Changing sex at the same relative body size

Similar forces may select for gender switching across taxa in all animals with this facility.

Sex change occurs in a variety of animals, including fish, echinoderms, crustaceans, molluscs and polychaete worms[1]. Here we show that the relative timing of sex change is surprisingly invariant across all animals: 91–97% of the variation in size at sex change across species can be explained by the simple rule that individuals change sex when they reach 72% of their maximum size. This suggests that there is a fundamental similarity across all animals, from a 2-mm-long crustacean to a 1.5-m-long fish (Fig. 1), in the underlying forces that select for sex change.

Evolutionary theory suggests that sex change is favoured when the reproductive success (fitness) of an individual varies with its age or size, and the relationship differs between the sexes[1–4]. In this case, natural selection favours individuals who begin life as the sex whose fitness increases more slowly with age, and then change to the other sex when they are older. Although this idea is well established, the theory is hard to test quantitatively because the underlying trade-offs, such as the relationship between age and fitness, are difficult to measure.

Even without such data, quantitative predictions can be made by using a dimensionless approach[5]. Specifically, the evolutionarily stable age of sex change depends upon several dimensionless properties: k/M, $\alpha \times M$ and δ, where k is relative growth rate (Bertalanffy coefficient), M is adult mortality rate, α is age at first breeding, and δ is a coefficient relating male fertility to size (male fertility correlates with L^{δ}, where L is size). Populations with the same values of these dimensionless quantities are predicted to have the same relative size at sex change[5], defined as average size at sex change (L_{50}) divided by maximum size (L_{max}).

Several studies have indicated that k/M and $\alpha \times M$ may be invariant within and even across taxa[2,6]. Consequently, the same relative size at sex change is predicted whenever δ is also invariant, which might be expected within species or between closely related species. Consistent with this, an invariant relative size at sex change has been found across populations of a shrimp[5] and across fish species[7].

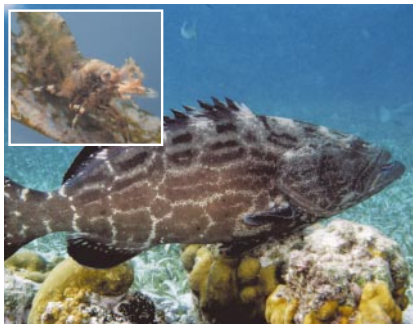

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Figure 1 Little and large: the black grouper (1.5 m) and the shrimp *Thor manningi* (2 mm, inset) switch sex at the same relative size.

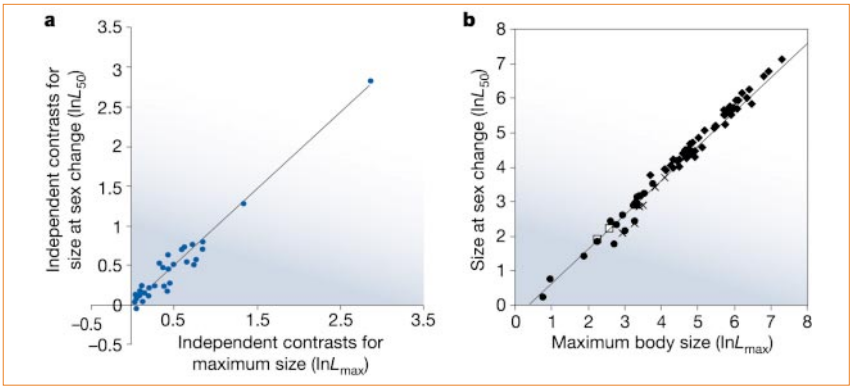

Figure 2 Animals change sex at the same relative body size. **a,** Log–log plot of independent contrasts for L_{50} against L_{max}, where these are the average size and the maximal body size, respectively, at sex change. The slope is fixed at unity and is driven through the origin, as required for the analysis of independent contrasts[9] ($r^2 = 0.908$, $n = 38$ independent contrasts). The ordinary least-squares (OLS) slope is 0.97 ± 0.05 (95% CI; $r^2 = 0.955$). **b,** Log–log plot of L_{50} against L_{max} for a wide range of sex-changing species, each shown as an independent data point. Data are split by taxa: asterisks, Echinodermata; circles, Crustacea; diamonds, Chordata; crosses, Mollusca. The regression has a slope fixed at unity, giving an intercept of -0.32 ± 0.05 (95% CI; $r^2 = 0.97$, $n = 77$ species). The OLS slope is 1.05 (95% CI) 0.03, with an intercept of –0.55 (95% CI) 0.07 (r^2=0.98). The mean relative size at sex change (L_{50}/L_{max}) is 0.72 (95% CI: 0.67–0.77), indicating that individuals change sex when they reach 72% of their maximum size. Size (L_{50} and L_{max}) was measured in millimetres before logarithmic transformation.

We investigated the degree of invariance in the relative size at sex change across all sex-changing animals (Fig. 2), using data from 77 species of fish, echinoderms, crustaceans and molluscs. If the relative size at sex change is invariant, then a plot of $\log(L_{50})$ against $\log(L_{max})$ would give a slope of 1.0. We first analysed our data using the method of independent contrasts[8,9], based on a composite phylogeny of sex-changing animals. This analysis gave a slope of 0.97 ± 0.05 (95% confidence interval, CI), which is not significantly different from 1.0 ($t_{37} = 1.2$, $P > 0.1$; Fig. 2a). The amount of variance in size at sex change explained by this regression was 95.5%, and remained high (90.8%) when we forced the regression to have a slope of 1.0.

We then analysed our data using species as independent data points. This analysis gave a slope of 1.05 ± 0.03 (95% CI), which is significantly greater than 1.0 ($t_{75} = 3.3$, $P < 0.01$; Fig. 2b). However, this difference reflects the extremely low residual/error variance and is of negligible biological importance, as shown by the fact that forcing the regression to have a slope of 1.0 causes little change in the amount of variation in sex-change size explained by the regression (it dropped by only 1.8%, to 96.7%). Consequently, 91–97% of the variation in mean size at sex change across species can be explained by the simple rule that individuals change sex when they reach 72% (95% CI: 67–77%) of their maximum size.

The amount of variation that can be explained across species in the mean size at sex change is not increased by taking into account life-history variables or taxonomic differences. We tested whether possibly important life-history variables[1–4,7] and taxonomic groupings could significantly improve the relationship between $\log(L_{50})$ and $\log(L_{max})$.

In possibly important life-history variables[1–4,7], there was no significant effect on the direction of sex change (intercept: $F_{1,73} = 1.4$, $P > 0.1$; slope: $F_{1,73} = 0.3$, $P > 0.1$; $n = 77$ species) or for the presence of individuals who mature early as the second sex (termed 'early maturers'; intercept: $F_{1,64} = 0.02$; $P > 0.1$, slope: $F_{1,64} = 0.02$, $P > 0.1$; $n = 68$ species in which the presence or absence of early maturers has been identified). Considering taxonomy, there was no significant difference between the groupings of Chordata, Crustacea, Echinodermata and Mollusca (intercept: $F_{3,69} = 2.7$, $P > 0.05$; slope: $F_{3,69} = 1.9$, $P > 0.1$).

Our results indicate that the relative size of sex change is invariant across all animal species. They suggest that there is a fundamental similarity across animals in the trade-offs and fitness functions associated with sex change. This is remarkable, given the huge variation across species in life-history details such as mating system, sex-change mechanism and size[1,3,7]. They also suggest a relative invariance in parameters such as $\alpha \times M$ (refs 2, 6), which determine the evolutionarily stable size at sex change[2].

The problem of when to change sex is formally equivalent to many other problems in life-history theory, such as when individuals

adjust the sex of their offspring in response to environmental conditions[1,2,4,10], suggesting that the dimensionless approach could be applied widely. More generally, our results demonstrate the usefulness of the dimensionless approach, in that it can allow quantitative tests of evolutionary theory in cases where the difficulty of measuring the underlying trade-offs has constrained previous tests to merely qualitative status.

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Ancient materials

Analysis of a pharaonic embalming tar

Details of mummification techniques used in dynastic Egypt have emerged from writings in subsequent ancient texts, in which the application of oils (*kedros*, *cedrium*) derived from the cedar tree have been described by Herodotus (490–425 BC)[1] and by Pliny the Elder (AD 23/24–79)[2]. But scholars have since argued that these products were prepared from juniper trees and not from cedar[3] — an assertion that is widely accepted by Egyptologists[4] but which has never been verified by chemical analysis. Here we use gas chromatography to analyse the constituents of a sample of unused entombed embalming material from 1500 BC at a site in Deir el-Bahari, Egypt, and find that its components probably originated from the cedar tree. We also identify one component, guaiacol, as having notable preservative properties.

In ancient Egypt, the deceased were mummified in the hope that this would ensure their eternal survival. The process included removal of the internal organs, followed by desiccation and embalming of the carcass[5,6]; occasionally cosmetics were applied[7] as a wealth of different compounds. Active enzymes have recently been isolated from embalmed bones from pharaonic Egypt[8].

We prepared a methanolic extract from unused embalming material (Fig. 1) found near the mummy '*Saankh-kare*', from the eighteenth dynasty, 1500 BC, at Deir el-Bahari. Gas chromatography of the extract showed no evidence of diterpenoid or triterpenoid resin components, but phenolic compounds (such as cresols, xylenols, guaiacols (2-methoxyphenols)), naphthalenes and azulenes were present (see supplementary information).

The phenolic and naphthalene derivatives probably originated from smouldering wood, with the methoxyphenols arising from lignin-degradation products that resulted from the pyrolysis of soft coniferous wood[9]. Dimethoxyphenols (syringols), on the other hand, are additionally formed by heating the hard wood of deciduous trees[10]. The presence of guaiacols without syringols in the embalming material strongly supports an origin from coniferous wood.

The brown embalming resin also contains sesquiterpenoid components, which are normally found in a fluid known as 'cedar oil'. This oil is prepared by extraction with organic solvents of wood from *Cedrus atlantica* and also includes junipene, cadalene, cadinatriene (calamene), cuparene and α-curcumene. Given the prevalence in the resin of guaiacols from coniferous wood-tar oils, our findings indicate that the embalming material was prepared from cedar trees.

The liquid probably originated from the water-containing fraction that is exuded before the tar from dry-distilled cedar wood. In his *Naturalis Historia*, Pliny the Elder[2] describes the technology: "*The wood of the tree is chopped up and put into ovens and heated by means of a fire packed all round outside. The first liquid that exudes flows like water down a pipe; in Syria this is called 'cedar-juice'* [in Latin: *cedrium*], *and it is so strong that in Egypt it is used for embalming the bodies of the dead.*" The application of a liquid cedar product is also described by Herodotus[1].

There is an unfortunate tradition of confusion between cedar and juniper trees, which has led to the erroneous assignment of Pliny's *cedrium*. In today's terminology as well as in ancient times, some juniper trees that are not cedar are still called cedar — for example, the American red cedar (*Juniperus virginiana)* and the Mediterranean 'little cedar' (*Juniperus oxycedrus*). In this context, our comparative investigations show that the oils or tars from juniper trees contain high amounts of cedrol or cedrene, respectively, neither of which was found in the *Saankh-kare* embalming material.

The surfaces of mummies embalmed with liquid tars or other liquid resinous materials are essentially free of contaminating microorganisms. The sealing effect of the embalming agents on the bone surface preserves the enzyme alkaline phosphatase inside for thousands of years[8]. We therefore tested the contribution of phenolic derivatives, which in the embalming agent originate from a smouldering process, and monoterpenes, which occur naturally in resinous balm, to this biochemical conservation process.

Guaiacol, *p*-cymene, limonene and α-pinene were tested for their effect on preserving alkaline phosphatase activity in a model embalming process, in which porcine bones were coated with one of these compounds for 35 days at room temperature. We found that guaiacol was the most effective preservative as the enzyme's specific activity was 12 times higher than that in an untreated bone control and exceeds the activity recovered from bones treated with monoterpenes (cymene has no effect; α-pinene and limonene seem to promote enzyme degradation) or with disinfectants such as phenols (see www.pci.chemie.uni-tuebingen.de/weser/supp_info.htm).

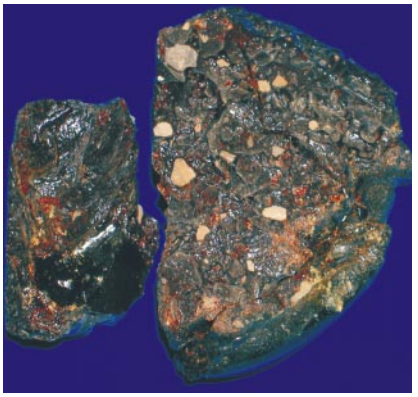

Figure 1 Unused embalming material ('*cedrium*') entombed with the mummy *Saankh-kare* (from 1500 BC and cemetery field 26225 at Deir el-Bahari in Egypt), at present located at the Egyptian Art Department of the Metropolitan Museum of Art, New York. The dark-coloured parts are organic resin; the pink and lighter-coloured inclusions orginate from mineral-based impurities.

We conclude that liquid tars in general are most efficient in the mummification process. The methyl- and ethylguaiacol identified in the *cedrium* of Deir el-Bahari are characteristic representatives of wood creosotes (from the Greek '*kreas*', or flesh, and '*soter*', preserver). Creosotes are nowadays used for rapid smoke-drying of meat. Thus, these outstanding conserving properties confirm the statements of Herodotus and Pliny that a "strong" liquid was used for embalming in ancient Egypt.

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