localized domain walls separating standing-wave regions with different but well-defined wavevectors can also be observed (Fig. 2). Thus, localization phenomena found earlier in one-dimensional vibratory systems[6] can be generalized to higher dimensions.

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1. Brillouin, L. *Wave Propagation in Periodic Structures* (McGraw-Hill, New York, 1946).
2. Ashcroft, N. W. & Mermin, N. D. *Solid State Physics* (Saunders, Philadelphia, 1976).
3. Yablonovitch, E. & Gmitter, T. J. *Phys. Rev. Lett.* **63,** 1950–1953 (1989).
4. Montero de Espinosa, F. R., Jiménez, E. & Torres, M. *Phys. Rev. Lett.* **80,** 1208–1211 (1998).
5. Chou, T. *Phys. Rev. Lett.* **79,** 4802–4805 (1997).
6. Denardo, B. *et al. Phys. Rev. Lett.* **68,** 1730–1733 (1992).
7. Cross, M. C. & Hohenberg, P. C. *Rev. Mod. Phys.* **65,** 851–1112 (1993).
8. Torres, M. *et al. Chaos Solitons Fractals* **5,** 2089–2093 (1995).

Genetic flexibility of plant chloroplasts

The chloroplast genome is thought to be monomorphic, or genetically uniform within individual plants[1]. But a single plant cell may contain several hundred chloroplasts, each containing up to 900 copies of DNA[2], so there is a huge potential for accumulating and maintaining mutations. I found that the chloroplast genome of common groundsel, *Senecio vulgaris*, is polymorphic for a point mutation that confers resistance to triazine herbicides. Moreover, this polymorphism can vary within and among different leaves of a single plant.

Common groundsel is an annual weed found almost all over the world. It is strongly self-fertilizing, and was the first species to develop resistance to triazine herbicides[3]. To assess the level of chloroplast DNA polymorphism within individual plants, I used six different portions of each of five leaves of seven plants of various origin to analyse the frequency and distribution of a point mutation in a chloroplast gene that confers resistance to triazine herbicides (Fig. 1). I used the polymerase chain reaction to amplify a 277-base-pair-long chloroplast DNA fragment of the *psbA* gene spanning the point mutation conferring triazine resistance[4].

Restriction analysis of the amplified sequence using *Mae*I resulted in two fragments of 123 and 154 base pairs in triazine-resistant individuals, and in three fragments of 35, 88 and 154 base pairs in susceptible individuals. The 123-base-pair restriction fragment is therefore diagnostic for triazine resistance and the 88-base-pair fragment indicates susceptibility; polymorphism of chloroplast DNA is observed if fragments of both 88 and 123 base pairs occur together within a leaf sample. Sequencing of the amplification product confirmed the restriction pattern (GeneBank accession number, AF061287; data not shown).

Abundant polymorphism was evident in all but the phenotypically resistant plants (Fig. 1). The pattern of polymorphism in the crosses (S × R, R × S) indicates maternal inheritance. Paternal leakage seems to be infrequent, as the R × S plants were sixth-generation backcrosses, indicating that there is stable transmission of polymorphic states to the progeny. The level of polymorphism in *S. vulgaris* is variable between plants ($P = 0.0001$ among all plants, as well as among the polymorphic plants only; nested analysis of variance on arcsin square-root-transformed proportions[5]). Variation between different leaves is observed in some plants (NL2, $P = 0.0001$; S × R, $P = 0.0005$; UK3, $P = 0.0164$) but not others (the monomorphic R and R × S; S, $P = 0.2991$; CH, $P = 0.0705$). Large values for residual mean squares in polymorphic plants show that considerable variation also occurs within single leaves (data not shown; see Fig. 1). Many samples showed variable amounts of additional fragments, such as a 186-base-pair fragment typically found in triazine-susceptible genotypes of other weeds[4], indicating several other polymorphisms. Because the samples originated from different countries, chloroplast DNA polymorphism seems to be a widespread characteristic of this plant.

Heteroplasmy (the existence of more than one type of chloroplast within an individual) may be attributed to somatic mutation or biparental inheritance, and is believed to sort out within one or very few generations[6]. Together with the fact that the point mutation that confers triazine resistance is associated with considerable fitness costs[7], this should lead to a rapid loss of heteroplasmic states from plants. However, the resistant genotype is not rapidly eliminated from populations, even after periods without any triazine treatment, as can be seen from the genotype of the 'susceptible' laboratory-reared S line, which, since its collection in the field in 1973 and after several cycles of reproduction, still carries a considerable degree of polymorphism (Fig. 1). Assuming that paternal inheritance is infrequent in this strongly selfing weed, this indicates that transmission of the polymorphic chloroplast DNA state to the progeny can be maintained over many generations. The fitness costs of the point mutation that confers triazine resistance may therefore only be important if the level of polymorphism exceeds a certain carrying capacity.

In *Drosophila*, the evolution of heteroplasmic states of mitochondrial DNA types is affected by their fitness with respect to selective forces[8]. Within-plant polymorphism of chloroplast DNA has the potential for environmentally imposed selective

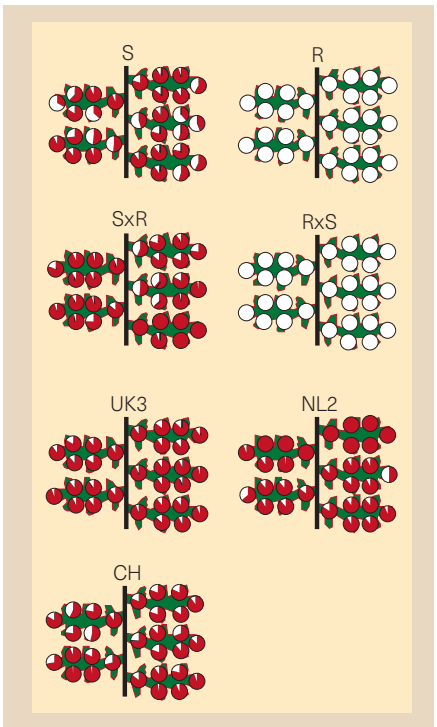

Figure 1 Analysis of chloroplast DNA polymorphism. Samples of *Senecio vulgaris* (ssp. *vulgaris* var. *vulgaris*) originated from Switzerland (CH), the Netherlands (NL2), Britain (UK3)[10] and from four inbred lines from the western United States: R (triazine-resistant parental biotype), S (triazine susceptible parental biotype), R × S (triazine-resistant sixth-generation backcrossed biotype with R cytoplasm and S nuclear genome) and S × R (triazine-resistant sixth-generation backcrossed biotype with S cytoplasm and R nuclear genome)[11]. Each leaf sample consisted of a small leaf disc (~2.4 mm^2) punched out of six leaf positions of each of the first five leaves using the tip of a disposable Pasteur pipette, homogenized in 100 μl lysis buffer (20 mM Tris, pH 7.4, 20 mM EDTA, 2 M NaCl), heated three times for 5 min at 85 °C, vortexed and centrifuged. Then 3 μl extraction solution was used with fluorescently labelled primers to amplify part of the *psb A* gene, and 4 μl amplification product was digested with *Mae*I as described for several weeds[4]. Two to four restriction analyses were performed on each amplification product, and 2 μl digested DNA was analysed. Possible effects of star activity of the restriction enzyme were tested by digesting different concentrations of purified amplification products. Levels of chloroplast DNA polymorphism for individual leaf samples are shown as the median of the restriction analyses of the amplification products of the ratio between the quantity of the 88-base-pair fragment (red) and the quantity of the 123-base-pair fragment (white), based on fragment peak amplitudes. Because the undigested 277-base-pair fragment found in many analyses may be due to either incomplete digestion or a resistant genotype lacking the 154-base-pair restriction site, the index probably underestimates the relative frequency of resistant genotypes.

change in chloroplast gene frequency within the lifespan of an individual plant. For example, a polymorphic plant that survives herbicide treatment will probably have eliminated susceptible chloroplasts, and will be insensitive to further herbicide treatment. If this process is completed before the plant starts to develop seeds, its progeny will carry only the resistant chloroplast DNA genotype and may therefore be completely resistant to herbicides. Such a phenotypic effect has been found in *Chenopodium album*, in which sublethal treatment with atrazine of plants with intermediate resistance resulted in resistant seeds[9]. This within-plant selection between different chloroplast DNA types will occur whether polymorphism results from rare bipaternal inheritance or stable transmission.

Chloroplast DNA polymorphism therefore provides an additional level of selection that gives plants a powerful mechanism by which they can adapt rapidly to specific environments. This mechanism may in part be responsible for the very rapid evolution of triazine resistance in *S. vulgaris*.

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1. Yu, W. & Spreitzer, R. J. *Proc. Natl Acad. Sci. USA* **89,** 3904–3907 (1992).
2. Bendich, A. J. *BioEssays* **6,** 279–282 (1987).
3. Ryan, G. F. *Weed Sci.* **18,** 614–616 (1970).
4. Cheung, W. Y. *et al. Plant Mol. Biol. Rep.* **11,** 142–155 (1993).
5. Sokal, R. R. & Rohlf, F. J. *Biometry* (2nd edn) (Freeman, San Francisco, 1981).
6. Birky, C. W. Jr *Science* **222,** 468–475 (1983).
7. Jasieniuk, M., Brûlé-Babel, A. L. & Morrison, I. N. *Weed Sci.* **44,** 176–193 (1996).
8. De Stordeur, E. *Heredity* **79,** 615–623 (1997).
9. Darmency, H. in *Herbicide Resistance in Plants* (eds Powles, S. B. & Holtum, J. A. M.) 263–297 (Lewis, London, 1994).
10. Wyss, G. S. Thesis, ETH Zürich (1997).
11. McCloskey, W. B. & Holt, J. S. *Plant Physiol.* **92,** 954–962 (1990).

Are vent shrimps blinded by science?

The exploration of deep-sea hydrothermal vents has depended on the use of manned submersibles, which are invariably equipped with high-intensity floodlights. But the eyes of many deep-sea crustaceans, which are exquisitely adapted for the dim conditions at such depths, can suffer permanent retinal damage as a result[1–3]. We suggest that the use of floodlights has irretrievably damaged the eyes of many of the decapod shrimps (family Bresiliidae) that dominate the fauna at vents on the Mid-Atlantic Ridge[4].

We collected *Rimicaris exoculata* and *Mirocaris* (*Chorocaris*) *fortunata* shrimps at the Rainbow and Lucky Strike sites, respectively, using the submersible *Nautile* during the AMORES Marvel cruise of RV *Atalante* in August 1997. We captured the animals by using a suction pump in floodlight illumination and brought them to the surface in a blacked-out Perspex chamber, which provided limited protection against surface light exposure.

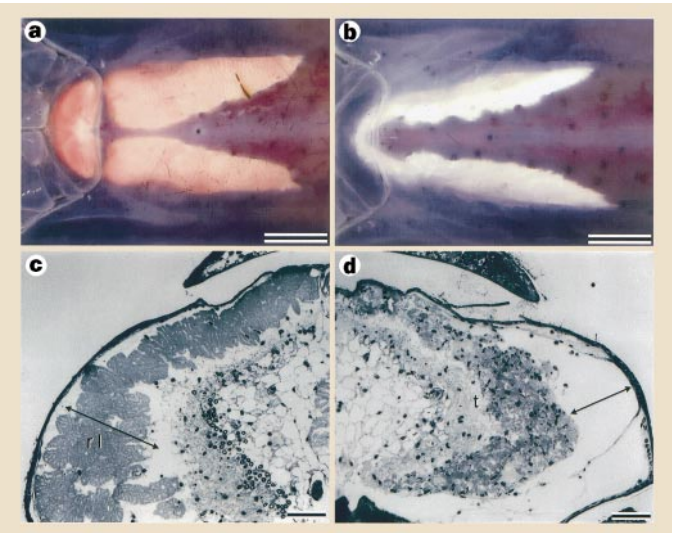

Figure 1 Eyes of deep-sea shrimp. **a, b,** The dorsal surface of live *Rimicaris exoculata* showing variations in the thoracic eye: **a,** the pink-eyed type, possibly coloured by rhodopsin; **b,** the white-eyed type, apparently with only the reflective tapetum. **c, d,** Sections through resin-embedded specimens of *Mirocaris fortunata*. **c,** In pink-eyed specimens there is an extensive rhabdom layer (rl, arrow) extending beneath the carapace from the midline to the lateral margin. **d,** In white-eyed specimens there is no rhabdom layer (arrow), although the tapetum (t) remains undamaged. Scale bars: **a, b,** 1 mm; **c, d,** 100 μm.

The thoracic eyes of some individuals were pink, with a smooth outline and regularly dappled appearance (Fig. 1a), whereas others were a matt chalky white, often with dark areas or streaks in the otherwise featureless reflector (Fig.1b). We examined the morphology of pairs of specimens of both *R. exoculata* and *M. fortunata* with pink and white eyes, each pair taken from the same sample. The pink-eyed specimens show the normal extensive rhabdom (photoreceptor) layer[5,6], although there is some evidence (confirmed by electron microscopy) of recent damage to the microvilli (Fig. 1c). The white-eyed specimens of both species show severe breakdown, often with complete loss of the rhabdom layer (Fig. 1d).

The Rainbow site was discovered in 1994 by remote physicochemical sampling without illumination[7]. The submersibles *Alvin* and *Nautile* first visited the active vents in July 1997. We suggest that the retinal damage observed in white-eyed *R. exoculata*, collected just one month later, was caused by the lights used during these surveys. The shrimps swarm over the vent chimneys and are illuminated by any vehicle working at the active region. The Lucky Strike sites have been visited many times, and we believe that the differences in our Lucky Strike specimens of *M. fortunata*, most of which had white eyes, have the same cause.

The eye structures of vent shrimps have varying degrees of abnormality, usually ascribed either to poor fixation or to light damage during collection[5,6,8]. *Alvinocaris*, for example, apparently has no rhabdoms[9], but this may be a consequence of previous encounters with a submersible, rather than being a specific adaptation. The only cases where damage has not been observed are those of juvenile specimens taken by trawling in midwater well above the vents[10]. These shrimps would not have been subject to previous floodlighting.

The rate of onset of retinal pathology is slow enough for the structure of the retina to be relatively unaffected over a period of hours (as can be seen from our illuminated pink-eyed specimens) but rapid enough for dramatic deterioration to occur in the few weeks between the initial visits to the Rainbow site and our capture of the *R. exoculata* specimens. There is at present no means of working at the vents without causing this damage, so every vent population visited will already have been exposed to it.

We have established an associative link but not a causal one. Confirmation of these conclusions will require study of the eyes of shrimp captured at the first visit to any new vent site and, ideally, an *in situ* time series of light-exposed specimens from the same site. Meanwhile, any behavioural observations at previously visited vent sites may relate to shrimp that are already blind.

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1. Loew, E. R. *Proc. R. Soc. Lond. B* **193,** 31–44 (1976).
2. Nilsson, H. L. & Lindstrom, M. *J. Exp. Biol.* **107,** 277–292 (1983).
3. Shelton, P. M. J., Gaten, E. & Chapman, C. J. *Proc. R. Soc. Lond. B* **211,** 217–236 (1985).
4. Segonzac, M., de Saint Laurent, M. & Casanova, B. *Cahiers Biol. Mar.* **34,** 535–571 (1994).
5. O'Neill, P. J. *et al. Vis. Neurosci.* **12,** 861–875 (1995).
6. Kuenzler, R. O. *et al. J. Mar. Biol. Assoc. UK* **77,** 707–725 (1997).
7. Klinkhammer, G. P., German, C. R. & Rudnicki, M. D. *Geophys. Res. Lett.* **23,** 2979–2982 (1996).
8. Van Dover, C. L. *et al. Nature* **337,** 458–460 (1989).
9. Wharton, D. N. *et al. J. Mar. Biol. Assoc. UK* **77,** 1097–1108 (1997).
10. Gaten, E. *et al. Biol. Bull.* **194,** 267–280 (1998).