibal' morph that has specialized oral structures to facilitate the ingestion of conspecifics12,13. Cannibals are induced facultatively by high densities of conspecifics13,14.

The potential exists in nature for cannibal-morph larvae to eat relatives. Stomach content analyses of wild-caught cannibals indicate that they often consume conspecifics of their own size and smaller11,15. The occurrence of these two environmentally induced morphs enabled us to test whether kinship influences morphogenesis of alternative morphs that differ in their ability to harm relatives.

We randomly assigned similarly sized, two-week-old larvae from eight different sibships (three of which were cousins) to three different treatment categories: larvae were reared in groups of 16 with (1) siblings only, (2) equal numbers of siblings and nonsiblings (some were cousins, others were non-kin), or (3) one sibling and two larvae from each of the seven other sibships (Fig. 1). This manipulation mimicked a natural situation. Larvae are surrounded solely or primarily by siblings in ponds in which only a few females oviposited, whereas larvae are surrounded primarily by non-relatives in ponds where numerous females oviposited (unpublished observation). Inclusive fitness theory predicts that larvae in the former setting should be less likely than those in the latter setting to develop into cannibals.

Cannibal morphs were indeed significantly more likely to develop in mixed-brood groups than in pure-sibship groups (Table 1). No more than one cannibal was produced per aquarium. In aquaria containing relatively few larvae, cannibals
have the effect of inhibiting other larvae from developing into cannibal morphs\(^1\), either through chemical cues or by diminishing the food supply, thereby making it less profitable for another larva to develop into a cannibal. Thirty-one of 77 (40\%) pure-sibship tanks produced a cannibal morph, as opposed to 33 of 40 (83\%) two-sibship tanks, and 34 of 39 (87\%) eight-sibship tanks. After we had statistically controlled for differences among sibships in the probability of producing a cannibal morph, we found that larvae in both the two- and eight-sibship treatments were significantly more likely to develop the cannibal morphology than were their siblings reared in pure-sibship treatments (Table 1). Thus, increased consanguinity in the larval environment decreases a larva's probability of becoming a cannibal. Even small differences in kinship environment affected morphogenesis. The difference between observed and expected probabilities of an individual becoming a cannibal morph was significantly greater in the eight-sibship treatment (mean ± s.d. = 0.38 ± 0.34; \(N = 39\)) than in the two-sibship treatment (0.26 ± 0.35; \(N = 40\); \(P < 0.0001\), two-tailed Mann–Whitney test).

Presumably, a larva that becomes a cannibal later in life threatens its siblings less than one that becomes a cannibal early in life. Thus, in addition to influencing the probability of a larva becoming a cannibal morph, kinship environment might affect the timing of the cannibal's initial expression.

The cannibal morphology was expressed significantly earlier in the eight-sibship treatment than in the one-sibship treatment (Fig. 2). Indeed, the mean time at which the cannibal morphotype developed in the eight-sibship treatment was earlier than in any of the eight sibships when they were reared alone. Thus, increased consanguinity in the larval environment also increased the age at which a larva became a cannibal. Interestingly, the cannibal morphotype developed significantly earlier in the two-sibship treatment containing non-kin and siblings than in the two-sibship treatment containing cousins and sibs (Fig. 2). This again illustrates that small differences in kinship environment influenced morphogenesis.

It may be argued that these differences between pure and mixed-sibship treatments were not due to differences in kinship

**TABLE 1  Effect of kinship environment on development of the cannibal morphology**

<table>
<thead>
<tr>
<th>Sibships</th>
<th>Number of replicates</th>
<th>Probability of producing a cannibal morph (number of aquaria in which a cannibal developed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>One sibship</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC,16</td>
<td>10</td>
<td>0.20 (2)</td>
</tr>
<tr>
<td>LC,21</td>
<td>9(^*)</td>
<td>0.89 (6)</td>
</tr>
<tr>
<td>LC,23</td>
<td>10</td>
<td>0.70 (7)</td>
</tr>
<tr>
<td>S,4</td>
<td>8(^*)</td>
<td>0.13 (1)</td>
</tr>
<tr>
<td>S,5</td>
<td>10</td>
<td>0.30 (3)</td>
</tr>
<tr>
<td>OA</td>
<td>10</td>
<td>0.40 (4)</td>
</tr>
<tr>
<td>WP</td>
<td>10</td>
<td>0.20 (2)</td>
</tr>
<tr>
<td>So</td>
<td>10</td>
<td>0.40 (4)</td>
</tr>
<tr>
<td>Two sibships</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LC,16,LC,21</td>
<td>10</td>
<td>1.00 (10)</td>
</tr>
<tr>
<td>LC,16,LC,23</td>
<td>10</td>
<td>0.70 (7)</td>
</tr>
<tr>
<td>LC,21,LC,23</td>
<td>10</td>
<td>1.00 (10)</td>
</tr>
<tr>
<td>S,4, S,5</td>
<td>10</td>
<td>0.60 (6)</td>
</tr>
<tr>
<td>Total</td>
<td>(33)</td>
<td>(22.58)</td>
</tr>
<tr>
<td>Eight sibships</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All sibships</td>
<td>39(^*)</td>
<td>0.87 (34)</td>
</tr>
</tbody>
</table>

* Derivation of expected probability of producing a cannibal morph: the expected probability that a larva in each mixed-sibship tank would become a cannibal morph was calculated from the observed probabilities in the constituent pure-sibship groups. The observed probability that any given larva from a sibship would become a cannibal morph when reared with only siblings was \(P_s = \frac{1}{4} = \frac{1}{N_s}\), where \(P_s\) is the observed probability in Table 1 of sibship \( j \) producing a cannibal morph for example, \(Q_{c.s.s.} = 1 - \frac{1}{2} = 0.75\) where \(Q_s\) is observed probability that a larva from sibship \( j \) would become a cannibal morph when reared with siblings only. \(S\) is the number of sibships per tank (2 or 8). \(N_s\) is the number of larvae from sibship \( j \) per tank (8 or 2). Because no more than one cannibal was produced per tank, we derived the expected probability that a cannibal morph would develop in sibships (each containing \(N\) larvae) by subtracting from one the expected probability that those sibships would not produce a cannibal morph \((1 - O_s)^N\).\(^{13}\) Sibships OA, WP and So were not used in the calculation of the expected probabilities for the two-sibship treatment because they were not represented in this treatment. Statistics for each mixed-sibship treatment (that is, two and eight sibships per tank), the observed and expected number of aquaria in which a cannibal morph developed were compared with a one-sample \(x^2\) test. All four two-sibship combinations were pooled for analysis because they did not differ significantly in the difference between observed and expected probabilities.

\(^{13}\) See Fig. 1 legend for notation used for study animals.

\(^{14}\) Four tanks were excluded from analysis because all animals died from disease; mortality was low in the other tanks.
per se. Instead, siblings may have been more similar in size than
non-siblings, as in certain frog tadpoles. If so, the benefits of
becoming a cannibal might have been greater in mixed-sibship
groups, because size disparities may facilitate cannibalism.

Four lines of evidence argue against this hypothesis. First, all
larvae were similar in size initially, and individuals from different
broods maintained similar growth trajectories, as evidenced by a
lack of significant variation in snout-vent length (SVL) of
randomly selected larvae from different sibships at the end of
the experiment (tadpoles: $F_{26, 26} = 0.71, P = 0.59$, $N = 40$
larvae from five sibships; cannibals: $F_{22, 21} = 1.16, P = 0.36$, $N =$ 27
larvae from seven sibships). Second, the size-variation hypothesis
cannot explain why cannibals developed earlier in treatments
containing siblings and non-kin than in those containing
siblings and cousins (Fig. 2). If anything, cannibals differed
more in size (mean difference in SVL between cannibals was
5.00 ± 5.71 mm; $N = 34$ pairwise combinations) than did non-kin
(mean difference in SVL between non-kin was 4.41 ± 4.27 mm;
$N = 224$ pairwise combinations). Third, at the end of the
experiment, the proportion of cannibals produced in a brood was
not positively correlated with the coefficient of variation in
SVL of individual larvae from that sibship ($r = -0.20, P = 0.70,$ $N = 6$
sibships, 105 larvae). Fourth, of 20 four-week-old typicals from
sibships LC-16 and LC-23 (mean SVL, 25.85 ± 0.26 mm) that
were housed individually with 15 smaller siblings (mean SVL,
18.21 ± 0.14 mm), none developed the cannibal morphology,
and even after consuming their siblings. By contrast, individuals
from these sibships that were housed with equal-sized siblings
and maintained under identical conditions occasionally
developed into cannibals (Table 1). Indeed, the benefits of
becoming a cannibal may be greater in groups of similar, not
dissimilar, size, because the cannibal phenotype enables
salamanders to eat conspecifics of their own size. In sum, the
size-variation hypothesis does not explain why larvae reared in
mixed-brood groups were significantly more likely to develop
the cannibal morphology than were their siblings reared in
pure-sibship groups.

We hypothesize that expression of the cannibal morph is
influenced by sibship-specific olfactory signals, which these
larvae use to cannibalize more distant relatives in preference to
close kin (D.W.P., P. Sherman and J.P.C., manuscript
submitted). Sibship-specific chemical cues seem to cause certain
frog tadpoles to grow faster in water conditioned by kin as opposed
to non-kin. Indeed, tadpoles of several anuran species
grow larger when reared in pure-sibship groups than when reared
in mixed-sibship groups.

Various studies have demonstrated that kinship environment
influences an organism’s behaviour and that social
environment can influence morphogenesis. It is unknown, however,
whether kinship affects morphogenesis in other species that
produce alternative cannibalistic and non-cannibalistic
morphs or ‘fighter’ and ‘non-fighter’ morphs, but there are some intriguing possibilities. For instance, different species of similarly sized parasitic wasps may attack the same caterpillar.

Larvae of species in which each female typically lays a single egg per host tend to develop formidable fighter mandibles, whereas larvae of species in which each female typically lays many eggs per host (and whose larvae therefore share their host with many siblings) lack these structures. Thus, although lethal rivalry can arise even among close relatives, kin-mediated morphogenesis may provide a general mechanism to reduce this conflict.

Received 28 October 1992; accepted 4 February 1993.


ACKNOWLEDGEMENTS. We thank R. Reeve for deriving the expected probabilities in Table 1; H. Reeve, P. Sherman and U. Moller for comments on the manuscript; J. Rush and L. Nenadzic for laboratory assistance; and the White Mountain Apache Indian tribe and the Arizona Game and Fish Department for collecting permits. This work was supported by an NSF postdoctoral fellowship to D.P. and by a grant from the NSF to J.C.