Third cousin removed?

Prehistoric land-use and lake acidity
Pathogenesis of atherosclerosis
Diamond films take shape
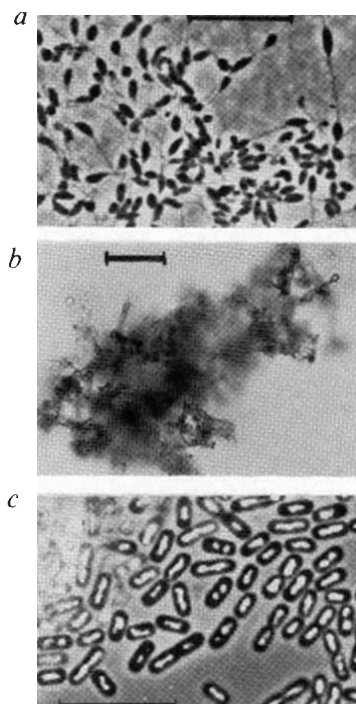
JOBS IN Japan

Labelling techniques
PRODUCT REVIEW

FIG. 2 Phase contrast photomicrographs of iron-oxidizing phototrophic bacteria. a, Rhodomicrobium-like isolate grown with succinate; b, Rhodomicrobium-like isolate grown with ferrous iron; c, gas vesicle-containing freshwater isolate grown with ferrous iron. Scale bars, 10 μm.

did not change. No growth occurred in media without iron added.

These results show that ferrous iron oxidation requires only photosystem I in anoxygenic phototrophs, and that anaerobic ferrous iron oxidation was therefore possible before the evolution of oxygenic two-step photosynthesis. These findings, together with the recently discussed photochemical ferrous iron oxidation[4-6], again question the assumption that the appearance of Fe(III) oxides in banded iron formations is always an indicator of the appearance of free oxygen. Anoxygenic phototrophic bacteria as isolated in the present study may have contributed to early banded iron formation at Archaean times before oxygen became significant as an oxidant. Such bacteria may still be of ecological significance as a means of regeneration of a widespread oxidant[23,24] in anoxic low-sulphur littoral sediments. □

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Kinship affects morphogenesis in cannibalistic salamanders

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INCLUSIVE fitness theory predicts that organisms can often increase their fitness by helping relatives[1]. Indeed, many animals modify their behaviour towards kin in a fashion consistent with theory[2-4]. 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 phenotypes[5-9]. We tested this hypothesis by examining whether consanguinity affected the probability that structurally distinctive cannibal morphs[5,10] 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-sibship 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 morphotypes[5,10]: 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 conspecifics[10-12]. Cannibals are induced facultatively by high densities of conspecifics[13,14].

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 smaller[11,15]. 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 oviposit, whereas larvae are surrounded primarily by non-relatives in ponds where numerous females oviposit (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[13], 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 morphotype 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 $\pm$ 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 siblings (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
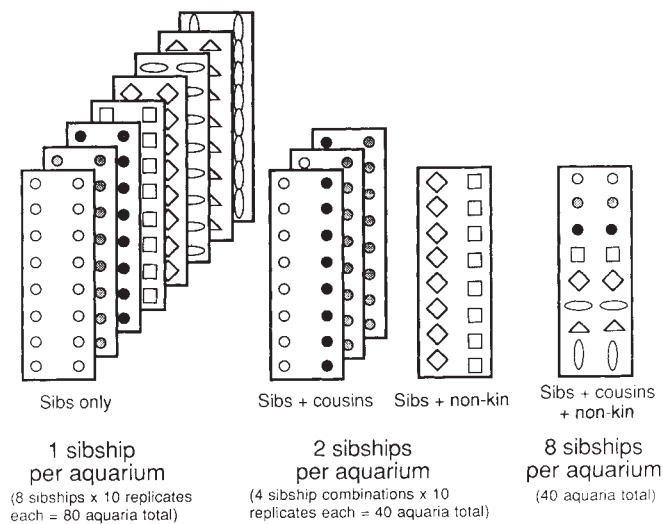

FIG. 1 Diagrammatic representation of the experimental design used to assess effects on morphogenesis of kinship. Each rectangle represents an aquarium containing 16 larvae, which are indicated by different geometric shapes (similar shapes shaded identically, siblings; similar shapes shaded differently, cousins; different shapes, non-kin). Study animals: we studied larvae from eight sibships whose parents or grandparents were caught in the White Mountains of Arizona, USA from five different ponds: Lower Cottonwood (LC), Snow (S), Old Apache (OA), South (So), and Wildcat Point (WP). Two sibships were captive-bred offspring of pairs collected from OA, So, and WP. Two sibships were captive-bred offspring of pairs collected from S, but were probably unrelated to each other owing to the large number of adults breeding in this pond. The other three sibships were offspring of captive-bred animals whose grandparents had been collected from LC and whose mothers were sisters. Thus, members of these three sibships were first cousins. Treatment groups: 160, 22-litre aquaria were each filled with 16 litres dechlorinated tap water. Two-week-old larvae (16 larvae per aquarium; tankmates differed in snout-vent length by no more than 5%) were distributed randomly[13] among three treatment groups: (1) one sibship per aquarium; (2) two equally represented sibships per aquarium; or (3) eight sibships per aquarium. The range of within-group relatedness across treatments was within the range of estimates from natural ponds based on observations of numbers of breeding adults per pond. The rearing density also was within the range of larval densities in the field[14]. We followed the rearing procedures outlined in ref. 13. Morph assignments: the experiment was ended at seven weeks because cannibals are unlikely to develop after this time[13]. Tanks were coded by number. Without knowledge of which treatment group was in an aquarium, two persons independently scored each animal as being of the typical or cannibal morphotype using criteria described in refs 5 and 10. Morph assignments were always unambiguous.

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

Sibship(s)*	Number of replicates	Probability of producing a cannibal morph (number of aquaria in which a cannibal developed)		P
		Observed	Expected	
One sibship				
LC-16	10	0.20 (2)	—	
LC-21	9†	0.89 (8)	—	
LC-23	10	0.70 (7)	—	
S-4	8†	0.13 (1)	—	
S-5	10	0.30 (3)	—	
OA	10	0.40 (4)	—	
WP	10	0.20 (2)	—	
So	10	0.40 (4)	—	
Two sibships				
LC-16, LC-21	10	1.00 (10)	0.704 (7.04)	
LC-16, LC-23	10	0.70 (7)	0.819 (8.19)	
LC-21, LC-23	10	1.00 (10)	0.513 (5.13)	
S-4, S-5	10	0.60 (6)	0.222 (2.22)	
Total		(33)	(22.58)	0.0008
Eight sibships				
All sibships	39†	0.87 (34)	0.490 (19.11)	0.0001

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 $O_j = 1 - \sqrt[16]{1 - p_j}$, where p_j is the observed probability in Table 1 of sibship j producing a cannibal morph (for example, $O_{LC-16} = 1 - (1 - 0.2)^{1/16} = 0.014$). The expected probability of a cannibal morph developing in each mixed-sibship group was $E = 1 - \prod_{j=1}^{S} (1 - O_j)^{N_j}$, where O_j 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), and N_j 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 S sibships (each containing N larvae) by subtracting from one the expected probability that those S sibships would not produce a cannibal morph (($(1 - O_1)^{N_1} \times (1 - O_2)^{N_2} \times \cdots (1 - O_S)^{N_S}$). 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 χ^2 test. The four two-sibship combinations were pooled for analysis because they did not differ significantly in the difference between observed and expected probabilities ($H_{adj} = 6.78$; d.f. = 3; $P = 0.0792$; Kruskal–Wallis test).

* See Fig. 1 legend for notation used for study animals.

† Four tanks were excluded from analysis because all animals died from disease; mortality was low in the other tanks.
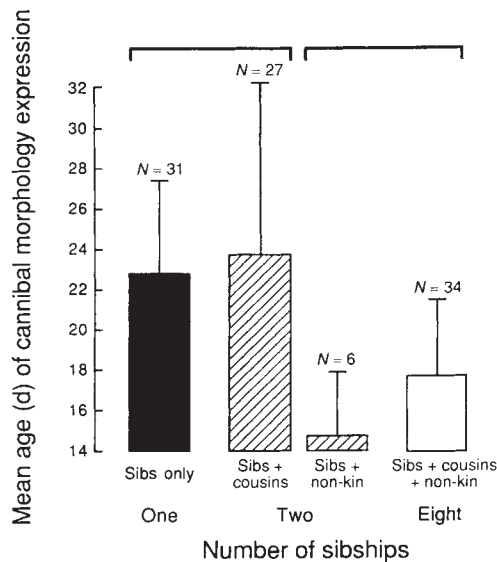
FIG. 2 Effect of kinship environment on larval age when the cannibal morph was initially expressed. Treatment group (number of sibships) had a highly significant effect on the time when a larva first developed the cannibal morphology (H_{adj} = 24.98; d.f. = 3; P < 0.0001; Kruskal–Wallis test). Groups under different brackets were significantly different (P < 0.005), whereas groups under the same bracket were not (Kruskal–Wallis multiple comparisons test[27]). Numbers above bars indicate the number of cannibal morphs produced per group.

per se. Instead, siblings may have been more similar in size than non-siblings, as in certain frog tadpoles[16]. 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 (typicals: $F_{4,36}$ = 0.71, P = 0.59, N = 40 larvae from five sibships; cannibals: $F_{6,21}$ = 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, cousins differed more in size (mean difference in SVL between cousins was 5.00 ± 5.71 mm; N = 84 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, 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[12]. 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[17]. Indeed, tadpoles of several anuran species grow larger when reared in pure-sibship groups than when reared in mixed-sibship groups[17-20].

Various studies have demonstrated that kinship environment can influence an organism's behaviour[21] and that social environment can influence morphogenesis[22]. It is unknown, however, whether kinship affects morphogenesis in other species that produce alternative cannibalistic and non-cannibalistic morphs[6-9] or 'fighter' and 'non-fighter' morphs[23,24], 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[25]. Thus, although lethal rivalry can arise even among close relatives[24-26], kin-mediated morphogenesis may provide a general mechanism to reduce this conflict. □

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