

When the American sea sturgeon swam east

A colder Baltic Sea greeted this fish from across the Atlantic Ocean in the Middle Ages.

The two species of Atlantic sea sturgeon on either shore of the North Atlantic, *Acipenser sturio* in Europe and *A. oxyrinchus* in North America, probably diverged with the closure of the Tethys Sea and the onset of the North Atlantic Gyre 15–20 million years ago, and contact between them was then presumably precluded by geographic distance. Here we present genetic, morphological and archaeological evidence indicating that the North American sturgeon colonized the Baltic during the Middle Ages and replaced the native sturgeon there, before recently becoming extinct itself in Europe as a result of human activities. In addition to representing a unique transatlantic colonization event by a fish that swims upriver to spawn, our findings have important implications for projects aimed at restocking Baltic waters with the European sturgeon.

A. sturio once occurred abundantly in

rivers in regions from the Black Sea right up to the Baltic, but is now reduced to a relict population in southern France. *A. oxyrinchus*, however, is still found in rivers that run into the Atlantic from the Gulf of Mexico to Quebec.

We studied centuries-old museum specimens ($n=453$; see supplementary information) and contemporary specimens of *A. sturio* ($n=467$) and *A. oxyrinchus* ($n=4351$) from almost all known populations. To investigate their molecular genetics, we compared the same two DNA fragments, one of about 200 base pairs from the mitochondrial D-loop¹ and the other of about 230 base pairs from nuclear DNA flanking the microsatellite Aox-23 (ref. 2).

Two *A. sturio* and 39 *A. oxyrinchus* mitochondrial DNA haplotypes were found that had 22 fixed differences between species. Fourteen archived specimens from the European Atlantic and the North, Adriatic

and Mediterranean seas showed the expected *A. sturio* haplotypes. But, surprisingly, ten archived specimens from the Baltic and one from the Oste River (North Sea) carried the *A. oxyrinchus* haplotype A (Fig. 1).

In the nuclear DNA, three consistent differences distinguished each species. Archived specimens with haplotype A carried *A. oxyrinchus* genotypes, whereas all other archived specimens had *A. sturio* genotypes. Nuclear sequences revealed no evidence of hybridization. Estimations based on a molecular clock³ indicate that the Baltic *A. oxyrinchus* originated from a single founding matrilineage (haplotype A) during the past 1,910 years (50% confidence interval).

Examination of the morphological differences associated with the two species^{4,5} revealed that archived Baltic sturgeon had a closer affinity with *A. oxyrinchus* than with *A. sturio*. In addition, archived Baltic specimens showed alveolar sculpting on their scutes — as seen in *A. oxyrinchus* — and not the tubercular-radial sculpting displayed by *A. sturio*⁵.

Re-examination of 972 scutes from 9 archaeological sites (Fig. 1b) indicated that *A. sturio* colonized Baltic waters some time after the Pleistocene (about 3,000 years ago), followed by *A. oxyrinchus* about 1,800 years later (see supplementary information). Both species were found in deposits in Ralswiek, Island of Rugia, suggesting that there had been a remarkable species shift from *A. sturio* to *A. oxyrinchus* between 1,200 and 800 years ago in the Baltic.

The subsequent decline of *A. sturio* and the establishment of a sustained *A. oxyrinchus* population in European waters during the Little Ice Age might have been due to selection for the hypothermal conditions that characterize their likely Canadian sources: *A. oxyrinchus* spawns between 13.3 and 17.8 °C (ref. 6), whereas *A. sturio* does not spawn below 20 °C (ref. 7).

Our findings have implications for restocking the Baltic Sea with sturgeon (HELCOM-Decision 18/97 project), an endeavour that has so far stagnated owing to the very limited availability of *A. sturio*. Attempts to reintroduce the North American *A. oxyrinchus* could also be hindered because this cold-water-tolerant species might no longer flourish in today's warmer Baltic waters, which would better suit the thermal tolerance of the European sturgeon.

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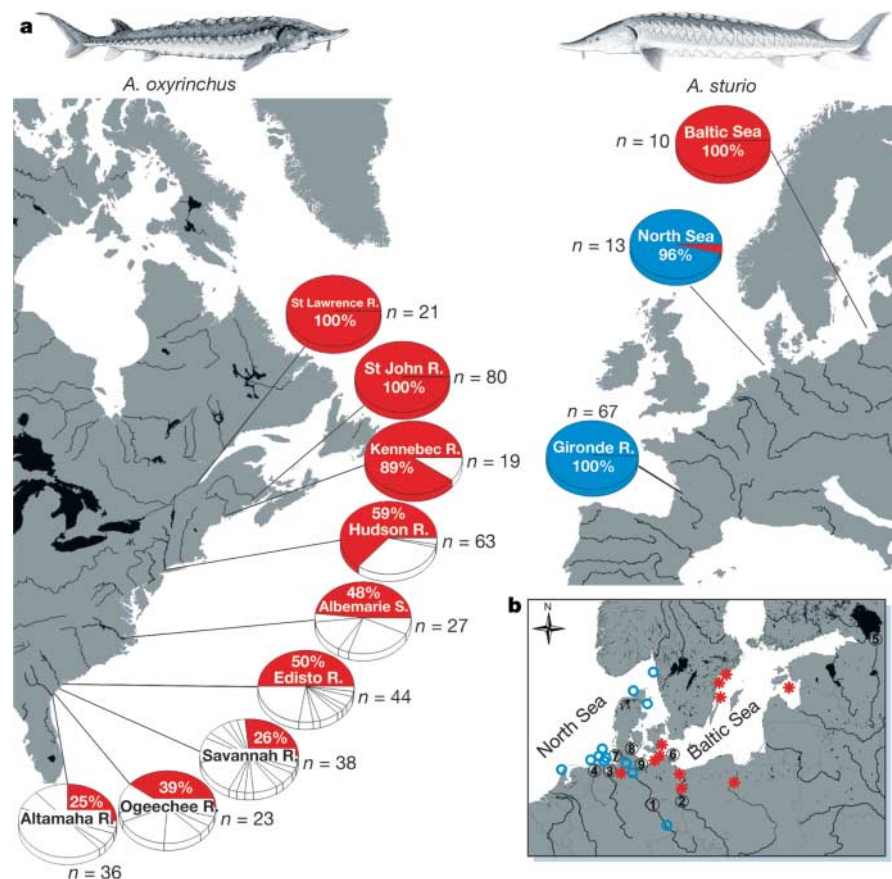


Figure 1 Geographical distribution of two lineages of mitochondrial DNA haplotypes found in Atlantic sea sturgeon from North America and Europe. **a**, Map (not to scale) showing sampling localities, number of sturgeon genetically analysed, and the distribution of *Acipenser oxyrinchus* haplotype A (red), all other *A. oxyrinchus* haplotypes (white) and *A. sturio* haplotypes (blue). The frequency of modal haplotype A increased with latitude among *A. oxyrinchus*. **b**, Localities of archival sampling sites (haplotypes: red, *A. oxyrinchus*; blue, *A. sturio*) and of archaeological data: 1, Elbe River (Niedergörne); 2, Oder River (Lossow); 3, Weser River (Feddersen-Wierde); 4, Ems River (Jegum); 5, Lake Ladoga; 6, Ralswiek (Island of Rugia); 7, Eider River (Elisenhof); 8, Spey River (Schleswig); 9, Lübeck (Germany).

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Supplementary information accompanies this communication on Nature's website.

Competing financial interests: declared none.

Food chemistry

Acrylamide is formed in the Maillard reaction

Reports of the presence of acrylamide in a range of fried and oven-cooked foods^{1,2} have caused worldwide concern because this compound has been classified as probably carcinogenic in humans³. Here we show how acrylamide can be generated from food components during heat treatment as a result of the Maillard reaction between amino acids and reducing sugars. We find that asparagine, a major amino acid in potatoes and cereals, is a crucial participant in the production of acrylamide by this pathway.

Products of the Maillard reaction are responsible for much of the flavour and colour generated during baking and roasting. An important associated reaction is the Strecker degradation of amino acids by

these intermediates (Fig. 1), in which the amino acid is decarboxylated and deaminated to form an aldehyde.

We investigated whether this reaction could provide a possible route to acrylamide. The amino acid asparagine should be a particularly suitable reactant as it already has an amide group attached to a chain of two carbon atoms. We therefore performed a series of Maillard reactions between glucose and asparagine, as well as with other amino acids that do not have the correct carbon backbone for acrylamide (Fig. 1).

Significant quantities of acrylamide (221 mg per mol of amino acid) were found when an equimolar mixture of asparagine and glucose was reacted at 185 °C in phosphate buffer in a sealed glass tube. The temperature dependence of acrylamide formation from asparagine indicates that this is favoured above 100 °C and that very high temperatures are not necessary (Fig. 2). In similar reactions with glucose and glycine, cysteine or methionine at

185 °C, no acrylamide was detected (detection limit, 0.5 mg mol⁻¹). Glutamine and aspartic acid gave only trace quantities of acrylamide (0.5–1 mg mol⁻¹).

When a dry mixture of asparagine and glucose was reacted at 185 °C (that is, without buffer solution), only 25 mg mol⁻¹ acrylamide was formed. Although the dry reaction is a realistic system with which to simulate the later stages of baking and toasting of food, it is less efficient because the reactants are incompletely mixed in the absence of a solvent. Trace quantities of acrylamide were produced under these conditions from glutamine and aspartic acid, but not from any of the other amino acids apart from methionine, which yielded 5 mg mol⁻¹.

To test for the involvement of Strecker degradation in the the production of acrylamide, we used 2,3-butanedione instead of glucose in these reactions (butanedione is one of several dicarbonyl compounds formed in the Maillard reaction). Acrylamide was produced when asparagine was allowed to react with butanedione both in a dry system (40 mg mol⁻¹) and in buffer (63 mg mol⁻¹). Heating asparagine on its own at 185 °C did not produce acrylamide, confirming the requirement for the dicarbonyl reactant and Strecker degradation.

Again, there was no significant production of acrylamide in either system from butanedione and the other amino acids, with the exception of methionine (6 mg mol⁻¹ in the dry system). The Strecker aldehyde formed from methionine is methional, but acrolein can also be formed, together with ammonia: subsequent oxidation of acrolein to acrylic acid followed by amidation could then generate acrylamide (Fig. 1). However, this reaction might be limited by its requirement for ammonia, which reacts readily with carbonyls and other Maillard intermediates.

The almost exclusive formation of acrylamide from asparagine could explain the occurrence of acrylamide in cooked plant-based foods, such as cereals and potato, which are rich in this particular amino acid⁴. In potato used for the manufacture of potato crisps, the dominant free amino acid is asparagine (940 mg kg⁻¹, representing 40% of the total amino-acid content⁵); in wheat flour it is present at 167 mg kg⁻¹, corresponding to 14% of the total free amino acids (our unpublished results), and a high-protein rye variety contains 173 mg kg⁻¹ (18% of the total free amino acids)⁶.

Our findings indicate that Maillard reactions involving asparagine can produce acrylamide and might explain the increased concentrations of acrylamide in certain plant-derived foods after cooking.

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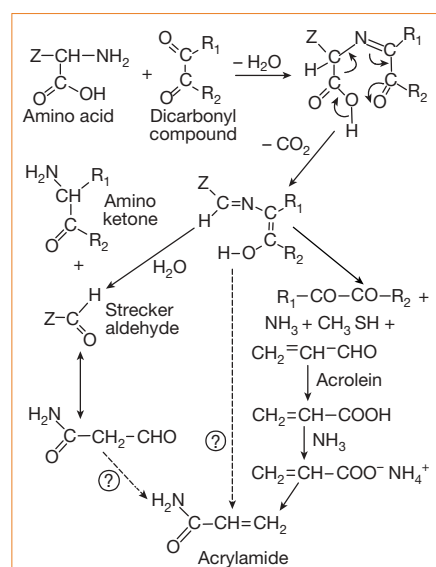


Figure 1 Proposed pathways for the formation of acrylamide after Strecker degradation of the amino acids asparagine and methionine in the presence of dicarbonyl products from the Maillard reaction. In asparagine, the side chain Z is -CH₂CONH₂; in methionine, it is -CH₂CH₂SCH₃.

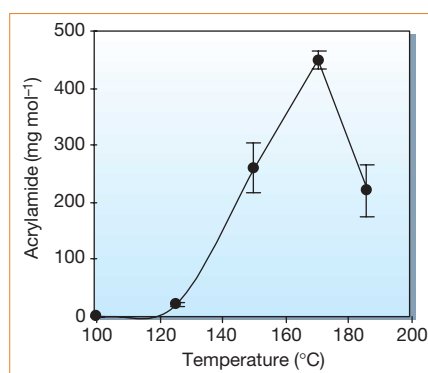


Figure 2 Temperature-dependent formation of acrylamide (mg per mol of amino acid) from asparagine (0.1 mmol) and glucose (0.1 mmol) in 0.5 M phosphate buffer (100 ml, pH 5.5) heated in a sealed glass tube for 20 min. Error bars represent standard deviations (n=3). Acrylamide produced in the reaction was extracted with ethyl acetate and analysed by gas chromatography with mass spectrometry after derivatization to 2,3-dibromopropanamide⁷, using 2-methylacrylamide as the internal standard. Selected ion monitoring was used to detect the analytes, with m/z 150 and 152 for acrylamide and m/z 120 and 122 for methylacrylamide. The presence of acrylamide in selected samples was confirmed in full mass spectra.

Animal behaviour

Fair refusal by capuchin monkeys

Brosnan and de Waal¹ report that capuchin monkeys show evidence of a sense of fairness or 'inequity aversion' because they rejected a less preferred reward when they saw a partner monkey receive a preferred reward for the same task. However, this does not show that monkeys are averse to inequity, only that they reject a lesser reward when better rewards are available. There are risks inherent in seeking anthropomorphic explanations for non-human behaviour.

In the 'inequality test', the monkeys refused to exchange a token for a cucumber slice (non-preferred reward) on 43% of trials when they saw a partner monkey receive a preferred grape reward for the same effort. However, in the 'food control' condition, in which the partner was not present, these same monkeys were just as likely to refuse the cucumber slice when they saw a grape placed where the partner normally sat (49% refusals). There can be nothing inequitable about receiving a non-preferred reward if nobody is receiving anything better. In the food-control condition, the monkeys are refusing the non-preferred reward simply because they can see that a better reward is potentially available. This is therefore the most parsimonious explanation for their refusal to accept the non-preferred reward when they see another monkey receive a better one.

Brosnan and de Waal¹ reject this reward-availability explanation for two reasons. First, in a third condition (the 'effort control' condition), where monkeys saw their partner receive a grape without having to exchange a token, the monkeys were more likely to refuse the cucumber slice than in the food-control condition. On its own, the comparison of the effort-control and food-control conditions is in the direction required by a fairness account. But fairness cannot account for the equally large difference between the effort-control and inequality-test conditions.

The basis of Brosnan and de Waal's second reason for rejecting the reward-availability explanation is in their Fig. 2, which seems to show an increasing trend of non-exchange for the two conditions in which another monkey was present (inequality test and effort control) and a decreasing trend of rejections in the food-control condition where no other monkey was present. Their Fig. 2 shows mean rejections for the first 10 and last 15 trials (not, as stated in the paper, the first 15 and last 10 trials; Brosnan and de Waal, personal communication) averaged across two sessions.

When the cumulative rate of rejections is

represented across all trials of both sessions for Brosnan and de Waal's monkeys, we find that there is no overall increase in rejection rate in the inequality-test and effort-control conditions, and that the rate does not decline across sessions in the food-control condition (results not shown).

Although explanations of animal behaviour in anthropomorphic terms are notoriously prone to imprecision², if 'fairness' or 'inequity aversion' mean anything in this context, they surely imply that individuals reject rewards more often when they see another receive a better reward than when the better reward is simply in view with no one else there to consume it. The very similar levels and patterns of cucumber rejection in the inequality-test and food-control conditions therefore contradict an account based on fairness or inequality.

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Brosnan and de Waal reply — We have shown¹ that animals compare their own rewards with those of others, and accept or reject rewards according to their relative value. Our aim was not to demonstrate that capuchin monkeys make a human response to inequality, but rather to elucidate evolutionary precursors to inequity aversion. We use this term as in ref. 2 — "people resist inequitable outcomes; that is, they are willing to give up some material pay-off to move in the direction of more equitable outcomes" — and specifically focus on "disadvantageous inequity aversion"². The monkeys in our experiment could not change the reward division, and hence could not actively avoid inequality, but we wanted to determine whether they would at least recognize inequality if subjected to it. We found that the capuchins reacted negatively, refusing to complete the interaction.

It is unlikely that inequity aversion appeared *de novo* in humans. It almost certainly evolved because individuals who responded to inequality disadvantageous to themselves increased their relative fitness compared with those who did not. We recognize several potential evolutionary precursors to disadvantageous inequity aversion (S. F. B., H. C. Schiff and F. B. M. de W., manuscript in preparation). First is the ability to recognize that rewards and efforts differ between individuals, which is also required for social learning, a skill present in capuchins³. Second is the propensity to react if another individual receives a better reward for a specific task. Third is sacrifice to alter another individual's outcome.

Our study mainly concerned the second ability, showing that capuchin monkeys react negatively when another individual gets a better reward for the same or less effort on a specific task. This finding suggests that precursors to inequity aversion are present in animals from which our lineage split millions of years ago. Although capuchins may be reacting somewhat differently from adult humans, we have still learned something about the behaviour's possible evolutionary trajectory.

Regarding the cross-cultural study, the lowest mean offer by a proposer in the ultimatum game was 26% of the total, whereas the lowest modal offer was 15%, both by the Machiguenga of Peru⁴. Such relatively high offers would not seem to be consistent with completely selfish individuals who lack any conception of fairness⁵.

As stated earlier¹, although the mere presence of a higher-value reward affects the capuchins, their reaction is not the same as when a conspecific receives the higher-value reward. To ignore the differences between the inequality test and the food-control test is unwarranted. Our Fig. 1 does not permit any conclusions about the effect of the food-control test and was not used for this purpose; it is the data in our Fig. 2 that inspired our claim.

The frequency of refusals across trials increases when a partner receives the reward and decreases when a reward is merely visible. The conservative statistic we chose did not allow significance ($P < 0.05$)¹, but we have since subjected these data to a comparison of the slopes of the linear regressions across trials for each test⁶. This re-analysis shows that refusals in the food-control test decrease across time, whereas those in the inequality test and effort-control condition increase ($F_{2,69} = 28.71, P < 0.001$). Our subjects therefore discriminate between a situation in which higher-value food is being consumed by a conspecific and one in which such food is merely visible, intensifying their rejections under only the former condition.

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Corrigendum

When the American sea sturgeon swam east

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Nature **419**, 447–448 (2002).

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