

fixation which resulted in increased diurnal fluctuations in acidity. At other times, stomata remained closed throughout the day and the small diurnal changes in acidity observed in these conditions were assumed to correspond to nightly fixation of respiratory CO₂ only. In no conditions did stomata open in the light. This pattern of CO₂ uptake would involve only PEP carboxylase in the assimilation of atmospheric CO₂, and from this a stable δ¹³C ratio of the magnitude observed in C₄ species would be predicted. Such a ratio was observed in this experiment (Fig. 1). Statistical analysis of the results indicated that the δ¹³C ratios of *O. acanthocarpa* and *O. bigelovii* were correlated with neither the time of year nor the diurnal change in titratable acidity. The correlation between δ¹³C value and diurnal fluctuation in titratable acidity for *O. basilaris* was significant (P=0.05). But, the range of δ¹³C values observed for all species was within the limits of observation and C₄ species⁵. In *O. basilaris* and *O. bigelovii* the δ¹³C ratios measured in pith and cortex samples were not significantly different from the bulk value for the tissue. In other experiments (our unpublished data), although carbon fixed at night was initially found localised in the cortex, radial equilibration with the pith occurred in a few days.

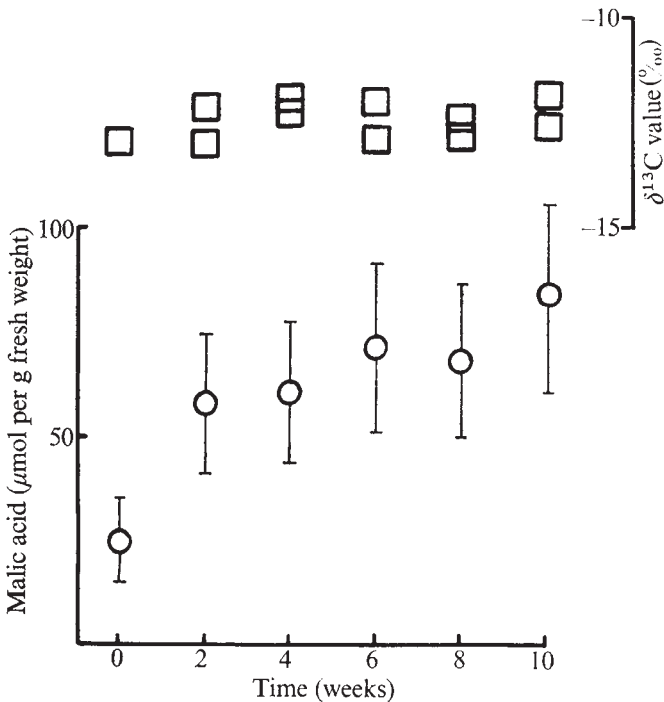


Fig. 2 Response of morning *O. bigelovii* malic acid levels and δ¹³C values to irrigation in autumn. A natural clone of plants was irrigated with 6–10 mm of water every 2 weeks. Correlation coefficient between δ¹³C values and time was 0.412. Mean and standard error of the δ¹³C values was -12.6 ± 0.137. Vertical bars indicate 95% confidence limits for the malic acid estimation.

The sensitivity of the δ¹³C ratio to environmental change was further examined in a clone of *O. bigelovii* which was subject to regular irrigation in autumn, when the cooler days and improved water status of the plants was expected to promote daytime CO₂ fixation⁸, and therefore a decline in the δ¹³C ratio. The results, presented in Fig. 2, indicate that although the production of malic acid at night increased markedly, the δ¹³C ratio remained stable at a high value. The data presented here therefore indicate that the δ¹³C ratio of the plants studied is relatively insensitive to short term seasonal changes in natural desert conditions. The data also confirm that no exogenous CO₂ is fixed in the light in this environment. These experiments imply

then, that the varying δ¹³C ratios observed in wild populations of cactus^{1,9} are a result of long term environmental differences rather than seasonal effects. This result gives credence to the use of such ratios for the reconstruction of past climates⁹.

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Real-time delayed tracking in flies

A DARK target on a bright background is fixated¹ and tracked^{2,3} by a flying fly. The delay (dead time) in the fly's behavioural response to a change in the retinal image of the target is very small (about 20 ms)³⁻⁵. An artificial increase of this delay may give useful insight into the kind of control systems used by the tracking fly. Comparable psychophysical tracking experiments have been performed. For instance, when a televised display of a person's own behaviour in (closed-loop) pursuit tracking is delayed, his performance, as measured by time on target, is seriously impaired⁶. It is possible to delay the fly's response in artificial closed-loop conditions, and we have therefore used the effects of delayed visual feedback on tracking performance to test quantitatively a theoretical description of the visual orientation behaviour of flies^{1,7,8}.

A female fly (head fixed to the thorax) was attached to a fast torque meter, in the centre of a cylindrical panorama, as described before¹. The torque signal is transduced into angular displacement of the 'panorama' through an analog simulation of the flight dynamics. In this way it is possible to simulate a free flight situation, in so far as the fly can track a target moving in the horizontal plane. In our experiments the moving target consisted of a black vertical stripe. The angular speed of the target was determined by a zero-mean Gaussian noise added to the torque signal of the fly. The torque, *F*, of the fly was delayed in steps of 78 ms by means of a standard tape recorder. The experimental situation is described by the following equation

$$[\theta\ddot{\psi}(t) + k\dot{\psi}(t)] + k\omega(t) = -F(t - \epsilon) \quad (1)$$

where the angular error $\psi(t)$, represents the actual position of the target on the fly's retina. The terms in brackets on the left give the analog approximation of the flight dynamics (θ is the moment of inertia of the fly, *k* represents a rotational

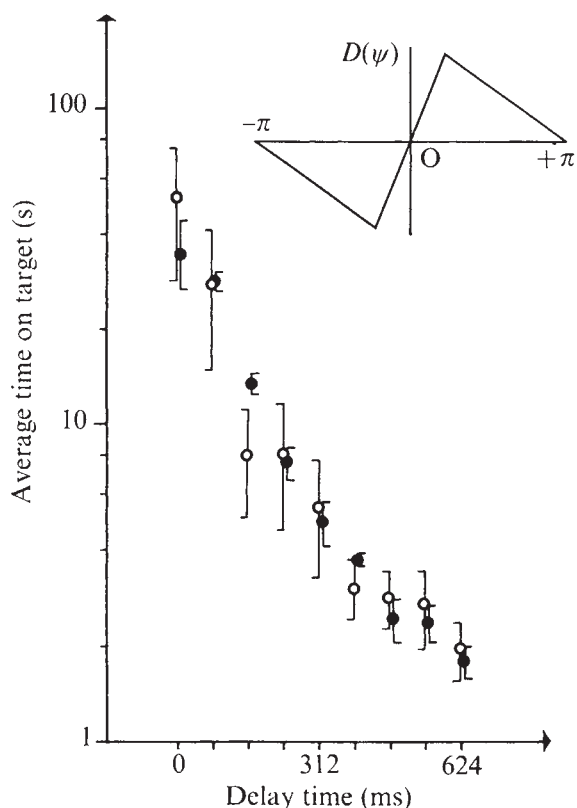


Fig. 1 Tracking performance under delayed response. The data points represent the mean time on target for six female flies, *Musca domestica* L. (○) and for four analog simulations of equation (1) (●). The vertical bars indicate the standard deviation of the mean. The inset shows the response function $D(\psi)$ which gives the ψ -dependent 'attractiveness' of a black stripe for the fly. $D(\psi)$ and the other parameter values used in the simulation are the same as those measured by Reichardt¹. With these values, equation (2) without the terms $N(t)$ and $k\omega(t)$ shows oscillatory solutions for $\epsilon \geq 156$ ms. Correspondingly, the associated linearised equation becomes unstable. $\omega(t)$, the target angular velocity, is a Gaussian zero-mean process in all experiments, with a flat spectrum up to 15 Hz and an r.m.s. amplitude of about 50° s^{-1} . The width of the stripe was 2° , and its vertical length about 60° .

friction constant). $\omega(t)$ is the zero-mean Gaussian process representing the angular speed imposed on the target in our experiment. The average time on target T was measured as a function of the artificial delay ϵ . The object was considered to be lost by the fly when the angular error $\psi(t)$ between the direction of flight of the fly and the object reached $\pm 170^\circ$.

In tracking behaviour, an increasing delay impairs performance: the greater the delay, the smaller is T (Fig. 1). The relationship between delay and time on target does not seem to fit a simple function. Psychophysical experiments, on the other hand, seem to show a simple inverse linear relationship between T (on a logarithmic scale) and magnitude of delay⁸. The orientation behaviour of flies and various tracking situations can be described quantitatively by a phenomenological theory^{2-4,7-14}, which leads to the equation

$$\theta\ddot{\psi}(t) + k\dot{\psi}(t) + k\omega(t) = -D[\psi(t)] - r\dot{\psi}(t) + N(t) \quad (2)$$

where the right hand side describes the instantaneous torque of the fly. $N(t)$ is a zero-mean Gaussian, band-limited random process, independent of visual input; $r\dot{\psi}$ represents a velocity-dependent, direction-sensitive optomotor response; $D(\psi)$ is a nonlinear anti-symmetrical function describing the fly's response to the stripe position (inset Fig. 1 and legend). All these terms have been characterised quantitatively^{1,3,4,7}.

If the right hand side is delayed by ϵ , equation (2) should represent our experimental situation. An approximate analytical

solution in T has been obtained⁹ in the case $\epsilon = 0$ but seems otherwise extremely difficult. Therefore, to check the validity of the model, we carried out an analog simulation of equation (2), using the parameter values measured by Reichardt¹. T was measured in the same conditions and with the same experimental arrangement as was used for the fly (Fig. 1). In the simulation we neglected the internal delay time of the fly (20 ms), and our results are consistent with previous evidence^{3,7} that it can indeed be neglected.

The satisfactory agreement between the two sets of data of Fig. 1 supports the validity of the hypothesis underlying the phenomenological theory. Not only do the quantitative values of T as a function of ϵ agree within the limits of experimental error, but also the autocorrelations of the process $\psi(t)$ for the various values of ϵ are similar. In summary, equation (2), in addition to explaining various orientation situations quantitatively, also accounts for the delayed tracking described here. An important consequence is that the range of validity of the terms $D(\psi)$ and $r\dot{\psi}$, representing the fly's visual induced response, extends through the rather extreme tracking conditions of our experiment. In particular, the function $D(\psi)$, which to a great extent determines the tracking performance, seems to be an almost invariant property of the visual control system of the fly.

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Determination of polarity in the amphibian limb

ACCORDING to the classical work of Harrison and his followers¹⁻⁶, the anteroposterior polarity of amphibian limbs consists of a 'molecular polarisation' of the cells which has been acquired by the late gastrula stage of development. I here present evidence that the polarity of the anteroposterior pattern is caused at a later stage of

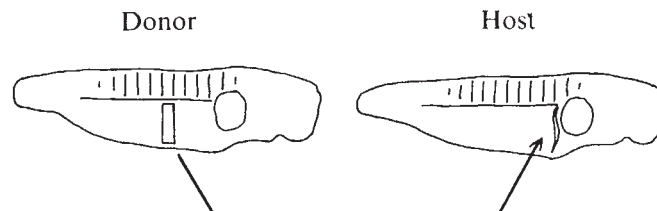


Fig. 1 Diagram of the reduplication-inducing graft described in the text. Operations were performed with electrolytically sharpened tungsten needles. The embryos were in half-strength Holtfreter solution containing 1/3,000 MS222 (Sandoz). The grafts were kept in place for 1-2 h with glass bridges. Embryos were kept in half-strength Holtfreter until the wounds had healed (usually overnight) and then transferred to 1/10 × Holtfreter containing 1/10,000 Nystatin (Squibb) and 1/2,000 Sulphadiazine (May and Baker). After hatching, larvae were kept in tap water which had been left to stand for 24 h. They were fed on brine shrimps (California Brine Shrimps Inc.) until all of the limb cartilages had developed, which takes about 4 weeks at 18°C .