Organic chemistry of embalming agents in Pharaonic and Graeco-Roman mummies

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Chemical treatments were an essential element of ancient Egyptian mummification. Although the inorganic salt natron is recognized as having a central role as a desiccant[1], without the application of organic preservatives the bodies would have decomposed in the humid environment of the tombs[2]. The nature of the organic treatments remains obscure, because the ancient Egyptians left no written record of the process. Secondary textual evidence for mummification is provided by Herodotus[3], Diodorus Siculus[4], Strabo[5] and Pliny[6]. The most important account is that of Herodotus[3] (about 450 yr BC), although archaeological evidence shows that by this time the process had declined significantly and the best results had been achieved centuries before[7]. His account mentions myrrh, cassia, palm wine, 'cedar oil' (still widely disputed[8–10]) and 'gum'; however, it is vague with respect to the specific natural products used. Here we report the results of chemical investigations of a substantial collection of samples of tissues, wrappings and 'resinous/bituminous' materials from provenanced and dated Egyptian mummies. We focused on examples of the 'classic' mummy-making culture of the Pharaonic or dynastic period, from which we can begin to track the development of mummification chronologically.

Studies of the organic materials used in mummification were carried out earlier in the last century (for example, see refs 11–13), but the analytical techniques available limited their ability to identify aged, complex organic mixtures (that is, the 'resins', spices, and so on). To gain a proper understanding of the mummification process and its development, it is vital to study securely provenanced and dated mummies using modern investigative chemical techniques. Only four such studies (which includes one performed in our own laboratory) have been carried out in recent

Table 1 Provenance and date of mummies, origin of balm samples and their chemical composition

Mummy¶	Date/age	Provenance	Sample location and description#	Inferred components of embalming 'resin'☆	Relative abundance (%)**
Male adult* ('Khnumnakht') 21471	1985–1795 yr BC (XII dynasty)	Rifeh	Resin/tissue/bandaging	Fat/oil[e] Proteinaceous material	90% 10%
Female adult† 1909.527	1650–1550 yr BC (XVII dynasty)	Qurna, Thebes	Resin/tissue from head of right tibia	Fat/oil[c] Coniferous pitch Balsam (?)	99% 1% Trace
Child (sex ?)† 1909.527	1650–1550 yr BC (XVII dynasty)	Qurna, Thebes	Unspecified bone and cartilage	Fat/oil[b] Coniferous pitch Balsam (?)	82% 1% 16%
Head (sex ?)‡ 1976.159.267	1550–1069 yr BC (XVIII–XX dynasties)	Thebes	Skin/resin from back of cranium	Fat/oil[g] A sugar/gum Coniferous resin Balsam (?)	95% 0.3% 2% 2%
Male adult§ ('Horemkenesi') Ha 7386	1069–945 yr BC (XXI dynasty)	Deir el Bahri, Thebes	Resinous material from left side of top of spine	Fat/oil[l]	100%
Female(?) adult‖ EA74303	1069–664 yr BC (XXI–XXV dynasties)	Thebes?	Resin from chest cavity	Fat/oil[k] Coniferous resin/pitch Balsam (?) Beeswax[n,s]	61% 6% 1.5% 31%
Female adult§ ('Neskhons') H 5062	945–715 yr BC (XXII dynasty)	Thebes	Resin-soaked outer wrapping from left side of neck	Plant oil[d] Coniferous resin Triterpenoid resin ? Balsam (?) Wax	82% 0.5% 4% 0.9% 11%
Male adult‡ ('Pedeamun') 1953.72	664–404 yr BC (XXVI–XXVII dynasties)	Thebes	Resin from top of cranium	Fat/oil[m] Coniferous resin Balsam (?) Beeswax[o,t]	61% 0.2% 1.5% 38%
Female adult† 1956.352	332–30 yr BC (Ptolemaic)	Thebes	Resin attached to linen thread on right ankle	Fat/oil[f] Pistacia resin Balsam (?) Beeswax[p,u]	6% 6% 0.4% 87%
Male adult (1)† 1911.2101	30 yr BC to AD 395 (Roman)	Hawara	Resin-soaked outer wrapping below right scapula	Fat/oil[i] Coniferous resin Balsam (?) Beeswax[q,v]	78% 16% Trace 6%
Male adult (2)† 1911.2102	30 yr BC to AD 395 (Roman)	Hawara	Resin from side/base of left foot	Fat/oil[a] Coniferous resin Balsam (?) Beeswax[r,w]	35% 37% 0.2% 27%
Male child (wrapped)† 1956.357b	30 yr BC to AD 395 (Roman)	Thebes	Resin on outer wrapping from area between calves	Fat/oil[h] Coniferous resin	90% 9%
Male child (unwrapped)† 1956.357c	30 yr BC to AD 395 (Roman)	Thebes	Resin from abdominal cavity (kidney area)	Fat/oil[j] Coniferous resin Balsam (?)	87% 13% 0.1%

Several samples were taken from all but one mummy (unwrapped male child). For example, 15 different samples were analysed from various parts of the body of Horemkenesi.
* Manchester Museum; † National Museum of Scotland; ‡ Liverpool Museum; § Bristol Museum; ‖ British Museum.
¶ Museum number.
# The term 'resin' denotes physical appearance and does not presuppose any chemical composition or biological origin.
☆ Superscript letters (a–w) refer to the histograms shown in Fig. 1.
** Percentage relative abundance based on absolute concentrations, calculated on the basis of internal standards added at the extraction stage. Compositions do not imply that they were the original formulations of the embalmers, owing to possible chemical changes over time.

times[14–17].

This study involves the first systematic chemical investigation of a collection of provenanced Egyptian mummies dating from the mid-dynastic period (*c.* 1,900 yr BC) to the late Roman period (AD 395), which encompasses the period at which the mummification process was at its peak (1,350–1,000 yr BC). We have used diagnostic marker compounds that are present in the original 'balms', are resistant to degradation and can be related to specific embalming agents. Gas chromatography with mass spectrometry (GC-MS) and thermal desorption (TD)- or pyrolysis (Py)-GC-MS facilitate the molecular separation and identification of the marker compounds.

Because of the probable nature, possible processing and burial history of embalming materials, both free and polymerized components are likely to be present. Therefore, this 'dual' approach allows the characterization and identification of both the free (solvent-extractable) marker compounds and the recognizable subunits of polymeric materials not amenable to the more conventional GC-MS approach[17]. Given the understandable sensitivity surrounding the study of mummies, the amount of sample required is an important consideration. Sequential TD-GC-MS and Py-GC-MS require very small sample sizes (< 0.1 mg), facilitating the virtually non-destructive analysis of valuable museum specimens.

A summary of the results of this study is given in Table 1. The main products seen in all mummies are degraded acyl lipids, which are derived from plant oils and animal fats (Fig. 1a–m). Where samples were taken directly from the bodies, these may well derive from endogenous body lipids, such as triacylglycerols from fats or cell-membrane phospholipids. But even after accounting for decay, for the most part these display non-human fatty-acid distributions. In cases where degraded fats or oils are, for example, seen in the bandaging and are not directly in contact with the body, they must derive from their intentional application as part of the embalming process.

In most cases, the fatty-acid distributions (Fig. 1) are indicative of plant origins; that is, they have a high abundance of $C_{16:0}$ compared with their abundance of $C_{18:0}$. But it is not possible to assign a precise plant origin on the basis of fatty acids alone, owing to degradation of the major unsaturated components; such degradation is evident from the presence of mono- and dihydroxycarboxylic acids, which are indicative of autoxidation. The presence of plant oils (and to a lesser extent animal fats) suggests that they were key ingredients in mummification, and were probably used as a less-costly base with which to mix and apply more exotic embalming agents to the bodies and/or wrappings.

Their widespread use indicates that the embalmers were aware of the special properties of unsaturated oils and fats that allow them to 'dry', or rather, to polymerize spontaneously[18] (later also appreciated by the oil painters of western Europe). This polymerization would have produced a highly crosslinked aliphatic network, which would have stabilized otherwise fragile tissues and/or textile

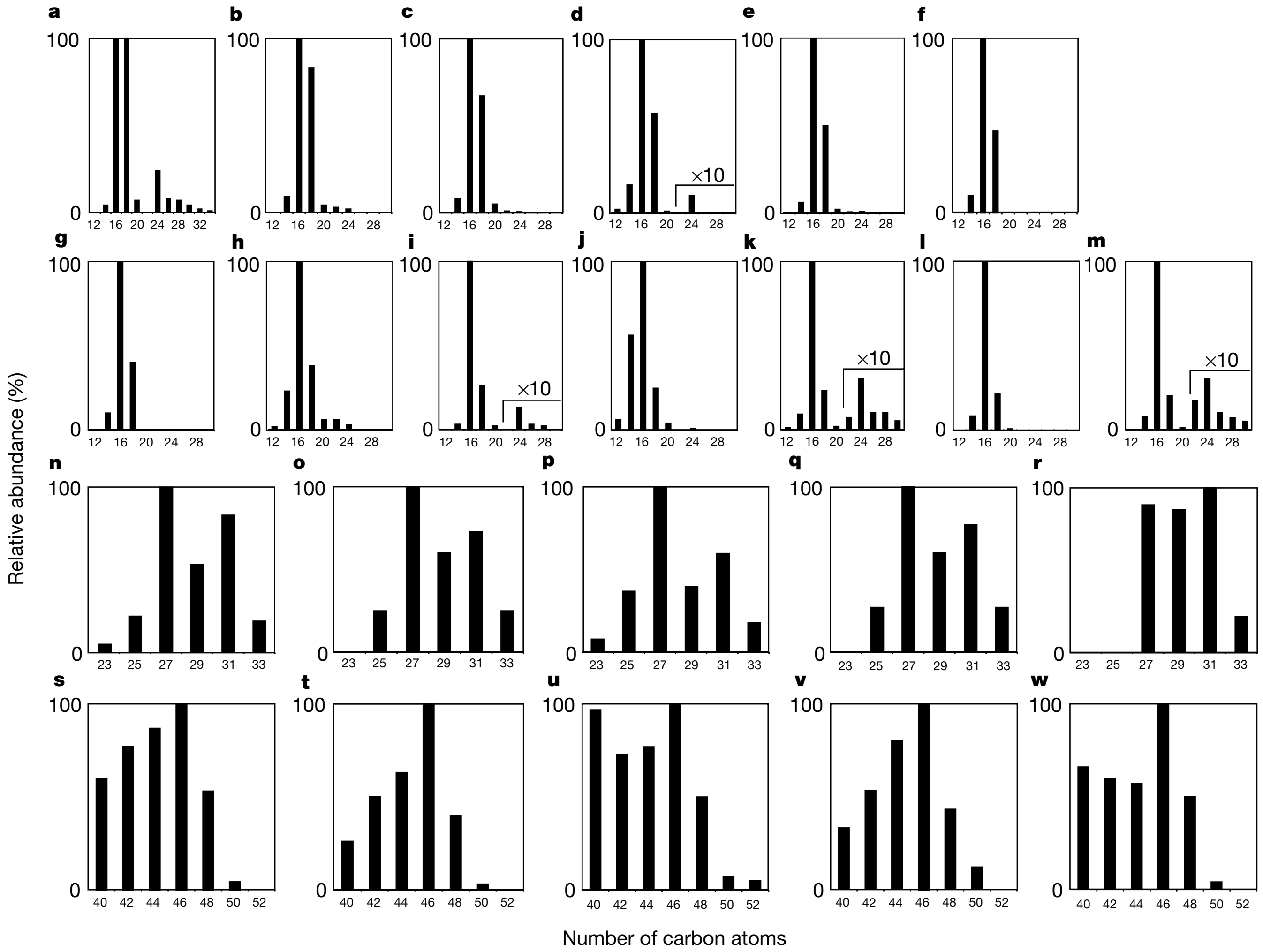

Figure 1 Chemical analyses of samples taken from the mummies in this study. Histograms show the distribution of fatty acids of equal carbon number in acid fractions (**a**–**m**; see Table 1), hydrocarbons of odd carbon number in neutral fractions from samples containing beeswax (**n**–**r**), wax esters of even carbon number in neutral fractions from samples containing beeswax (**s**–**w**).

wrappings against degradation by producing a physico-chemical barrier that impedes the activities of microorganisms.

The results from the wide date range of the mummies investigated reveal notable trends in the evolution of the use of other commodities (Fig. 2). For example, beeswax and coniferous resin clearly increase in their prominence through time, and are found in material taken both directly from the bodies and from the wrappings. Coniferous resin is identified by the presence of both functionalized and defunctionalized diterpenoid components. For example, 7-oxodehydroabietic acid and 15-hydroxy-7-oxodehydroabietic acid were usually the dominant diterpenoid components, and the normally abundant dehydroabietic acid was virtually absent.

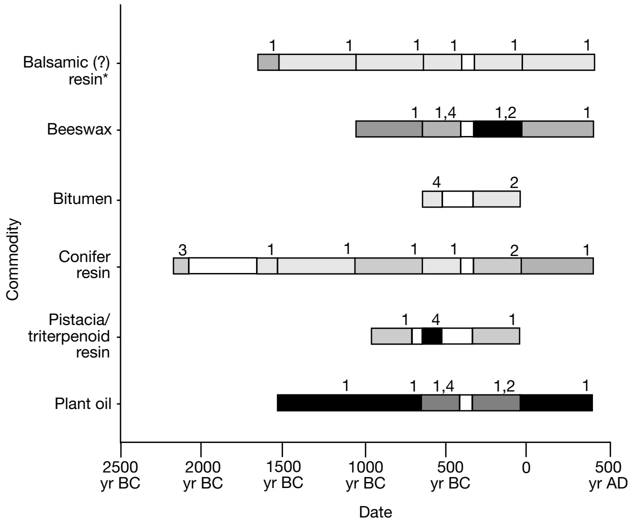

Figure 2 The use of organic commodities (preservatives/unguents) identified in chemical investigations of the 'balms' of provenanced and dated ancient Egyptian mummies. Grey scale reflects the relative abundance of a particular commodity found in the balm samples, with darker shades representing a higher relative abundance. Asterisk, tentative identification of balsamic resins; 1, data from this study; 2, data from ref. 14; 3, data from ref. 16; 4, data from ref. 15.

Although coniferous resins were clearly used in the embalming process at least as early as 2,200 yr BC (VI dynasty)[16], their use becomes most apparent in later periods; both the tissues and the wrappings of mummies from the Roman period (30 yr BC to AD 395) contain appreciable quantities (up to 37%) of coniferous diterpenoids. The increasing use of coniferous resin suggests that the embalmers may have become aware of the ability of specific natural products to inhibit microbial degradation by means of mechanisms (physico-chemical barriers and antimicrobial action) analogous to their protective roles in the plants from which they derived.

A significant trend is also seen in the use of beeswax, which is characterized chemically by alkanes (C_{25}–C_{33}) (Fig. 1n–r), wax esters (C_{40}–C_{50}) (Fig. 1s–w) and hydroxy wax esters (C_{42}–C_{54}). It first appears notably later than coniferous resin, with its positive identification in a resinous coating taken from the chest cavity of a female mummy of the Third Intermediate Period (XXI to XXV dynasty; 1,069–664 yr BC). In a sample taken from 'Pedeamun', a XXVI dynasty (664–525 yr BC) mummy, the solvent-soluble extracts comprise 38% beeswax (Fig. 3). But an even greater amount was found in a very extensive black 'resinous' layer from a female mummy of Ptolemaic date (332–30 yr BC) (Fig. 4), in which beeswax made up 87% of the solvent extractable components; in other words, this treatment was essentially beeswax mixed with a small amount of *Pistacia* resin (see also below). The choice of beeswax was clearly motivated by its hydrophobic/sealant and antibacterial properties, notwithstanding its symbolic significance[19].

Other components of the embalming 'resin' are triterpenoids and hydroxyaromatic acids, which are diagnostic of plant products. The

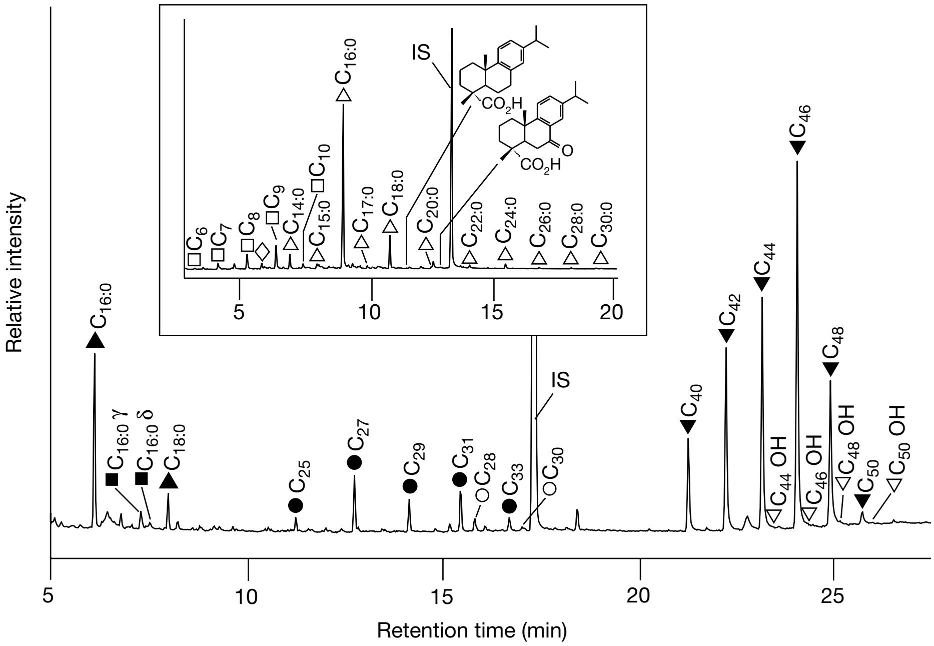

Figure 3 Reconstructed total-ion chromatogram of the trimethylsilylated neutral fraction (after solvent extraction and fractionation) of 'resin' from the top of the cranium of the Late period male adult 'Pedeamun', XXVI to XXVII dynasty (664–404 yr BC). Peak identities (*x* indicates carbon chain length): filled triangles, $C_{x:0}$ indicates saturated fatty acid methyl esters; filled squares, $C_{x:0}$ indicates saturated lactones (carbon chain length *x* is followed by the position of the lactone ring); filled circles, C_x indicates alkanes; open circles, C_x indicates alkanols; filled inverted triangles, C_x indicates wax esters; open inverted triangles, C_x OH indicates hydroxy wax esters; IS, internal standard (*n*-tetratriacontane). Inset displays a reconstructed total-ion chromatogram of the trimethylsilylated acid fraction (after solvent extraction and fractionation) of this sample. Peak identities: open triangles, $C_{x:0}$ indicates saturated fatty acids; open squares, C_x indicates α,ω-dicarboxylic acids; open diamond, vanillic acid. Also shown are the structures of two diterpenoid acids identified: dehydroabietic acid and 7-oxodehydroabietic acid. IS, internal standard (*n*-heneicosanoic acid).

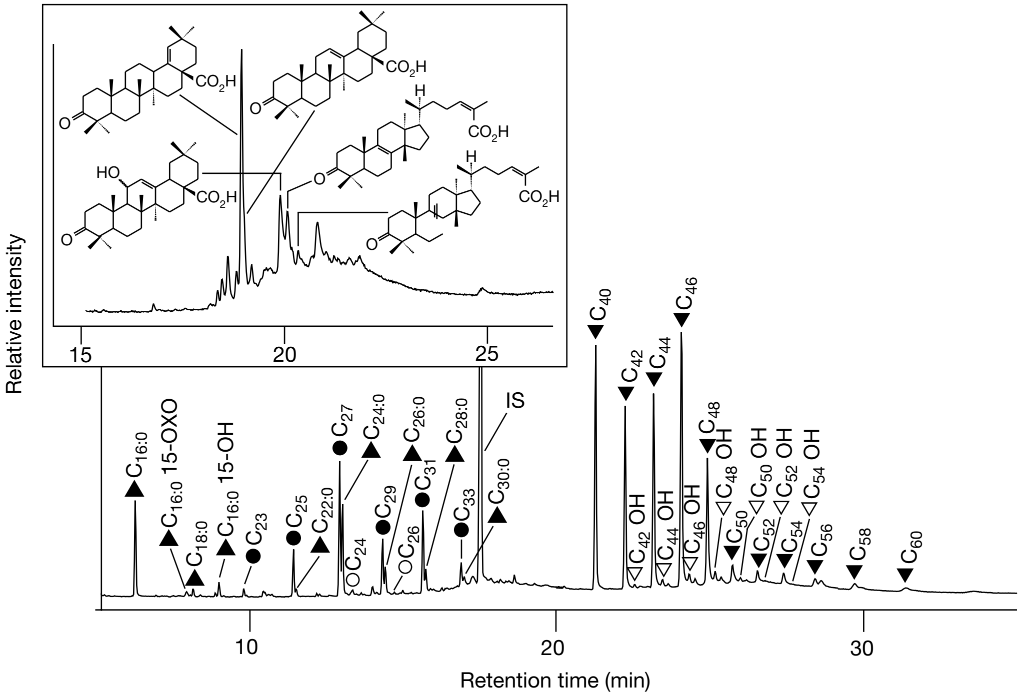

Figure 4 Reconstructed total-ion chromatogram of the trimethylsilylated neutral fraction (after solvent extraction and fractionation) of 'resin' attached to linen thread on the right ankle of a female adult from the Ptolemaic period (332–30 yr BC). Peak identities (x indicates carbon chain length): filled triangles, $C_{x:0}$ indicates saturated fatty acid methyl esters; filled circles, C_x indicates alkanes; open circles, C_x indicates alkanols; filled inverted triangles, C_x indicates wax esters; open inverted triangles, C_x OH indicates hydroxy wax esters; IS, internal standard (n-tetratriacontane). Inset displays a reconstructed total-ion chromatogram of the trimethylsilylated acid fraction (after solvent extraction and fractionation) of this sample, showing the structures of the *Pistacia* triterpenoids identified: moronic acid, oleanonic acid, hydroxyoleanonic acid, isomasticadienonic acid and masticadienonic acid.

former include the isomasticadienonic, masticadienonic, moronic and oleanonic acids that are diagnostic of the presence of *Pistacia* resin[18] and are found in the Ptolemaic female mummy (Fig. 4). The *Pistacia* content is significant, constituting 6% of the extractable lipids. Triterpenoid acids, including a prevalence of dammaranes that can occur in highly degraded *Pistacia* resins[20], were present in significant quantities (4%) in the wrappings of the XXII dynasty (945–715 yr BC) mummy 'Neskhons'. However, the absence of moronic acid (which is used often to characterize *Pistacia*) leaves its precise botanical origin uncertain.

It is less easy to assign an origin to the hydroxy aromatic acids, but these are clearly plant derived. Although sawdust was often used to pack the cavities of mummies, the derivation of these acids from lignin is unlikely as there were no detectable lignin building-block compounds (methyl, ethyl, n-propyl and vinyl guaiacols) in any of the Py-GC-MS analyses. Present in ten of the mummies, their distribution is generally dominated by 4-hydroxy-3-methoxybenzoic acid with smaller amounts of p-hydroxybenzoic acid, which is consistent with oxidation of either unsaturated C-3 side chains (either carboxyl or hydroxyl substituted) in the aromatic benzoate ester of balsamic resins or ferulic acid—a principal component of *Umbelliferae*[21].

The absence of the precursor compounds is not unexpected, given the susceptibility of these to the oxidation that is prevalent in mummies. Whereas low abundances of the components may originate from dissolved organic matter in the Nile water used in washing the bodies, higher concentrations, such as 16% in the XVII dynasty child mummy, would be consistent with a plant-resin treatment. The choice of these latter natural products by the ancient embalmers again points to their recognition of the antibacterial properties[22] of these substances and hence their vital role in the mummification process.

Our results show that a mixture of commodities, with a compositional diversity greater than reported previously, was used. The use of drying oils was clearly widespread, with coniferous resins and beeswax increasing in importance with time. Notably, despite the frequent and rather indiscriminate use of the term 'bitumen' in connection with mummification, the idea that its general use in embalming has now been proved "unequivocally"[23] cannot be supported. In repeated searches, the steranes and hopanes characteristic of petroleum bitumens[14,24] were not detected, which leads us to the conclusion that natural bitumens were not used in these mummies.

Furthermore, given that beeswax generally constitutes the greater proportion of the organic materials rather than the resins, the term embalming 'wax' has at least equal validity to so-called 'resin'. In this respect it is especially interesting to note that the Coptic (directly derived from the ancient Egyptian language) word for wax is in fact 'mum'[25]. These results clearly show that, despite statements to the contrary[10,23], we do not know all there is to know about Egyptian mummification, and caution needs to be exercised when making assumptions about the materials that the ancient Egyptian embalmers might have used. ☐

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Local interactions predict large-scale pattern in empirically derived cellular automata

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An important unanswered question in ecology is whether processes such as species interactions that occur at a local scale can generate large-scale patterns seen in nature[1,2]. Because of the complexity of natural ecosystems, developing an adequate theoretical framework to scale up local processes has been challenging. Models of complex systems can produce a wide array of outcomes; therefore, model parameter values must be constrained by empirical information to usefully narrow the range of predicted behaviour. Under some conditions, spatially explicit models of locally interacting objects (for example, cells, sand grains, car drivers, or organisms), variously termed cellular automata[3,4] or interacting particle models[5], can self-organize to develop complex spatial and temporal patterning at larger scales in the absence of any externally imposed pattern[1,6–8]. When these models are based on transition probabilities of moving between ecological states at a local level, relatively complex versions of these models can be linked readily to empirical information on ecosystem dynamics. Here, I show that an empirically derived cellular automaton model of a rocky intertidal mussel bed based on local interactions correctly predicts large-scale spatial patterns observed in nature.

Mussel beds characterize many temperate, rocky intertidal shores throughout the world[9], and can exhibit complex patterning. This patterning is thought to result from the interplay between interactions among sessile species and external disturbance agents, particularly large waves[10–15]. Along the Pacific coast of North America, California mussels (*Mytilus californianus*) seem to interact locally with other organisms in two ways. First, mussels invade bare rock or displace species adjacent to them by overtopping their neighbours as they grow, and by creeping into adjacent areas by means of sequential attachment of new byssal threads in uncolonized areas as old byssal threads in colonized areas break or become detached[12]. Second, because mussels attach to each other with byssal threads, when waves dislodge a mussel from the rock that mussel pulls its neighbours along with it, thereby transmitting the disturbance through the mussel bed in much the same way as forest fires propagate when sparks from a burning tree ignite neighbouring trees[12]. Wave disturbance also acts differentially on mussel beds of different ages, with beds of old, multi-layered mussels being more prone to disturbance, owing to the high ratio of mussel mass per unit attachment area to the rocks, and to the high fraction of byssal threads attached to other mussels, rather than the rock[12,14,15]. Other sessile species and their mobile associates colonize the bare rock created by disturbance events, and exhibit a complex set of interactions among themselves and with mussels[10,12,13,16–19]. Because of its rapid dynamics and macroscopic organisms of modest size and mobility, the rocky intertidal zone is an unusually tractable model system in ecology for experimental investigations and for readily observing spatial and temporal dynamics in a complex natural ecosystem[19]. These features make mussel beds particularly suitable for the purpose of developing and testing different versions of models with empirical parameters, such as cellular automata.

Data on the dynamics of mussel beds were collected over a six-year period (May 1993 to May 1999) at 1,400 fixed points in the mussel bed of Tatoosh Island in Washington State[20]. The data were taken from 14 quadrats (60 × 60 cm) positioned with fixed corner

Figure 1 Photograph of spatial patterning in the mussel bed of Tatoosh Island, Washington.