

Colour-enhancing protein in blue petals

Spectacular morning glory blooms rely on a behind-the-scenes proton exchanger.

The coloration of blue flowers depends on the production of the appropriate anthocyanin pigments, the presence of metal ions and co-pigments, and the vacuolar pH¹. An increase in vacuolar pH enhances blue coloration^{1,2}, but little is known about the proteins responsible for raising the vacuolar pH. Here we show that a mutant purple-flowering Japanese morning glory (*Ipomoea nil*) plant that carries a recessive mutation in the gene encoding a vacuolar Na⁺/H⁺-exchanger is unable to increase its vacuolar pH to create the normal bright blue petals.

In the morning glory *Ipomoea tricolor*, reddish-purple buds unfurl into blue flowers. As the bloom opens, the vacuolar pH in the flower epidermis increases from 6.6 to 7.7 (ref. 2). The colour is very similar to that of *I. nil*, and the flowers of both plants contain the same anthocyanin as their principal pigment^{2,3}.

Ipomoea nil plants bearing the purple-mutable (*pr-m*; Fig. 1a) allele in the Purple (*Pr*) locus have purple flowers with blue sectors⁴ (Fig. 1a) owing to recurrent somatic mutation from the recessive *pr-m* to the blue Purple-revertant (*Pr-r*; Fig. 1b) allele. We have produced germinal revertants (heterogenotes, *Pr-r/pr-m*) that have blue flowers (Fig. 1b) and, after self-pollination, we chose pairs of siblings carrying either the *pr-m* or *Pr-r* allele homozygously. We found no difference in the pigment composition of the *pr-m* and *Pr-r* lines, but the pH of the sap from the *Pr-r* flower epidermis was higher than that in *pr-m* by about 0.7, indicating that the *pr* mutant fails to increase the vacuolar pH.

As *Tpn1*-related transposons are a common spontaneous mutagen in *I. nil*⁵, we used a transposon-display procedure⁶ to identify the *Pr* gene. Sequencing of *Pr* complementary DNA (accession no. AB033989) revealed that it contains an open reading frame that is highly homologous to the *Arabidopsis* and rice vacuolar Na⁺/H⁺-exchangers^{7,8} and that it complements the yeast *nhx1* mutation⁷ (our unpublished results), confirming that the *Pr* gene encodes a vacuolar Na⁺/H⁺-exchanger. We named this transporter InNhx1 (for *I. nil* Nhx1).

The *Pr* gene itself (accession no. AB033990) comprises 15 exons and a *Tpn1*-related element, *Tpn4*, which is integrated into its first exon in the *pr-m* lines (Fig. 1c). This integration of *Tpn4* generates a 3-base-pair target duplication, and the germinal revertants studied here contain footprints generated by *Tpn4* excision (Fig. 1d). Moreover, blue sectors of the variegated flowers

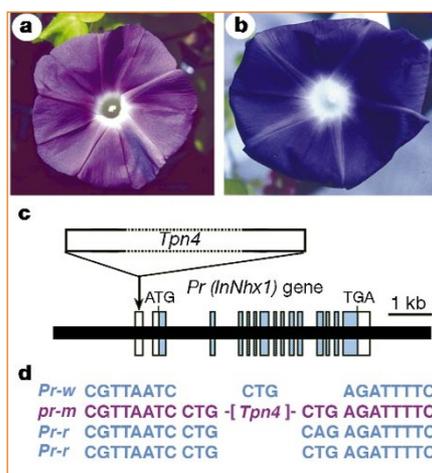


Figure 1 *Ipomoea nil* flower phenotypes and the structure of the *Pr* gene. **a**, Mutable line (*pr-m*). **b**, Germinal revertant (*Pr-r*). **c**, The *Pr* gene. White and blue boxes represent the *Pr* exons and open reading frame, respectively. **d**, Sequences at the *Tpn4* insertion site in the *Pr-r* (wild type), *pr-m* and *Pr-r* lines.

contain footprints that result from somatic excision of *Tpn4*, whereas we found no evidence of such excision in the purple regions of these flowers in six different *pr-m* lines.

Materials science

Ti₃SiC₂ has negligible thermopower

Apart from superconducting materials below their critical transition temperature, all other materials subjected to a temperature gradient will develop an electromotive force or (absolute) thermopower which is itself a function of temperature. We have discovered that the thermopower of Ti₃SiC₂ is essentially zero over an extended temperature range (from 300 to 850 K). This material should allow the thermopower of other substances to be determined directly at high temperatures, a measurement that has so far been impossible.

Characterization of single-phase, bulk dense samples of Ti₃SiC₂ has revealed that this ternary carbide possesses a unique combination of properties^{1–5}. It has high elastic moduli and easy machinability¹, good thermal and electrical conductivity^{2,3}, together with excellent oxidation properties⁴ and fatigue resistance⁵, as well as being tough and tolerant to damage⁵. We have now investigated another property of this remarkable material, namely its absolute thermopower, Θ , over the temperature range 300 to 850 K.

To our knowledge, the *Pr* gene encoding InNhx1 is the first gene to be identified that increases the vacuolar pH in petals in order to confer blue coloration.

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We measured the absolute thermopower and electrical resistivity, ρ , simultaneously on the same specimen of single-phase Ti₃SiC₂ in the 300–850 K temperature range, as described^{6,7}. The 4-probe d.c. resistivity of

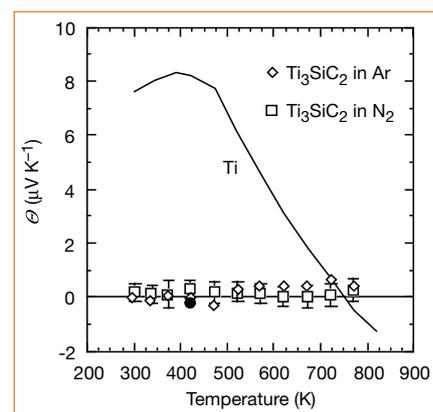


Figure 1 Temperature dependence of thermopower, Θ , of Ti₃SiC₂. Two different techniques were used to confirm the reproducibility of the data⁶: a heat pulse and steady state (solid circle at 423 K) in two different atmospheres (nitrogen or argon). Each datum point was measured at least ten times and the error bar represents one standard deviation, which was always less than 0.3 $\mu\text{V K}^{-1}$. The thermopower as measured was subsequently corrected against the absolute thermopower of the platinum leads¹² to obtain the absolute thermopower of Ti₃SiC₂. The curve representing Θ of polycrystalline Ti is from ref. 8.

Ti₃SiC₂ is metallic-like and can best be represented against temperature T (in degrees Kelvin) as $\rho = (0.224 \pm 0.002)[1 + (0.00371 \pm 0.0003)(T - 300)] \mu\Omega \text{ m}$, which is lower than that of titanium metal and in good agreement with previous results⁵.

The temperature dependence of Θ is shown in Fig. 1. The data are in good agreement with each other, irrespective of measurement technique or atmosphere, supporting their reproducibility and reliability. The absolute thermopower may best be estimated as $\Theta = 0.18 \pm 0.22 \mu\text{V K}^{-1}$.

This shows that Θ is practically zero within $\pm 0.22 \mu\text{V K}^{-1}$ in the temperature range examined. Based on this result, we would expect Θ to remain zero even outside the 300–850 K range. Included for comparison in Fig. 1 is Θ of titanium metal⁸, which is like most other solids in that its thermopower is a function of temperature.

The reason for this vanishingly small thermopower is not entirely clear. There is little doubt, however, that like Ti and some other transition metals^{9,10}, both electrons and holes are involved in the transport properties. It is only by having oppositely charged particles that Θ can vanish¹¹. Our results imply that the thermoelectric contributions by electrons and holes delicately cancel each other out over a wide temperature range. Hall coefficient measurements indicate that it also fluctuates between positive and negative values⁷; that is, it is also approximately zero. This, together with the present results, suggests that the concentration, mobility and heat of transport values of the electrons are identical to those of the holes over the temperature range investigated.

So far, all thermopower measurements — including ours — have had to be corrected for by adding the absolute thermopower of the lead wires. Such corrections can, if not carefully made, introduce errors. The benefit of using a material with a thermopower of zero over an extended temperature range to measure the thermopower of other substances is obvious. Ti₃SiC₂ might also find an application as leads for high-temperature thermocells.

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Biomechanics

Asymmetric toes aid underwater swimming

The unique morphology of the toes of the great crested grebe (*Podiceps cristatus*), which are asymmetrically lobed with a narrower skin flap on the lateral side of the toe, enables these birds to swim very efficiently. Here we study video recordings of a diving grebe and stroboscopic pictures of its moving feet and conclude that the bird uses a hydrodynamically lift-based (power) stroke to propel itself underwater, with the separated toes functioning as multiple slots to increase the lift-to-drag ratio. The asymmetric lobes are an adaptation for self-stabilization of the toes during the power stroke, and the toes themselves act as separate hydrofoils, each producing lift and each being twistable individually under hydrodynamic load.

Three-dimensional kinematics reveal that during the power stroke, the grebe's feet move through an arc upwards and medially, but not backwards relative to the still water, while the spread toes are twisted and the lateral, fourth, toe is leading the movement. A large lift force is then produced that is directed forwards — providing a large thrust component — and a smaller drag force is directed outwards and downwards. The propulsion is thus based primarily on a hydrodynamically lift-producing leg and foot stroke (our unpublished results), in contrast to the drag-based locomotion assumed previously^{1,2}.

Lift-based paddling is quicker and more energy-efficient than the drag-based propulsion used by diving birds such as

anseriforms. These swimming movements can also be compared to asymmetric hovering in flying animals³, as the feet may instantaneously accelerate a flow through the circular sweep made by the feet.

The function of the grebe's toes can be compared to that of flight feathers in several respects³. The separated toes most probably function as multiple slots, thereby increasing the lift-to-drag ratio during swimming and probably also the maximum lift⁴. This is dependent on the bird using a lift-based power stroke. Furthermore — as our kinematic analyses suggest (Fig. 1a) — the toes must be spread and staggered in height in relation to the oncoming water during the power stroke.

The asymmetric lobing of the grebe's toes seems to be an adaptation to self-stabilize the toes during the power stroke, like the primary-feather asymmetry of flying birds⁵. The asymmetry of the grebe's toes at one quarter of the distance from the tips is close to what is found in handwing feathers. In both, the axis of rotation (at the toe phalanges and feather shaft) lies at about 1/5–1/3 of the chord length from the leading edge (Fig. 1b), with the greatest asymmetry on the toe leading the movement during the power stroke (toe IV), as would be expected from the pressure distribution of multiple slots⁶.

The toes may act as separate hydrofoils, each producing lift and each twistable individually in the nose-down direction under hydrodynamic load. Separated feathers, acting as separate airfoils, can be twisted more than the entire, unseparated wing, with higher local angles of attack and lift coefficients^{3,7}.

In the grebe, the rotation of the foot produces a speed gradient along the toes, with increasing velocity from base to tip. Increasing asymmetry towards the tips of the toes may allow the grebe to twist the toes passively and keep favourable angles of attack along the entire length of the toes during the power stroke.

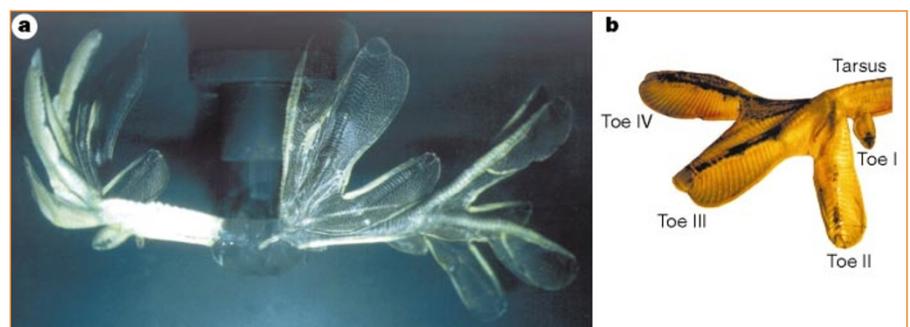


Figure 1 The great crested grebe's separated toes produce lift. **a**, Stroboscopic pictures of the grebe's right foot as it is moved mechanically in a still-water tank, simulating the power stroke. The foot is moved from right (first part of the power stroke) to left (last part of power stroke) in the figure, which shows five different phases. Note the staggering and twisting of the toes in the picture on the left. **b**, Dorsal view of the same foot as shown in **a**. The asymmetry of the toes is most pronounced on the fourth toe, which is leading the movement during the power stroke, and least on the second. The second and third toes in particular show increasing asymmetry towards their tips. Photographs by L.C.J.