PCR identification of black caviar

SIR — Sturgeons (family Acipenseridae) and paddlefishes (family Polyodontidae), well known as producers of caviar, are threatened by unregulated overfishing, dams eliminating access to spawning grounds, and pollution[1–3]. Three species inhabiting the Volga River–Caspian Sea basin — the beluga sturgeon (*Huso huso*), Russian sturgeon (*Acipenser gueldenstaedti*) and sevruga (*A. stellatus*) — are particularly vulnerable because of the great demand for their eggs. According to the estimate of the oldest European caviar trading company Dieckmann & Hansen, in 1995 the international market need was 450 tonnes of black caviar, whereas the legal production of caviar in Russia and Iran was 228 tonnes. Even more telling are figures from the US Commerce Department indicating that caviar imports have increased by 100% since 1991. As things stand, a mere 30 grams of beluga caviar costs $69.50 at Petrossian in New York City.

As a result, the population sizes of commercial sturgeon species have fallen dramatically during the past few years[1–3]. Indeed, it has been suggested[4] that "the sturgeon's only chance of survival may be in captivity."

Traditionally, caviar dealers have relied on crude factors such as egg size and the appearance, smell, texture and colour of the roe to identify the species of a particular shipment of caviar. Protein electrophoresis has been used for species diagnosis of caviar, but it requires a large quantity of eggs and is not always accurate[5–7]. Here we describe the use of the polymerase chain reaction (PCR) for species diagnosis, which allows accurate and relatively inexpensive identification using single eggs.

We examined all extant acipenseriform species for portions of mitochondrial cytochrome *b*, 16S ribosomal DNA and 12S rDNA nucleotide sequences, as well as the variation among individuals from the three commercial caviar species. The data include several unique nucleotide positions that can be used to identify species within this group. We designed several primers specific for diagnostic nucleotide substitutions for each of the three commercial Russian sturgeon species mentioned above. Details of the primer sequences will be presented elsewhere. We designed the primers by the PCRASS method[8] so that a high-stringency PCR reaction with primers specific for a given species gives a positive reaction (a band of the correct length on an agarose gel) and nonspecific primers give a negative reaction (see figure).

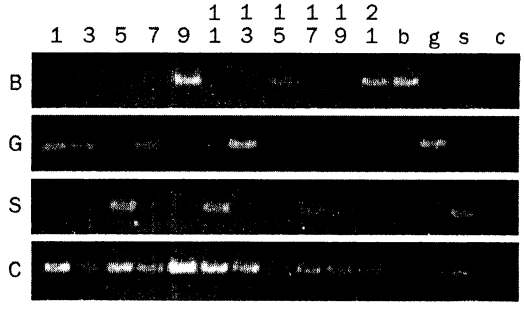
The table shows the results of our survey of 23 commercially available lots of caviar purchased in reputable Manhattan gourmet food shops and two brought from Russia. We identified five misrepresentations among the New York samples. The labels on the Russian lots did not identify the species (lots 18 and 19 in the table). Three of the misrepresentations are alarming in the light of the fragile status of the substituted fish[1]. The appearance of Siberian sturgeon, *A. baerii*, caviar (lot 12) is of concern because poaching in Siberia has increased dramatically. Before 1991, the regulated annual catch of this species was 200–300 tonnes for all Siberian rivers. In 1994 in the Ob River alone, the illegal catch of Siberian sturgeon was about 250–300 tonnes[9]. Our data (lot 4) also show that caviar of the Amur River sturgeon, *A. schrencki*, which is on the IUCN Red List of threatened animals, and ship sturgeon, *A. nudiventris* (lot 3), are sold in New York. This species is already extinct in the Aral Sea and is rapidly declining in the Caspian Sea[1].

For the members of the order Acipenseriformes, species identification is essential for survival. The use of our method to identify the origin of caviar samples could assist international conservation and legal efforts to save what little is left of the commercial sturgeon populations and help to protect the other species that will

SPECIES DESIGNATIONS FOR 25 COMMERCIALLY AVAILABLE LOTS OF CAVIAR

Caviar lot no.	Designation by supplier (trade name)	Diagnosis
1	American sturgeon	Osetra*
2	Caspian beluga	Beluga
3	Caspian osetra	Ship sturgeon*
4	Eastern beluga	Amur River sturgeon*
5	Caspian sevruga	Sevruga
6	Fresh beluga malossol	Sevruga*
7	Fresh ossetra malossol	Osetra
8	Fresh sevruga malossol	Sevruga
9	Caviar Russ (beluga)	Beluga
10	Russian caviar (osetra)	Osetra
11	Sevruga malossol	Sevruga
12	Beluga malossol	Siberian sturgeon*
13	Osetra malossol	Osetra
14	Sevruga malossol	Sevruga
15	Beluga malossol	Beluga
16	Osetra malossol	Osetra
17	Sevruga malossol	Sevruga
18	Sturgeon caviar	Osetra
19	Russian caviar	Sevruga
20	Sevruga malossol	Sevruga
21	Beluga prime	Beluga
22	Oscetra malassol	Osetra
23	Beluga malossol	Beluga
24	Ossetra malassol	Osetra
25	Sevruga malossol	Sevruga

Most lots were identified unambiguously. Lots marked with an asterisk indicate inaccurate labelling by the supplier. Lots 4 and 12 gave negative results in our experiments and were identified by sequencing the positive-control PCR fragment. Lots 1, 9, 13 and 16 were identified both by the PCRASS method and by sequencing the positive-control PCR fragment. Lot 3 gave a positive reaction for one of our *A. gueldenstaedti* primer pairs, but a negative signal for the other and was subsequently diagnosed by sequencing. Ship sturgeon is the common name for *A. nudiventris*, Amur River sturgeon the common name for *A. schrencki*, and Siberian sturgeon the common name for *A. baerii*. There are three different spellings for osetra on suppliers' labels (Osetra, Oscetra and Ossetra). Malossol means 'lightly salted'.



Performance of the PCR assay on DNA isolated from single eggs of commercially available caviar. Initially, DNA from five to ten individual eggs was isolated[10] for each commercial lot. We show only one representative from each odd-numbered lot, as all eggs from a lot gave the same results. The odd numbers (1–21) refer to the commercially available lots to which we gave arbitrary numbers. Results for even-numbered lots are not shown, but are reported in the table. PCR products using beluga- (panel B), sevruga- (S) and osetra- (G) specific primer pairs are shown in this figure. A fourth primer pair was used as a positive control and was designed to amplify all sturgeon samples (panel C). Note that none of the lots gave positive reactions for more than one of the diagnostic primer pairs (B, G or S). These results are verified using other primer pairs and, in some cases, the double-stranded fragment produced by the positive control (panel C) for these four lots was purified and sequenced using an ABI Model 373 automated sequencer. Several positions in this fragment are diagnostic for other species, and these nucleotide positions were used to type these four lots (table). Sample 3, although showing a positive reaction in panel G, did not show a positive reaction for the second *A. gueldenstaedti* primer pair. The control PCR product was therefore sequenced and used to diagnose this lot of caviar. Samples 4 and 12 were the only samples that were negative for all three primer pairs but positive for the control primers. Control PCR of these three lots of caviar as well as lots 1, 9, 13 and 16 were GeneCleaned and sequenced to determine the source species used for making these caviars (see table).

no doubt be more widely substituted for the disappearing commercial caviar species. At present, these commercial species are not included in the appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the only international agreement on endangered species trade. CITES will consider placing all sturgeon species in the appendices at a meeting (23–27 September 1996) in Pruhonice, Czech Republic. The enforcement of such a listing depends largely on the ability to diagnose these species accurately. Our method will give wholesalers an alternative to the crude identification methods they use now and give consumer groups a weapon to ensure accurate labelling.

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Red/blue chaotic power spectra

SIR — Cohen[1] has reported that the time series of chaotic, single-species ecological models have blue power spectra rather than the reddened ones associated with natural populations[2]. In other words, the dynamic behaviour of real populations is apparently dominated by longer-term trends, but population models, of one common type at least, fail to capture this crucial characteristic, being dominated instead by shorter-term responses. This calls into question both the usefulness of the models and the applicability of chaotic dynamics to natural systems. However, equivalent analyses (see Fig. 1) of chaotic metapopulation time series from explicitly spatial models (comprising coupled-map-lattices of $n \times n$ identical patches, describing host–parasitoid[3] or host–pathogen[4] dynamics, with the eight nearest-neighbouring patches linked by dispersal) give rise to distinctly reddened spectra — like the natural population data, but quite unlike the simpler models.

Note that the two-dimensional models within patches are dominated by short-term responses to population density (governed by 'biotic' mechanisms). This tends to undermine any suggestion[1,2] that the important contrast is necessarily between systems dominated by biotic factors (blue) and external, 'climatic' factors (red). Moreover, similar spectral analyses of chaotic but non-spatial two-dimensional models[5,6] still give rise to blue spectra. Thus, we judge that including the spatial dimension is crucial in reddening our power spectra, and that those of natural populations may still be explained (at least partly) by chaotic dynamics.

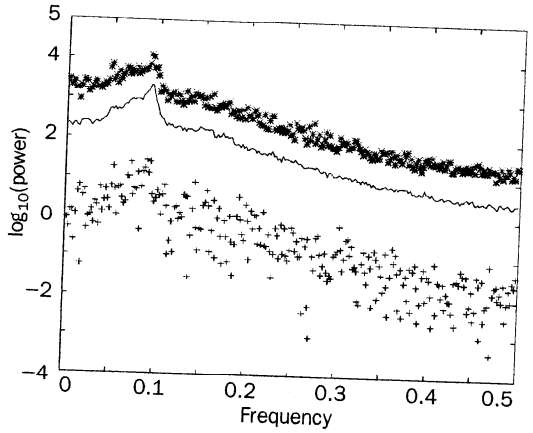

FIG. 1 Power spectral densities of the metapopulation of hosts in a spatially explicit host–parasitoid model[3] (similar portraits are observed for the host–pathogen model[4]). Parameter values, taken from ref. 4, are $r = 2$, $\sigma_1 = 0$, $\sigma_2 = 1$, $\Lambda = 1$, $\nu = 1$, $\mu_X = 0.2$, $\mu_W = 0.89$ and $n = 30$; similar results are obtained for larger values of n. In common with natural populations, the spectra are reddened, with greater power at low frequencies. (Scale on axes was chosen to allow comparison with ref. 1.)

We can also deduce why, biologically, space can produce reddened spectra. In the spatially explicit lattice, subpopulations respond quickly only to the density in their immediate neighbourhood. Hence, the concerted response of the whole metapopulation to its own total density can occur only on much longer timescales that allow initially local effects to spread and to influence the whole. This echoes, and puts flesh on, the complaints of empiricists that population density may be uninformative because individuals experience only local crowding.

Chaotic population dynamics, when generated by spatially explicit systems, may be important in explaining the reddened spectra seen in natural populations.

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SIR — We wish to add to the debate about colour in the power spectra of the complex dynamics of natural poplulations[1,2,7,8]. Power spectra of population trajectories are observed to be white (no dominating frequency) or red (dominance of low-frequency fluctuations[2,9]). Thus, low frequencies might be expected to dominate in population models producing chaos-like oscillations. But Cohen showed in various nonlinear models that high frequencies dominate in their chaotic fluctuations[1], making the power spectra blue. Here we report that adding delayed density dependence[10–12] to these population models reduces the dominance of high-frequency oscillations and either whitens or reddens their power spectra.

In a non-delayed density-dependent system, the population size P_t at time t defines the population size at time $t+1$ with a given growth rate r. We analysed the power spectra of chaotic trajectories of six of the eight models discussed by Cohen[1] by incorporating delayed density dependence into their dynamics. As an example, the Moran–Ricker discrete-time nonlinear dynamics[13,14], $P_{t+1} = P_t \exp[r(1 - P_t)]$, modifies into $P_{t+1} = P_t \exp\{r[1 - (P_t + cP_{t-1})]\}$. Of the models analysed by Cohen, we omitted the Verhulst model, known to produce white noise[1], and the Malthus–Condorcet–Mill model, in which delayed density dependence creates typically isolated spikes in spectra.

It is generally expected that the addition of time delays in population models will lower the stability of the dynamics. However, the consequences of delayed density dependence for the colour of the power spectra of chaotic population dynamics are not known. Adding delayed density dependence in the models can remove the dominance of high-frequency oscillations and can whiten or redden their power spectra (see Fig. 2), which brings them better into line with data from natural populations[9].

Simple population models have drawn attention to the possibility of complex dynamics in natural populations[7,8], but they may be of limited use in explaining the rich phenomena observed in dynamics of natural populations. Chaos in models of natural populations can be obtained in several ways, including periodic doubling (used in simple models[1]), quasiperiodicity and intermittency[15,16]. Depending on the way in which chaos is obtained, there may be fundamental differences in the power spectra of the population trajectories. For example, the quasiperiodic route, which strongly affects the power spectra of the population trajectory, occurs often in the context of species or population interactions, or in age–class interactions in a single population. Delayed density dependence may also produce the quasiperiodic route to chaos. Thus, when adding delayed density dependence to population models, we not only altered the assumptions on the density dependence, but also increased the dimension of the dynamics and moved to