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derms, inturned humeral head and reduced digits of *Junggarsuchus* indicate that the closest known relative of crocodyliforms was also the most highly adapted cursor. The consolidation of the crocodylian skull thus began well before crocodylians entered the water.

Methods

Order Crocodylomorpha Hay, 1930 *sensu* Walker, 1970[3]
Junggarsuchus sloani gen. et sp. nov.

Etymology. For the Junggar Basin in northern Xinjiang, *souchous* (Greek) meaning crocodile and for C. Sloan, who discovered the holotype.

Holotype. IVPP V14010, (Institute of Vertebrate Paleontology and Paleoanthropology, Beijing), the articulated anterior half of a skeleton including a nearly complete skull (Figs 2 and 3).

Locality and age. Lower part of the Shishugou Formation[18] at Wucaiwan, Altay Prefecture, Xinjiang. The lower Shishugou (also known as the Wucaiwan Formation) is considered late Middle Jurassic (Bathonian–Callovian)[19,20].

Diagnosis. Small (body length ~1 m) non-crocodyliform crocodylomorph with longitudinal concavity on ventrolateral surface of dorsally arched jugal, broadened dorsal edge of surangular, shallow fossa on distal edge of paroccipital process and part of squamosal, enlarged anterior maxillary teeth, well developed surangular foramen, retroarticular process lacking medial process and with broad dorsolateral and posteroventral flanges, shallow procoely in all preserved vertebrae, anteroposteriorly and distally elongate hypapophyses on posterior 4 cervical and anterior 4 dorsal vertebrae, broadened posterior border and sinusoidal dorsal edge on scapula, anteriorly directed humeral head and reduced deltopectoral crest, reduced metacarpal V not contacting carpus, no manus digit I, and no osteoderms.

Phylogenetic analysis

Phylogenetic relationships were analysed using a matrix of 55 characters from the skull and postcranial skeleton distributed among 17 taxa (see Supplementary Information). The taxa included four outgroups and three representatives of the Crocodyliformes. The matrix is derived from earlier studies[10] with 16 additional characters and the addition of *Junggarsuchus*, *Erpetosuchus*[21] and *Gobiosuchus*[22]. The data were analysed in PAUP*[23].

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Supplementary Information accompanies the paper on **www.nature.com/nature**.

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Cooperation and competition in pathogenic bacteria

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Explaining altruistic cooperation is one of the greatest challenges for evolutionary biology[1–3]. One solution to this problem is if costly cooperative behaviours are directed towards relatives[4,5]. This idea of kin selection has been hugely influential and applied widely from microorganisms to vertebrates[2–10]. However, a problem arises if there is local competition for resources, because this leads to competition between relatives, reducing selection for cooperation[3,11–14]. Here we use an experimental evolution approach to test the effect of the scale of competition, and how it interacts with relatedness. The cooperative trait that we examine is the production of siderophores, iron-scavenging agents, in the pathogenic bacterium *Pseudomonas aeruginosa*[15–17]. As expected, our results show that higher levels of cooperative siderophore production evolve in the higher relatedness treatments. However, our results also show that more local competition selects for lower levels of siderophore production and that there is a significant interaction between relatedness and the scale of competition, with relatedness having less effect when the scale of competition is more local. More generally, the scale of competition is likely to be of particular importance for the evolution of cooperation in microorganisms, and also the virulence of pathogenic microorganisms, because cooperative traits such as siderophore production have an important role in determining virulence[6,9,17–19].

Explaining altruistic cooperation is fundamental to understanding the main evolutionary transitions from single-celled organisms to complex animal societies[1]. The problem is why should an individual carry out an altruistic behaviour that is costly to perform, but benefits another individual or the local group? Hamilton's[4,5] kin selection theory provides an explanation for altruism between relatives: by helping a close relative reproduce, an individual is still passing on its genes to the next generation indirectly. This is encapsulated by Hamilton's rule[3,4], which states that an altruistic behaviour is favoured whenever $rb-c > 0$, where r is the genetic relatedness between the actor and the beneficiary, b is the benefit of receiving the altruistic behaviour and c is the cost of performing the behaviour. The theory of kin selection is well accepted, and variation in relatedness has been applied to explain variation in the level of altruistic cooperative behaviours in organisms ranging from microorganisms to vertebrates[2–10].

However, selection for altruism depends also upon the scale of competition (population demography or structure)[3,11]. There is a

large theoretical literature on this topic, initiated by the debate on whether limited dispersal (population viscosity) favours the evolution of altruism[2,3,11–14,20]. Although limited dispersal leads to a higher relatedness, r, between interacting individuals, which favours altruism, it can also lead to more local competition between relatives. This reduces the advantage of altruism because the increased fitness of the relative who receives the altruism is increasingly paid for by other relatives[13]. Frank[3] showed that this effect could be incorporated into Hamilton's rule, by allowing the scale at which competition occurs to vary from global, with no competition between relatives, to local, with potentially extreme competition between relatives. Specifically, he used an extra parameter, a, which is the proportion of competition that is local, and can vary from $a = 0$ (global competition) to $a = 1$ (local competition). This leads to an extended version of Hamilton's rule[3], which predicts that the
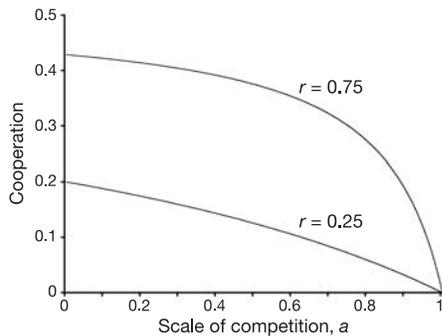

Figure 1 Scale of competition and kin selection theory. We have plotted the effect of the scale of competition on selection for an altruistic trait, from the incorporation of Frank's[3] scale of competition parameter, a, into a classic tragedy of the commons formulation[3,17]. This allows a simple and general graphical representation of the theoretical predictions. The y axis gives the evolutionary stable allocation of resources to a cooperative trait that is costly, but provides a benefit locally[3,25]. The scale of competition varies from global ($a = 0$) to local ($a = 1$). The different lines represent relatively high ($r = 0.75$) and relatively low ($r = 0.25$) relatedness. Higher levels of cooperation are favoured when relatedness is higher (higher r), and competition is more global (lower a). Furthermore, there is an interaction between scale of competition and relatedness: as competition becomes more local, the influence of relatedness on selection for cooperation is reduced. In the extreme, if $a = 1$, then competition is completely local and so kin selection cannot favour altruism[3,22]. The same conclusions can be reached using Queller's[11] approach of allowing for local competition, or with a model specifically developed for siderophore production[17].

scale of competition has two influences. First, as competition becomes more local (higher a), cooperation is selected against (Fig. 1). Second, there is an interaction between scale of competition and relatedness: as competition becomes more local, the influence of relatedness on selection for cooperation is reduced (Fig. 1). These effects occur because local competition leads to increased competition between neighbours and relatives, decreasing the kin-selected benefit of altruism[3,11,13,20].

Unfortunately, this problem is often ignored, across taxa ranging from microparasites[3,21] to cooperative breeding vertebrates[12], and Hamilton[2] left its importance as a major unsolved problem. To an extent this is because there is a lack of empirical evidence for how the scale of competition can influence selection for altruistic cooperative behaviours[12]. Empirical tests have been hindered because the amount of competition between relatives is usually confounded with relatedness; both increase with reduced dispersal[13,14,22]. Consequently, the best support for the role of scale of competition has come from other areas of social evolution and cases of extremely local competition, such as the favouring of female-biased sex ratios when males compete locally for mates[2,3,11,12,22]. An ideal experiment to address this problem would involve the independent manipulation of relatedness, r, and the scale of competition, a.

Here we use a bacterial system to carry out such a study, by experimentally testing the effects of the scale of competition and relatedness on the evolution of altruistic cooperation. Bacteria offer exceptional opportunities for addressing this problem because the effects of population demography on relatedness and the scale of competition can be disentangled, and because evolutionary responses can be followed over a reasonable timescale[9,23,24]. The system that we use is siderophore production in the opportunistic pathogen *P. aeruginosa*. Iron is a major limiting factor for bacterial growth because most iron in the environment is in the insoluble Fe(III) form, and, in the context of bacterial parasites, is actively withheld by hosts[15–17]. Siderophores are agents produced by bacteria in response to iron deficiency, that are released into the environment to scavenge insoluble and host-bound iron, making it available for bacterial metabolism[15–17]. Siderophore production is an altruistic cooperative trait that is costly for the individual but provides a local (group) benefit because other individuals can take up the siderophore–iron complex (see Methods)[15–17]. It is therefore termed a 'whole group' cooperative trait[25]. Importantly, *P. aeruginosa* mutants that do not produce siderophores have been seen to evolve both *in vitro* and in the lungs of cystic fibrosis patients[17,26]. Furthermore, the relationship between siderophore
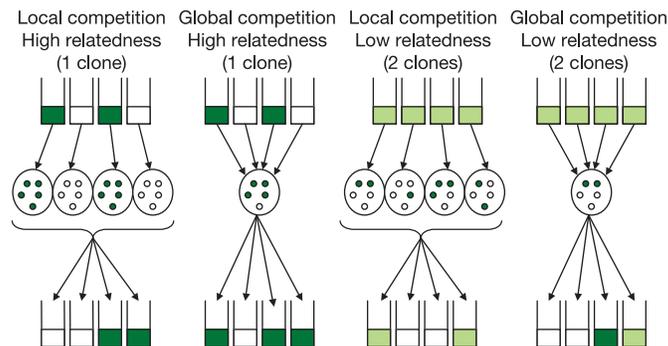

Figure 2 Experimental design. We varied relatedness between interacting individuals by initiating each subpopulation with either a single bacterial clone (relatively high r) or two bacterial clones (relatively low r). We varied the scale of competition by either mixing the cultures from all of the subpopulations in a treatment before plating, and then transferring random colonies from this single plate to initiate new subpopulations (relatively global competition, lower a), or by allowing every subpopulation in a treatment to provide equal numbers of colonies to the next generation (relatively local competition, higher a). We use dark green to symbolize the siderophore-producing cooperator, white to symbolize a cheater that does not produce siderophores, and light green for a mixture of cooperators and cheaters.

production and the growth rates of parasitic bacteria suggests that siderophore production plays a key part in determining parasite virulence, the damage to a host resulting from parasite infection[15–17,27].

We performed an experiment in which we independently manipulated relatedness and the scale of competition, and determined the consequences for the evolution of cooperation. We used a classic two factorial analysis of variance (ANOVA) design, and set up four treatments in which relatedness was either relatively high or low and the scale of competition was either relatively global or local (Fig. 2). Each treatment was replicated four times, giving a total of 16 (4 × 4) independent replicates. Each replicate contained one population divided into 12 subpopulations. The evolution of altruistic siderophore production was monitored by starting with a 50:50 overall ratio of cooperators and cheats, and monitoring how this changed over time. The cooperator is a wild-type strain that produces the primary siderophore, pyoverdin, and the cheater is a mutant strain that does not. These can be readily distinguished because pyoverdin is green, and so colours the wild-type cooperator colonies, whereas pyoverdin-minus cheater colonies are white.

To achieve relatively high relatedness (higher r) between individuals, we initiated each subpopulation with a single bacterial clone: in the first generation half the subpopulations in a treatment were initiated with the cooperator strain that produces pyoverdin, and the other half with the cheater strain that does not (Fig. 2). In contrast, we imposed relatively low relatedness (lower r) by initiating each subpopulation with two bacterial clones: in the first generation all of the subpopulations were initiated with a 50:50 mix of cooperators and cheaters. Note that relatedness is measured with respect to the 'altruist allele' (pyoverdin production), which is the relevant measure[3]. We imposed relatively global competition (lower a) by mixing the cultures from all of the subpopulations in a treatment before plating, and then transferring random colonies from this single plate to initiate new subpopulations (Fig. 2). This procedure allows productivity in a tube to determine the genetic contributions to subsequent generations, increasing the relative importance of global (between subpopulation) competition. In contrast, we imposed relatively local competition (higher a) by allowing every subpopulation in a treatment to provide equal numbers of colonies to the next generation. This removes the advantage of being in a more productive tube, and hence increases

the importance of local (within subpopulation) competition.

As expected from Hamilton's rule[4], cooperative siderophore producers were favoured in the higher relatedness relative to the lower relatedness treatments ($F_{(1,13)} = 73.6$, $P < 0.0001$; Fig. 3). This occurs in our experiment because the low relatedness treatments provide a relative advantage to cheating, by allowing cheaters to exploit siderophores produced by cooperators whenever a subpopulation contains both types. However, as explicitly predicted by Frank's[3] extension of kin selection theory (Fig. 1), the success of cooperators was also dependent on the scale of competition, and its interaction with relatedness. First, cooperators had an advantage under relatively global competition compared with relatively local competition ($F_{(1,13)} = 44.8$, $P < 0.0001$; Fig. 3). Global competition provides a relative advantage to cooperators, even when relatedness is low, because by chance there will be some subpopulations containing only cooperators, which are more productive and hence provide a greater contribution to the next generation. Second, there was a significant interaction between relatedness and the scale of competition, with relatedness having a relatively weaker effect when the scale of competition was more local ($F_{(1,12)} = 7.4$, $P = 0.019$; Fig. 3). The variation in cooperator success was associated with a significant increase in cooperator frequency (one treatment), a significant increase in cheater frequency (two treatments), and no significant change from starting conditions (one treatment) (Fig. 3). Overall, relatedness, the scale of competition and their interaction explained 94% of the variance in cooperator frequency.

Our results provide a clear experimental demonstration of how the scale of competition influences the evolution of altruistic cooperation, both in isolation and through its interaction with relatedness. The scale of competition could reduce selection for cooperation between relatives across a wide range of organisms[12]; for example, it is likely to be important in some cooperative breeding vertebrates[12], and could help explain the variation across species in the extent to which individuals preferentially help relatives[8]. We suggest that the scale of competition is likely to be of particular importance for the evolution of cooperation in bacteria and other microorganisms. This is because life cycles can involve stages with limited movement and hence relatively local growth and competition, but also relatively long distance dispersal stages[6,9,28]. Consequently, the overall scale of competition is likely to vary continuously across species, between local and global, depending on dispersal rates. For example, in the context of bacterial pathogens this variation will occur as a result of within-host growth (relatively local) and transmission to new hosts (relatively global)[3,21].

Many traits associated with increased growth and virulence in pathogenic bacteria seem to be altruistic and subject to kin selection. Here we have discussed siderophore production, but other examples include biofilms, Shiga toxins and immune suppression[17–19]. The link between cooperation and virulence could be exploited with new intervention strategies, and also suggests that virulence could evolve as a consequence of intervention[29]. For example, if the reduced transmission from a control programme led to more local competition (higher a), without significantly altering relatedness, then it would select for less cooperation and hence lower virulence. These consequences could be particularly important in pathogens such as *P. aeruginosa*, in which transmission can occur between infected sufferers of cystic fibrosis[30], and evolution can take place on such a short timescale relative to the duration of an infection[26]. □
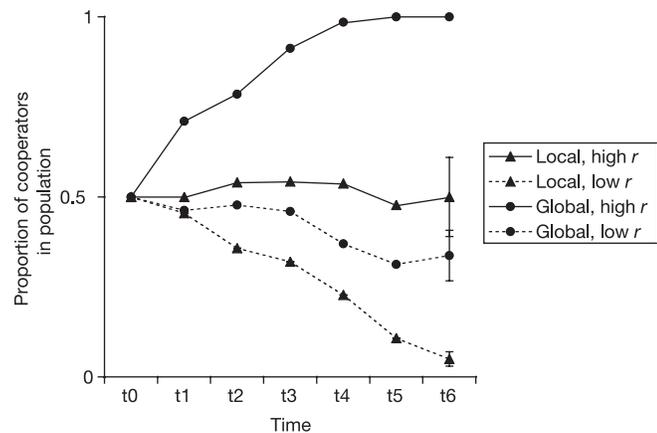

Figure 3 The evolution of cooperation in response to relatedness and the scale of competition. The proportion of cooperating individuals who produce pyoverdin siderophores is plotted against time. The different lines represent relatively high (solid line) and low (dashed line) relatedness. The different symbols represent relatively global (circle) and local (triangle) competition. Each of the four treatments was replicated four times, and standard errors are shown for the final time point. Time is measured as transfers, between which cultures were allowed to grow for 24 h. Cooperation is favoured by higher relatedness and more global competition.

Methods

Strains

Benefit and cost of siderophores

We determined that siderophore production is an appropriate cooperative trait for our experiment. We measured the growth rates of a wild-type strain that produces the primary siderophore, pyoverdin (cooperator), and a mutant strain that does not (cheater), either when alone or in mixed cultures where the other strain was present, and at a variety of iron availabilities. Both strains were grown overnight in 30 ml glass universals containing 6 ml standard King's medium B (KB) in an orbital shaker (200 r.p.m.), at 37 °C. Cell densities did not differ between strains when grown under these conditions ($n = 12$, 2-sample t-test: $t = 0.37$, $P > 0.2$). Sixteen universals containing Casamino acids medium (CAA; 5 g Casamino acids, 1.18 g $K_2HPO_4.3H_2O$, 0.25 g $MgSO_4.7H_2O$, per litre) media were inoculated with 10^6 overnight culture cells of either PA01, PA6609 or a 50:50 mixture of both, resulting in a total of 48 cultures. Sodium bicarbonate was added to each tube to create a 20 mM solution, necessary for effective chelator activity[27]. Four tubes inoculated with the three different bacterial populations (PA01, PA6609 and both) were exposed to three different iron-limitation treatments: $50\,\mu g\,ml^{-1}$, $100\,\mu g\,ml^{-1}$ and $200\,\mu g\,ml^{-1}$ of human apo-transferrin (Sigma), a natural iron-chelator, were added. Human iron chelators (such as transferrin) bind iron, preventing non-siderophore-mediated uptake of iron by bacteria. Siderophores are also strong iron chelators, and so compete with transferrin for iron. The tubes were incubated at 37 °C in a static incubator for 24 h. Cultures were then plated onto KB agar and relative densities were determined.

Siderophore (pyoverdin) production in these strains has the characteristics that make an appropriate cooperative trait for our study. First, there is a cost to individuals that can produce pyoverdin, as shown by monocultures of mutants being able to outcompete wild-type strains in an iron-rich environment ($F_{(1,6)} = 12.61$, $P = 0.01$). Second, when iron is limiting, siderophore production is beneficial, as shown by populations containing wild-type strains growing to a higher density than those where pyoverdin producers are absent ($F_{(1,21)} = 16.13$, $P = 0.0006$). Chelator concentration was also significant ($F_{(1,21)} = 12.45$, $P = 0.002$), but not the interaction ($F_{(1,20)} = 0.69$, $P = 0.42$). Third, individuals who do not produce pyoverdin are able to exploit the pyoverdin produced by the others, as shown by mutants growing to higher densities in the presence of wild type in iron-poor environments ($t = 6.37$, d.f. = 10, $P < 0.005$). Consequently, pyoverdin production is a costly altruistic trait that can potentially be exploited by cheaters.

Selection experiment

We independently manipulated relatedness and the scale of competition, using a classic two factorial ANOVA design. Replicates contained one population divided into 12 subpopulations. Each subpopulation was grown in a tube of CAA medium supplemented with 20 mM $NaHCO_3$ and $100\,\mu g\,ml^{-1}$ human apo-transferrin[27]. In high relatedness treatments, six tubes were inoculated with 10^6 cells from an overnight culture of either PA01 or PA6609 (1:1 overall ratio of altruist to cheater). Low relatedness treatments initially comprised 12 tubes inoculated with 10^6 cells of a 1:1 mix of both strains. Cultures were then grown for 24 h in a 37 °C static incubator, during which time approximately seven generations take place. Local competition cultures were then individually plated onto KB agar, whereas an equal volume from each culture within a global competition treatment were mixed together before plating. Plates were incubated for 24 h at 37 °C, and after determining relative frequencies of the two strains, 24 or 12 (low and high relatedness treatments, respectively) random colonies were separately inoculated into KB media and grown at 200 r.p.m. and 37 °C, overnight. CAA tubes were then inoculated with a total of 10^6 cells from these overnight cultures: low relatedness tubes were inoculated with one clone, and high relatedness with two. (Colonies were grown up in KB rather than used to inoculate the subsequent transfer directly, to control for the difference in size between altruistic and cheater colonies, and therefore starting densities). This selection procedure was repeated for six transfers, allowing a total of 42 (7×6) bacterial generations under experimental conditions. Every round of selection we counted the frequencies of altruistic and selfish bacteria growing on the agar plates, and the relative proportion inoculated into the next round. We used the proportion of cooperators inoculated into the next generation as the response variable in our analyses, and in Fig. 3. The whole experiment was repeated a further three times.

Analyses

We analysed our data using standard ANOVA methodology, as implemented in the package GLMStat 5.7.5 (Kagi Shareware). For all analyses on the proportion of cheats (pyoverdin-minus colonies) in the population, the proportion was arcsine square root transformed before analysis, and a normal distribution subsequently confirmed with a Shapiro–Wilkinson test. We present the analyses for the end of the experiment after six transfers.

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High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean

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The availability of nitrogen is important in regulating biological productivity in marine environments. Deepwater nitrate has long been considered the major source of new nitrogen supporting primary production in oligotrophic regions of the open ocean, but recent studies have showed that biological N₂ fixation has a critical role in supporting oceanic new production[1–7]. Large colonial cyanobacteria in the genus *Trichodesmium* and the