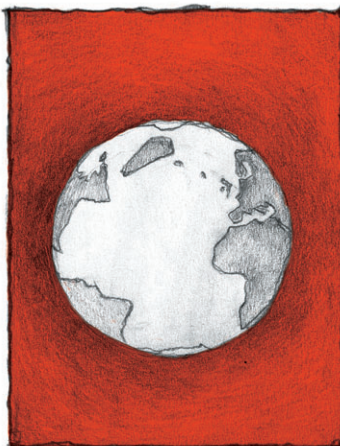


Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

ASEXUAL SELECTION



ROTIFERS PLAY 'DRY AND SEEK' TO EVADE PARASITES

Sex is a real drag. It costs a huge amount of energy, reduces the efficiency of gene transfer and spreads disease. Theoretically, it would be a lot more efficient just to reproduce asexually, but less than 1% of animal species make do without sex. The 'Red Queen' ('It takes all the running you can do, just to stay in the same place!') *Lewis Carroll, Through the Looking Glass*) hypothesis posits that organisms must constantly evolve just to maintain their fitness relative to other co-evolving systems. In this hypothesis, the main reason for the evolutionary success of sex is that it helps create genetic variability. This helps animals quickly develop adaptations against relentlessly co-evolving parasites and pathogens. Bdelloid rotifers (a class of small freshwater invertebrates) are the big exception to this rule. They have reproduced asexually for tens of millions of years and diversified into over 450 species. Bdelloid rotifers seem to be impervious to the Red Queen effect as asexuals and manage to stay ahead in the evolutionary arms race, but how do they cope with co-evolved enemies? Chris Wilson and Paul Sherman at Cornell University recently tested the idea that rotifers ditch co-adapted parasites simply by drying up and dispersing on the wind, and published their results in a recent edition of *Science*.

Wilson and Sherman started by measuring how long a population of Bdelloid rotifer clones (*Habrotrocha elusa*) can survive when infected by a fungal parasite (*Rotiferophthora angustispora*). When infected, the rotifers were wiped out after 13 days in water. Next, the team infected hydrated rotifer populations with fungi, dried the cultures out for several weeks and looked at how the rotifer and fungal populations recovered when re-hydrated. The fungi were able to recover and destroy rotifer populations when dried out for up to 3 weeks. However, after dry spells longer

than 3 weeks, the rotifers recovered but the fungi were almost non-existent. These results suggest that rotifers can evade parasites by simply outlasting them in dry conditions.

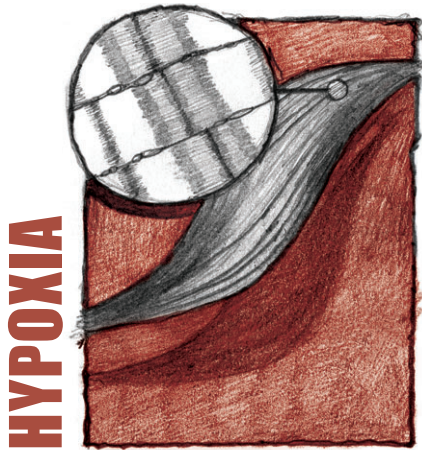
Knowing that rotifers are small enough to be carried as spores (tuns) by wind when dried out, the researchers wanted to find out whether rotifers could use this ability to evade fungal parasites in space. To test this, they grew cultures of rotifers and fungi, dried them out for a week and then put the dried out cultures in a wind tunnel. Collecting the rotifer tuns downwind, the team then rehydrated them. Over half of the rotifer populations tested were uninfected and started new fungi-free populations. When cultures containing rotifers and fungi were dried out for a week and rehydrated, the fungi quickly regained the upper hand in all cultures, so wind dispersal clearly increased the chances of rotifers shedding their fungal freeriders.

Wilson and Sherman provide strong evidence that spatiotemporal escape can help protect asexual animals from co-evolved enemies. The experiments are exceedingly simple, but very elegant; they will almost certainly evoke a 'Why didn't I think of doing that?' response from a large host of biologists. Most importantly, the work of Wilson and Sherman cuts right through the heart of a long-standing knot in evolutionary biology. The authors have discovered one way asexual rotifers can outrun the Red Queen.

10.1242/jeb.036608

Wilson, G. W. and Sherman, P. W. (2010). Anciently asexual bdelloid rotifers escape lethal fungal parasites by drying up and blowing away. *Science* **327**, 574-576.

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HYPOXIA SIGNALS FOR MORE MUSCLE CELLS

Adult phenotypes are the result of both genetic and environmental factors acting to program the form and function of embryonic tissues during development. Skeletal muscle development is known to be plastic for many vertebrate species and is affected by a variety of environmental factors such as temperature and hypoxia. The development of skeletal muscle tissue can be broadly divided into two phases, progenitor cell (myoblast) proliferation which increases the number of developing muscle cells and terminal differentiation which is marked by the expression of a definitive muscle cell phenotype. Both of these events are regulated by insulin-like growth factor (IGF) signaling through the same cellular receptor. Despite using the same receptor, cell proliferation and differentiation are mutually exclusive events in the development of a myoblast. Ren and colleagues at the University of Michigan set out to identify how the same signaling receptor can regulate these two processes and how environmental hypoxia might alter the outcome of IGF signaling in developing muscle cells.

Knowing that oxygen deprivation has a dramatic effect on muscle development, the team tested the effect of reduced oxygen on myoblast (muscle precursor) development. They exposed mouse myoblast cells to normoxia (20% O₂) and hypoxia (1% O₂) for up to 96 h in the presence or absence of IGF-II and measured cell number and the extent of cellular differentiation in the cultures. As hypoxia-inducible factor 1 α (HIF-1 α) regulates cellular responses to hypoxia, they also tested the possible effect of HIF-1 α activity in mediating the action of IGF-II under hypoxia by blocking HIF-1 α expression under normoxic and hypoxic conditions. Finally, the team assessed the role of three signaling cascades (Akt-mTOR, p38 and Erk1/2 MAPK), which are known to be associated with the IGF

receptor, using pharmacological inhibitors to block their activity under normoxia and hypoxia, and in response to IGF-II activation.

The team discovered that exposure to IGF-II caused the myoblasts to undergo differentiation (myogenesis – the expression of a skeletal muscle-specific cellular phenotype) under normoxic conditions, but blocked differentiation and promoted cell division under hypoxic conditions. This effect was mediated by HIF-1 α , because blockage of its activity caused myogenesis under normoxia and hypoxia. Pharmacological studies suggest that myogenesis is blocked by IGF signaling under hypoxia by suppression of the Akt-mTOR pathway. In addition, IGF signaling favored cell division under hypoxia by altering the activity of Erk1/2 and p38 MAPK signaling activities. Thus, this study illustrates that the same extracellular signal can initiate different intracellular signaling events depending on oxygen levels.

This study is one of the first to detail the mechanisms behind the effect of hypoxia on muscle cell development. The finding that hypoxia can alter IGF signaling through a differential interaction with HIF-1 α and specific downstream signaling cascades offers a powerful model for testing the physiological and ecological impacts of altered muscle development in the face of hypoxia. Exposure to hypoxia may inhibit muscle differentiation and favor myoblast proliferation during development. If hypoxic stress is encountered during critical periods of muscle development, this could have profound effects on the adult muscle phenotype and thus perhaps physiological performance and maybe even fitness.

10.1242/jeb.036590

Ren, H., Accili, D. and Duan, C. (2010). Hypoxia converts the myogenic action of insulin-like growth factors into mitogenic action by differentially regulating multiple signaling pathways. *Proc. Natl. Acad. Sci. USA* **107**, 5857-5862.

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BLUE FLASHERS: ATTRACTING FEMALES AND DETERRING PREDATORS

Similar modes of communication can be applied to confer very different messages based on specific ecological contexts. And depending on the message – either ‘stay away’ or ‘come hither’ – the effectiveness of the communication signals can be crucial for survival and/or reproduction and are, therefore, subject to selection and evolution. The hindwings of the pipevine swallowtail butterfly, *Battus philenor*, have brilliant blue iridescent patches on both the dorsal (upper) and ventral (lower) surfaces. Both sexes display the larger ventral patches early and late in the day when the butterflies are at rest, and evidence suggests these patches function as predator deterring aposematic (warning colouration) signals, advertising the distastefulness of these butterflies. The dorsal patches are smaller and are far more intense in the males, suggesting that they are sexual signals, flashed by males at females in flight around midday. Because of the different ecological contexts of these iridescent communication signals one would also expect differences in the evolutionary processes that established these iridescent wing patches.

Ron Rutowski and colleagues, from Arizona State University analysed the reflectance spectra of wild and lab-reared butterflies’ iridescent patches to test the differences in signals directed towards predators or potential mates. They made five predictions. (1) Ventral iridescence has greater intensity than dorsal iridescence because of lower light levels when warning off predators. (2) Ventral iridescence has longer wavelength hues because of more reddish light illuminating these patches in the early morning and late afternoon. (3) Ventral iridescence has greater angles of visibility than dorsal iridescence since the approach directions of predators are more variable than the in-flight directions when females view courting males. (4) Ventral

iridescent colours in males are less variable than dorsal iridescence as the ventral patches are selected by predation and the dorsal patches by sexual selection.

(5) Ventral iridescence (an anti-predator signal) is less dependent on rearing conditions than dorsal iridescence (a sexual signal).

Rutowski and colleagues tested their predictions using reflectance spectrophotometry and found that their data supported predictions 1 and 3. The ventral iridescence in both sexes, directed towards predators during low-light hours, was almost twice as intense and could be viewed over a wider range of angles than the dorsal iridescence, which is specifically directed toward females when the light is brighter.

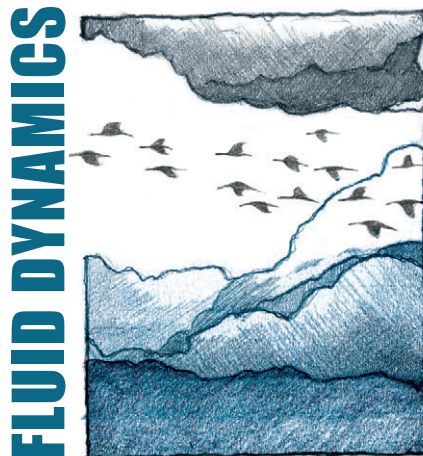
By contrast, predictions 2 and 4 were not supported. The ventral and dorsal hues did not differ, and the dorsal iridescence in males was not more variable than the ventral iridescence – contrary to the expectation that the sexual signals may vary to allow females to choose the best mate. In fact, the ventral and dorsal colour parameters were closely correlated, possibly because the developmental processes driving iridescent wing-scale production are coupled in both patches, constraining colour differentiation and variability.

Finally, evidence supporting the fifth prediction was weak. Compared with wild caught specimens, lab rearing generally improved the animals' condition (larger body masses and longer wings); however, the lab conditions were possibly too constant to effect differences in lab animals' colour condition. Surprisingly, though, the dorsal patches in wild caught males were more intense than in the lab reared animals. Food quality could explain this but the authors warn that these results emphasise the need to broaden the scope of investigation of iridescent signals. For example, the dorsal patches may not be sexual signals signifying male quality but could aid in species recognition, as *Battus philenor* shares its habitat with several other species that mimic its iridescent colour patterns.

10.1242/jeb.036582

Rutowski, R., Nahm, A. C. and Macedonia, J. M. (2010). Iridescent hindwing patches in the Pipevine Swallowtail: differences in dorsal and ventral surfaces relate to signal function and context. *Funct. Ecol.* doi: 10.1111/j.1365-2435.2010.01693.

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WHAT A DRAG

The power requirements of flight at the size and speeds of birds have long been of interest to biologists, and are increasingly studied by engineers considering small Unmanned Air Vehicles. Techniques for calculating power are generally based on those that have proved successful for the much larger, faster machines that we regularly fly in. However, fluids (including air) behave quite differently at different sizes and speeds, so a new range of experimental validation, and perhaps also theoretical formulation, may be required for flight on smaller scales. Geoff Spedding and John McArthur from the University of Southern California report measurements on wings under conditions appropriate for bird flight, and highlight some troubling departures from the assumptions inherent in conventional drag – and so power – analyses.

Three components contribute to conventional drag calculations. The first form of drag is associated with pushing air downwards to provide lift upwards and is known as 'induced' drag. Even without air viscosity ('stickiness'), this continuous acceleration of surrounding air requires power, and results in drag that is proportional to the square of the lift force. Well-designed wings do this efficiently, accelerating air as little as possible. However, this is never perfect, and the additional drag is accounted for in power calculations by a fudge factor, expressed either as a 'span efficiency' (<1) or its inverse, the 'induced-power correction factor' (>1) which, in the animal flight literature, is usually taken as close to 1.2.

Second, the duo begin to include the effects of viscosity. Even at zero lift, there is a small amount of drag. The exact value of this drag is often difficult to measure, even

under extremely controlled windtunnel conditions; it can be a thousand times smaller than the other forces to be measured. In the bird flight literature, this drag, in coefficient form, tends to take the value 0.02.

The difficulty really starts with a third drag relationship. This drag is again due to viscosity but, confusingly, tends to rise in proportion to the square of lift (just like the 'inviscid' induced drag component). It is therefore often convenient to include this effect as an altered fudge factor in the induced drag component, as an adjustment to the 'span efficiency' (or its inverse). But this mixing up of aerodynamic mechanisms for mathematical convenience can cause considerable confusion.

Spedding and McArthur highlight the issues using their detailed measurements on a wing – the Eppler 387. They demonstrate that, at the sizes and speeds appropriate for much of animal flight, the relationship between drag and the square of lift is really very poor and highly dependent on speed. This is not just 'stall' – with increasing angle of attack, lift can suddenly increase at the same time as drag decreases. The derived induced power factor (including the viscous adjustment) comes out at 3.7 – more than triple that usually adopted in bird flight calculations.

The authors used a smooth Eppler 387 in part because it is known for its weird characteristics at low speeds; it may very well be that some aspect of animal wings returns aerodynamic performance to lower drag and easier theoretical tractability. But whether this is the case, and which aspect this turns out to be, awaits further experimentation. So the animal flight community looks destined to continue with the '1.2ish, 0.02ish' rule of thumb, but be continually haunted by the possibility that the aerodynamic power calculations might not just be wrong, but very – and systematically – wrong.

10.1242/jeb.036574

Spedding, G. R. and McArthur, J. (2010). Span efficiencies of wings at low Reynolds numbers. *J. Aircr.* 47, 120-128.

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