Trifling variation in truffles

Of the ten species of European truffle (fungi of the genus *Tuber*, phylum Ascomycota), some have economic value because of their organoleptic properties (taste and perfume), in particular the black truffle (*Tuber melanosporum* Vitt.) and the summer and burgundy truffles[1,2]. The black truffle is mainly found in Spain, France and Italy (Fig. 1a), and it shows variation in several traits, including in its famous organoleptic properties, across this geographical range. Here we show that this variation probably results from environmental, rather than genetic, influences.

In an attempt to explain the variation in *T. melanosporum* across its geographical range and to study the distribution of genetic variability within and among populations, we analysed fruiting bodies (ascocarps) from different populations in France and Italy for random amplified polymorphic DNA (RAPD) and microsatellite polymorphism.

We found an extremely low level of polymorphism over the whole study area for both types of marker (Fig. 1b). This pattern contrasts dramatically with that of the sympatric summer truffle (*T. aestivum* Vitt., which forms a species complex with the burgundy truffle, *T. uncinatum* Chatin), which has distinct internal transcribed spacer alleles[3] and highly variable RAPD patterns[4].

The absence of heterozygotes in *T. melanosporum*, shown by study of microsatellites, is consistent with a very closed mating system, such as homothallism or even exclusive selfing. As the two putative haploid genomes from an ascocarp are very similar, we considered each variable RAPD band to be a distinct locus exhibiting two alleles (presence or absence of a band). The genetic models used subsequently showed no increase in genetic differentiation with geographic distance (Fig. 1c).

The low level of genetic diversity of the black truffle probably cannot be explained by its present large population size. In France, 1–3×10^4 kilograms of black truffles are now officially sold each year, and more than 10^6 kilograms per year were traded in the last century (the average weight of an ascocarp is about 30–40 grams).

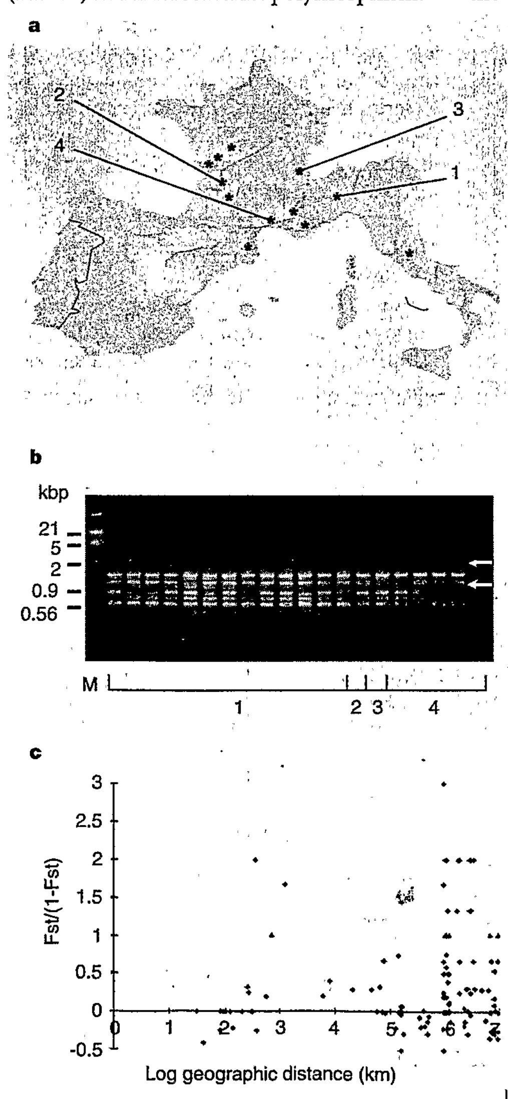

Figure 1 Geographic and genetic characteristics of the black truffle *Tuber melanosporum* Vitt. **a,** The western European geographical range (shaded area) of *Tuber melanosporum* and sampling localities (stars); numbers refer to samples shown in **b**. All truffles ($n=208$) were collected in natural habitats, except one sample which was collected in an artificial 'truffle-field'. **b,** Example of RAPD patterns obtained with the OPF-14 primer (Operon Technologies, Alameda) for truffles from four locations (numbers refer to **a**); arrows indicate polymorphic bands used. Sizes on left are in kilobase pairs. **c,** Analysis of isolation by distance according to ref. 7, using six RAPD loci (generated by primers OPF-11, OPF-13, OPF-14 and OPB-2) and the only two polymorphic microsatellite loci out of the nine assessed, $(GAGT)_{14}$ and $(GTTA)_{9}$, showing two alleles each. Computations were performed with Genepop version 3.1b (ref. 8). The increase in genetic differentiation with geographic distance was not significant (Mantel test, 10^5 permutations, $P>0.10$).

A population bottleneck probably occurred during the last, and coldest, glaciation, when the broadleaved forest of Europe was considerably reduced and restricted mainly to the Mediterranean coastal zone[5]. The black truffle ripens in winter (November–February), which probably contributed to its drastic reduction in population size and restriction to its southernmost limits during the glaciation, as ascocarps of contemporary *T. melanosporum* are susceptible to frost. The present low level of genetic variability in black truffle populations is consistent with such a bottleneck occurring 10,000 years ago, followed by a rapid colonization of southwestern Europe, which would also explain the absence of phylogeographic signals in the few polymorphic markers found. The 'glaciation hypothesis' would be strengthened if more southerly populations (in Spain or Italy) were found to show greater genetic diversity.

The seasonal behaviour of the summer and burgundy truffles, which ripen in spring and autumn, respectively, would have allowed them to sustain a larger geographical range and population size during the last glaciation, explaining the present high level of genetic variability of this species complex. Moreover, their current geographical range, extending further to the east and north (for example, to Poland and Sweden[6]), shows that they are more tolerant of colder climates.

Our results show that the morphological and organoleptic differences seen over the geographical range of the black truffles can probably be explained by environmental variation rather than by genetic factors. Research is needed to identify the environmental variables that affect the black truffle's perfume and taste, which are the objects of intense human interest.

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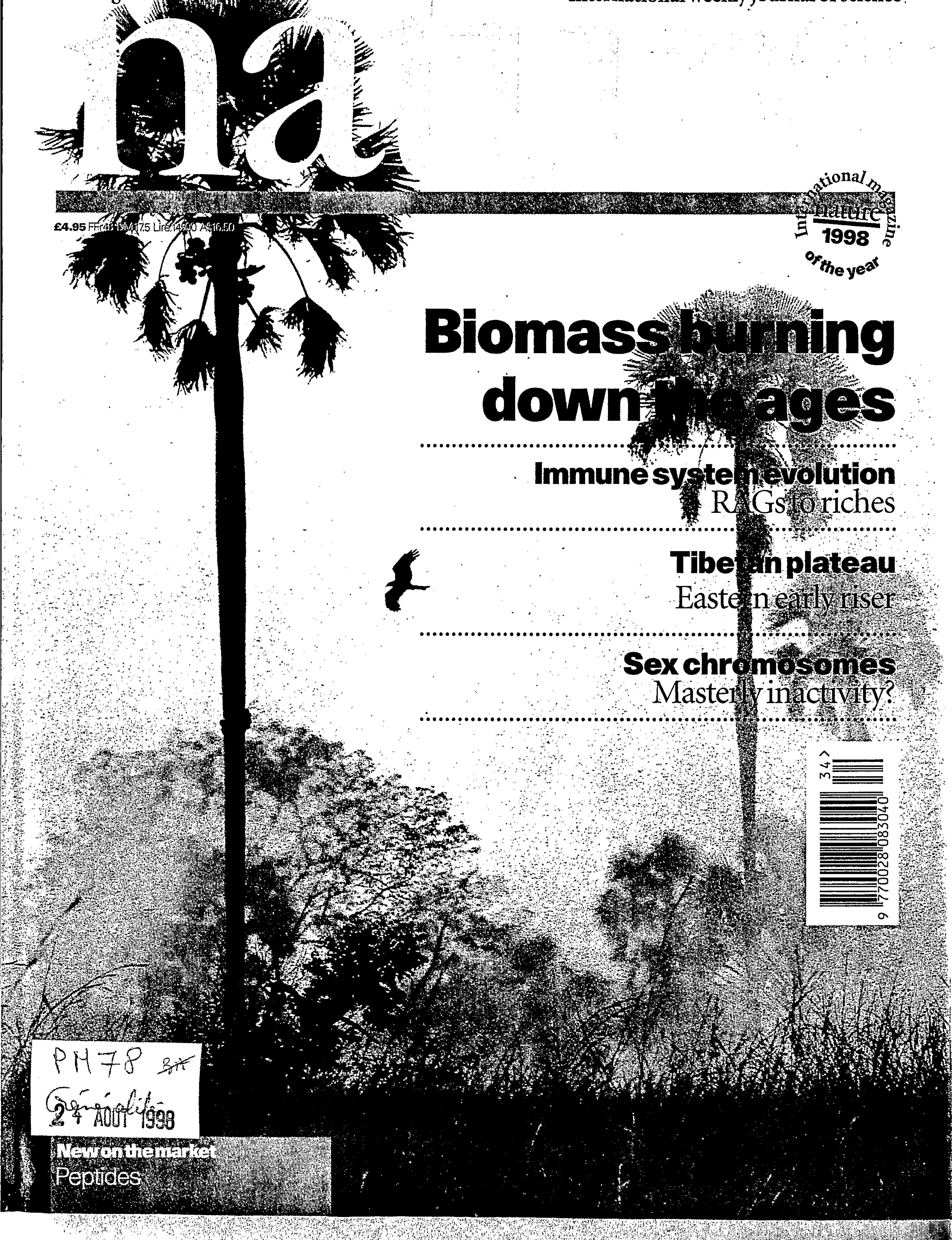

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