Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

AQUATIC OXYGEN

## OXYGEN SUPPLY INDEX: UNIFYING PHYSIOLOGICAL AND ECOLOGICAL APPROACHES

Compared with air, water contains about 33 times less oxygen and it diffuses ~300,000 times slower. Also well established is that oxygen availability in aquatic ecosystems varies with temperature and atmospheric pressure. As a consequence, it is an important ecological factor with wide-ranging influences, from animal body size to species distribution and diversity. However, expressing oxygen availability with a consistent metric has been problematic. Ecologists prefer to use oxygen availability in the context of solubility or concentration, whereas physiologists use partial pressure ($P_{O_2}$) as a metric. These two indicators are, however, not readily interchangeable and cause confusion. For instance, oxygen concentration correlates well with latitudinal body size in amphipods. Conversely, across a range of altitudes there are no clear size relationships, as one would expect given similar temperature changes across latitudes and altitudes. However, decreases in $P_{O_2}$ at increasing altitude do explain decreased invertebrate species richness. But this could also explain the absence of an altitudinal size relationship because reduced $P_{O_2}$ also means a reduction in the oxygen available to dissolve in water, even though lower temperatures allow for greater solubility. It is clear that when one metric is used as an indicator of ecological patterns without accounting for how it is affected by the other the potential for conflicting assessments in eco-physiology increases.

To address this issue, Wilco Verberk and co-workers from the School of Marine Science and Engineering in Plymouth, UK, set out to resolve this issue by returning to first principles. Using Fick's law of diffusion they derived an oxygen index that incorporates both concentration and $P_{O_2}$. They also included a third key factor – oxygen diffusivity ($D_{O_2}$). This gave them the oxygen supply index (OSI) in $\mathrm{mol\,m^{-1}\,s^{-1}}$ and incorporates solubility, $P_{O_2}$ and diffusivity as follows: OSI $\propto \alpha O_2 \times P_{O_2} \times D_{O_2}$. The OSI shows that oxygen exchange between organism and environment is driven by an interplay between solubility, pressure and diffusivity and can fully account for the prior inconsistencies observed when using these metrics in isolation. This also allows for temperature, which not only affect factors such as solubility but also determines the oxygen demands of ectothermic organisms. This enables the calculation of oxygen supply relative to demand, or relative OSI.

To test this new index, Verberk and colleagues re-analyzed previous data sets from prior published studies that demonstrated ecological patterns related to oxygen availability. In all cases, for body size and species richness indicators, OSI consistently was a better predictor. Their new index also accounted for effects of temperature and oxygen demand, and pointed to a counterintuitive assessment of latitudinal oxygen availability. Based on original assessments of temperature-dependent oxygen solubilities it was always assumed that polar waters had greater oxygen availability. The OSI shows that equatorial water actually has greater oxygen availability. Here, the lower solubility is off-set by increased diffusivity. But increased demand, due to warmer temperatures, results in a limit in oxygen exchange that affects maximum body size. Conversely, higher polar solubilities are off-set by reduced diffusivity, but the drastically reduced oxygen demand of animals does allow for increased maximal body size.

10.1242/jeb.063560

**Verberk, W. C. E. P., Bilton, D. T., Calosi, P. and Spicer, J. I.** (2011). Oxygen supply in aquatic ectotherms: partial pressure and solubility together explain biodiversity and size patterns. *Ecology* **92**, 1565-1572.

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MATING COSTS/BENEFITS

## MATING STRATEGIES FOR A CHANGING WORLD

When it comes to mating, it seems that you're damned if you do, and damned if you don't. Mating can transmit diseases, and mating partners might even injure or kill one another. Yet, for most animals, mating is necessary for reproduction, and mating with multiple partners can increase the number or quality of offspring. In this complicated world, what is the best strategy for maximizing the benefits while minimizing the costs of mating?

As it turns out, the situation is even more complicated for female red flour beetles (*Tribolium castaneum*): the costs and benefits of different mating strategies are dependent on environmental conditions. Red flour beetles have been maintained in the laboratory at 30°C for over 30 years, which amounts to over 350 beetle generations, so these beetles are well adapted to life at 30°C. However, life at 34°C is significantly more stressful for the beetles, so how do the relative costs and benefits of different mating strategies change at a stressful temperature? Vera Grazer and Oliver Martin, from the Swiss Federal Institute of Technology Zurich, decided to address this question by measuring the survival and reproductive success of female red flour beetles at different temperatures.

The researchers placed individual female beetles into small enclosures alone (virgin females that did not invest in reproduction), with a single male beetle (monogamous females that invested in reproduction with a single mate), or with multiple male beetles (polyandrous females that invested in reproduction with multiple mates) at both 30 and 34°C. After 1 week, the researchers removed the males from the enclosures, and monitored the females for an additional 9 weeks to assess their long-term survival, and to count the number of larvae that hatched for each female. Using this method, Grazer and Martin were able to quantify both the survival and reproductive success of females at both temperatures.

The duo found that at the standard 30°C temperature, the virgin red flour beetles had the highest survival, with intermediate survival in the monogamous beetles, and the lowest survival in the polyandrous beetles. In other words, at the red flour beetle's adapted temperature, reproduction became increasingly costly for the females as the number of mates rose. The team also found that at 30°C there was no difference in the number of larvae produced by monogamous and polyandrous beetles. Therefore, at the standard temperature, it is clear that mating with multiple males carries a survival cost and does not confer any reproductive benefits for females.

However, at 34°C, the survival differences disappeared. All of the 'hot' females survived for a reduced length of time relative to the beetles held at 30°C, and all of the 'hot' females survived for the same length of time, regardless of whether they were virgins, monogamous or polyandrous. What was even more exciting was that at 34°C the polyandrous beetles produced more larvae than monogamous beetles. Therefore, mating with multiple males does not carry an additional survival cost at higher temperatures, and results in increased reproductive success for female red flour beetles.

This experiment elegantly demonstrates that both the costs and the benefits of polyandry are dependent on environmental conditions for female red flour beetles. As climate change and anthropogenic activity increases, wild populations are faced with rapid environmental change and environmental conditions that are quite different from those that populations are adapted to. The results of Grazer and Martin's experiment therefore have wide-reaching implications for wild populations across the globe. In this changing world, mating strategies will need to adapt.

10.1242/jeb.063818

**Grazer, V. M. and Martin, O. Y.** (2011). Elevated temperature changes female costs and benefits of reproduction. *Evol. Ecol.* doi: 10.1007/s10682-011-9508-4.

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STATISTICS

## NEUROSCIENTISTS STUMBLE ON STATS

To make sense of variability in their data, biologists use statistical tests to determine whether experimental groups are significantly different from one another. The ability to determine statistical significance is a cornerstone of all biological research, and yet biologists of all ages often make fundamental errors in the ways they perform statistical comparisons. One of the most common mistakes is to conclude that two effects differ from one another when one group is significantly different from a control, but the other group is not. Sander Nieuwenhuis, Birte Forstmann and Eric-Jan Wagenmakers at Leiden University recently set out to quantify how often this mistake is made in neuroscience articles published in the world's leading scientific journals. They published their work in a recent edition of *Nature Neuroscience.*

Nieuwenhuis and colleagues first explain in detail the mistake itself. It tends to happen when neuroscientists want to claim that one effect is bigger or smaller than another effect compared with control data. To do this, they simply report that one effect is statistically significantly different from controls (i.e. there is a 95% probability that the effect has not arisen by chance, $P<0.05$), while another is not ($P>0.05$). On the surface, this sounds reasonable, but it is flawed because it doesn't say anything about how different the two effects are from one another. To do this, researchers need to separately test for a significant interaction between the two results in question. Nieuwenhuis and his co-workers sum up the solution concisely: '…researchers need to report the statistical significance of their difference rather than the difference between their significance levels.'

The team had an impression that this type of error was widespread in the neuroscience community. To test this idea, they went hunting for 'difference of significance' errors in neuroscience articles published in five very prestigious journals (*Nature*, *Science*, *Nature Neuroscience*, *Neuron*, *Journal of Neuroscience*). In total, they ended up evaluating the statistical tests used in over 500 neuroscience papers. They found that 31% of behavioural, systems, and cognitive studies contain situations where authors could potentially make an error. In half of these cases, authors made the mistake of not reporting the significance of differences within their data. The team then went on to look at cellular and molecular neuroscience articles published in *Nature Neuroscience* in 2009 and 2010. Incredibly, out of 120 articles sampled, not a single publication used correct procedures to compare effect sizes. At least 25 papers erroneously compared significance levels either implicitly or explicitly.

The work of Nieuwenhuis, Forstmann and Wagenmakers is a sobering self-evaluation. It shows clearly that a large number of neuroscientists at the very highest levels make basic errors in the way they statistically analyse data. To be fair, the group points out that many of the mistakes they found probably don't invalidate the main conclusions of the publications they examined. But this should not be seen as a reason for neuroscientists to be lax about 'stats'. Clearly, the community has a responsibility to make sure that all the conclusions they put in print are backed up by valid statistics. After all, small mistakes eventually add up.

10.1242/jeb.063826

**Nieuwenhuis, S., Forstmann, B. U. and Wagenmakers, E. J.** (2011). Erroneous analyses of interactions in neuroscience: a problem of significance. *Nat. Neurosci.* **14**, 1105-1107.

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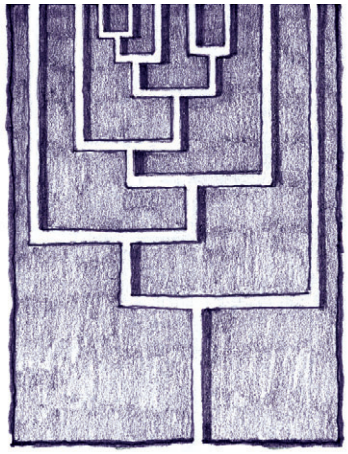
POPULATION ADAPTATION



## SEX AS A WEAPON AGAINST PARASITES

Sex is a deeply puzzling thing for evolutionary biologists. The problem is that biparental sex requires and produces males, who do not partake in the costs of childbearing, but do munch at the limited food supply of reproductive females. Indeed, if males were out of the picture, a population of purely asexual females would increase in number at twice the rate of its male-burdened counterparts. Why then do most animals and plants go to such a bother? Why suffer males?

One of the oldest answers to this question is that sex accelerates population adaptation in the face of change. Building on this idea, the Red Queen hypothesis proposes that sex allows plants and animals to stay one step ahead in their endless arms race against co-evolving parasites. New research by Levi Morran and colleagues at the University of Indiana in the USA provides the strongest experimental support yet for this idea, showing that parasites can tip the sexual balance, making males worth their weight.

Using experimental evolution, Morran and colleagues pitted the nematode worm *C. elegans* against its lethal natural parasite, the bacterium *Serratia marcescens*. Most *C. elegans* are hermaphrodites that reproduce by self-fertilization; however, about 20% of the time hermaphrodite worms reproduce by mating with rare males. When the researchers forced wild-type worms to co-evolve with their parasites for 30 worm generations the rate of biparental breeding in the population increased to nearly 90%. By contrast, when parasites infected mutant worms that were unable to mate with males, they drove the worms extinct within 10 generations. Thus sex, and lots of it, kept worms alive, while its absence led to their doom.

Now what about the parasites? Because bacteria and worms can be frozen and later reanimated, the team revived the ancestral parasite and host and compared them with their evolved descendants. Infecting the ancestral worms with the evolved parasites, Morran and his colleagues saw dramatic results; the evolved bacteria killed worms 2- to 3-fold more effectively than their ancestors. However, when the evolved bacteria infected co-evolved worms, they were no more effective at killing them than the ancestral parasites were at killing the original worms. That is, while parasites evolved to become nastier, the worms simultaneously evolved to resist them, both staying essentially where they were to begin with. This outcome is exactly what the Red Queen hypothesis predicts; both parasite and host have to keep running to stay in exactly the same place.

Lethal parasites exert very strong natural selection on their hosts, because hosts must either resist infection or die. This study shows that sex, or more specifically the presence of males, helps hosts run at a slightly faster speed than their co-evolving parasites. This advantage just about makes the cost of males bearable.

10.1242/jeb.063537

**Morran, L. T., Schmidt, O. G., Gelarden, I. A., Parrish, R. C., II and Lively, C. M.** (2011). Running with the Red Queen: host–parasite coevolution selects for biparental sex. *Science* **333**, 216-218.

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## CORRECTION: SEA SLUG SWIMMING SURPRISE

In the Outside JEB article by Maarten Zwart (doi: 10.1242/jeb.050021), the first author's name in the reference was incorrectly cited as Sakurai, S. The author's name is Sakurai, A., and the reference should read:

**Sakurai, A., Newcomb, J. M., Lillvis, J. L. and Katz, P. S.** (2011). Different roles for homologous interneurons in species exhibiting similar rhythmic behaviors. *Curr. Biol.* **21**, 1036-1043.