NEWS & VIEWS

SEX DETERMINATION

Some like it hot (and some don't)

David Crews and James J. Bull

There is a widely accepted theoretical explanation for why sex in some species is determined at the embryo stage by environmental factors such as temperature. That theory is now supported by experiment.

How the sex of offspring is determined seems simple enough if you don't look beyond ourselves. For humans, the system is genotypic: two X chromosomes, and you're female; an X and a Y, and you're male. There are plenty of variants of this system, but in many reptiles an entirely different mechanism applies: sex is determined by the temperature of the incubating egg, and clutches can be all-male, all-female or somewhere in between.

On page 566 of this issue[1], Warner and Shine answer a long-standing question about the evolutionary significance of sex-determining mechanisms in reptiles. Four decades ago, it was reported[2] that incubation temperature determined sex in the African red-headed rock lizard, an observation that seemed to fly in the face of evidence for sex chromosomes in several other reptiles[3]. Scientists soon realized that both types of sex-determining system were not only widespread in reptiles but also highly developed, and at the time they seemed to be mutually exclusive[4]. What has remained a puzzle is whether there is an adaptive benefit of temperature-dependent sex determination (TSD) and how that benefit might work. Using an Australian lizard, Warner and Shine find the long-sought evidence of an adaptive benefit of TSD.

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Figure 1 | The jacky dragon, *Amphibolurus muricatus*. This is a species of reptile in which the sex of the offspring is determined by the egg-incubation temperature, and was the experimental model chosen by Warner and Shine[1]. The jacky dragon's comparatively short lifespan of some 3–4 years makes it especially suitable for sex-determination research.

The main model suggested to explain the advantage of TSD, or of any sex determination in response to an environmental cue, takes an idea from Trivers and Willard[5]. This posits that in some circumstances a species will have greater reproductive fitness if the offspring are male instead of female, whereas in other circumstances the reverse is true. For example, if there is a premium on large size for male reproduction, an offspring deprived of food such that it will be born small and remain smaller than average throughout its life may have higher fitness as a female than as a male. From there, the argument for why sex should be environmentally determined is merely that, if there is a strong benefit to controlling offspring sex ratio to suit the circumstances, natural selection will favour a mechanism to do so[6].

In reptiles, sex is determined during the embryonic stage. For environmental sex determination to fit this model, something happening to the egg or embryo must carry over into adult fitness, and the effect must be one that works differently for males than females, so mere survival to hatching does not provide an explanation. The puzzle is that, because so much growth happens between hatching and maturity in a reptile, it would seem that all effects of embryonic temperature would be erased by adulthood.

There has been no shortage of ideas for how this model[6] could apply to reptiles, from supposing any of several direct effects of incubation temperature on egg-to-adult fitness, to allowing mothers to manipulate offspring sex ratio by choice of nest site[7]. However, until now all explanations have relied on inferences about fitness effects, not measurements.

By integrating several techniques for lab and field studies, and with a careful choice of study organism, Warner and Shine[1] show that incubation temperature affects lifetime fitness and does so differently for males and females. Their study organism was a short-lived lizard, the jacky dragon (Fig. 1). The use of a short-lived species is important because the differential effects of incubation temperature are expected to be strong in short-lived species, and not necessarily so in long-lived ones.

The next trick required a way of producing both sexes across a wide range of incubation temperatures. The theory holds that only the sex of relatively higher fitness should be produced at any one temperature, and indeed, TSD is often so extreme that only one sex is produced across a wide range of incubation temperatures. Thus a test of the theory requires producing both sexes at temperatures where one sex is normally absent. Male jacky dragons are produced in only a narrow, intermediate temperature range. So to produce males at high and low temperatures, Warner and Shine used the now-common method of applying chemicals to the egg that interfere

with steroid hormone biosynthesis, in this instance the aromatase inhibitor fadrozole[8].

Eggs were incubated in the lab at one of three temperatures (low, intermediate, warm), and the hatchlings were released into outdoor enclosures (about 30 lizards in each of 6 enclosures). The lizards were allowed to grow up, mate and produce offspring in these enclosures over a period of 3.5 years. To measure fitness — reproductive success — Warner and Shine established parentage of each of the offspring by genotyping DNA microsatellites. All offspring born in the enclosures were unambiguously assigned to specific parents, thus bypassing any indirect measures of presumed mating success and fecundity.

Lifetime reproductive success showed some surprises. For females, it was expected, first, that warmer incubation temperatures would lead to larger body size (because warmer temperatures lead to earlier hatching); and, second, that body size would correlate strongly with fecundity. Thus female fecundity should increase with incubation temperature. This compound expectation was only partly supported: female lifetime fitness was highest at the warmest temperature, but no appreciable fitness difference was found between the intermediate and low temperatures. For males, there was no obvious basis for prediction, but males from intermediate temperatures had appreciably higher fitness than males from low and warm extremes. In all, the fitness measures matched the theory, but most of the fitness effects of temperature defied intuition.

The study[1] provides directions for future work. The most important concerns the mechanistic bases by which incubation temperature affects male versus female fitness. There is accumulating evidence that incubation temperature in TSD lizards has a variety of behavioural, anatomical and physiological effects, including directly acting on brain development[9,10]. In addition, even though offspring are either male or female in terms of their gonads, hormone levels throughout life vary according to the individual's incubation temperature, further contributing to a gradation of attributes that translate into fitness differences within a sex caused by incubation temperature. To the extent that such interactions exist, TSD may have evolved to be somewhat self-reinforcing, in essence providing the basis for much of its own benefit. It will thus be interesting to solve the mechanistic link between temperature and fitness, to augment the observations that Warner and Shine have at last provided to resolve the riddle of reptilian sex determination.

There is also a wider picture to this line of research. It has been suggested that sex determination by temperature or other environmental factors is ancestral to genotypic sex determination, and that elements of TSD can be found in mammals[11]. Even in humans, conditions during gestation have lasting effects throughout life, with recent work indicating a connection with coronary disease, obesity, diabetes, cancer, cognitive dysfunction and infertility[12]. ■

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NANOMATERIALS

Golden handshake

John C. Crocker

Three-dimensional nanoparticle arrays are likely to be the foundation of future optical and electronic materials. A promising way to assemble them is through the transient pairings of complementary DNA strands.

One of the staple concepts of nanotechnology is that of 'growing' useful materials or devices by coaxing a random mixture of microscopic parts to assemble spontaneously into a desired structure. Versatile self-assembly schemes have been demonstrated that use DNA as the primary building material[1]. In this issue, two research teams, one led by Oleg Gang (Nykypanchuk *et al.*, page 549)[2] and the other by Chad Mirkin (Park *et al.*, page 553)[3], recount how they have built on the successes with DNA to aid the self-assembly of gold nanoparticles. Their technique should also work for other varieties of technologically exciting nanoparticles.

Progress in achieving the directed self-assembly of nanoparticles had been elusive, owing to one potentially daunting requirement: selective adhesion. Each microscopic part must be engineered so that it sticks only to the others it should abut in the desired final structure. In earlier experiments[4], nanoparticles were found to form ordered arrangements when a surrounding solvent was evaporated. In this case, however, the final structures depended sensitively on the particle chemistry and charge.

This is where DNA comes into its own. Particles carrying complementary strands of DNA selectively adhere to each other when the strands 'hybridize' to form the familiar DNA double helix. The final architecture is thus determined not by chemistry or charge, but by the lengths and nucleotide sequences of the DNA strands. That promises a versatile assembly scheme that might be used with particles of nearly any material to fabricate nanocomposites or 'metamaterials'[5] with unusual electronic and optical properties. The applications of such materials might include high-efficiency solar panels and lasers, super-resolution microscopes — and even coatings to render objects invisible.

Nykypanchuk *et al.*[2] and Park *et al.*[3] both start by grafting DNA to gold spheres of the order of 10 nanometres in diameter to give two populations of DNA-capped particles, A and B. Each sphere bears several dozen strands, and the ends of the strands on A-type and B-type particles are complementary. This configuration means that spheres of one type will selectively adhere to spheres of the other, but neither type of sphere will adhere to its own kind.

The authors mixed the A and B spheres in water. Under the right conditions, they found that the nanospheres were rapidly guided, as the DNA strands hybridized, to arrange themselves into well-ordered arrays. The resulting crystal had body-centred-cubic crystal symmetry, with A and B spheres taking up alternating locations in the lattice, so that each A sphere was surrounded by eight B spheres and vice versa (Fig. 1). Such a structure — known as a CsCl lattice after crystals of caesium chloride, which take the exact same form — provides the maximum possible number of A–B adhesion contacts.

Both Nykypanchuk *et al.*[2] and Park *et al.*[3] report that crystallization requires the DNA-binding regions to be connected to the gold spheres by flexible spacers, also made of DNA, that are roughly as long as the sphere diameter. Moreover, crystallization happens only at higher temperatures, at which the DNA binding strands are dynamic, continuously forming double helices and dissociating back into single strands.

The DNA in these experiments is being used in a fundamentally different way from its use in earlier DNA self-assembly techniques such as Ned Seeman's 'DNA tile' approach[1]. There, each constituent tile of the structure was made of interconnected DNA double strands. Each tile had one binding strand dangling from each corner, so that it could mate with neighbouring tiles. The structure of each tile was thus controlled at the molecular scale. The chemical process for attaching DNA strands to nanoparticles[2,3], by contrast, is essentially random,