Biodiversity

Seed germination in rainforest fragments

Habitat loss and fragmentation remain the greatest threats to the world's biodiversity[1]. The local extinction of plant species from habitat fragments is common[2], although the reasons for this are not fully understood. Fragmentation is known to influence both birth- and death-related processes[3], but the disruption of plant reproduction, especially pollination and seed production, is thought to be particularly important[4,5]. The effects of fragmentation on post-pollination processes such as seed dispersal and germination have rarely been explored experimentally[6]. Here I show that seeds planted in forest fragments are less likely to germinate than those in continuous forest. This finding can have negative demographic consequences because it reduces the emergence of seedlings.

Heliconia acuminata is an Amazonian understorey herb that is found in both the experimentally isolated rainforest fragments and the continuous forest reserves of Brazil's Biological Dynamics of Forest Fragments Project. During the 1998 fruiting season, which lasted from March to May, I collected 1,668 seeds from more than 200 plants in continuous forest. I then distributed these seeds to seven forest fragments (four fragments of one hectare and three of ten hectares) and three continuous forest sites. Seeds from each maternal plant were distributed evenly across sites to avoid confounding potential maternal effects with fragmentation-related effects.

In each site, seeds were planted every 10 metres along five 100-metre transects. At each location on the transect, a seed was placed on the ground, placed in a plastic cup planted into the ground and filled with local soil (preventing removal of seeds by ants), and placed in a cup buried in the same way but covered with a fine mesh (to prevent all seed predation and accumulation of leaf litter). Seeds were checked each month to see whether they had germinated. One year after planting, I compared the proportion of emerging seedlings in each treatment in each site by using two-way analysis of variance (ANOVA)[7].

Seeds planted in continuous forest were between three and seven times more likely to germinate than those in forest fragments ($P < 0.0001$; Fig. 1). Seeds in fragments suffered from a variety of edge effects, including hotter, drier conditions and increased light penetration[8,9], all of which can affect the cues required for germination. I also found that seeds protected with mesh from leaf-litter accumulation and seed predation were more likely to germinate (although not significantly so), with the improvement being more marked in fragments than in forest sites (2.4- to 4.2-fold compared with 1.8- to 1.9-fold; Fig. 1). This asymmetrical improvement indicates that the greater accumulation of leaf litter in fragments (resulting from slower decomposition[8]) could be partly responsible for preventing germination as seeds become buried. Higher seed predation in fragments is an unlikely explanation, as predation is extremely low in all sites (E.M.B., unpublished data) and germination rates were similar for seeds placed on the ground and protected from predation in plastic cups.

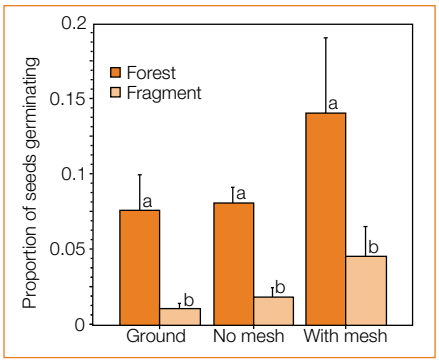

Figure 1 Mean proportion (± s.e.m.) of seeds germinating in continuous forest and fragmented sites in different experimental treatments. Differences in the proportion germinating in each habitat and treatment were assessed by ANOVA. The square root of the percentage germinating in each plot was arcsine-transformed, and habitat type and treatment were treated as fixed effects. The effect of habitat type was highly significant ($F_{1,24} = 22.623$, $P < 0.0001$), but that of planting treatment was not ($F_{2,24} = 2.336$, $P = 0.118$). The planting treatment × habitat type interaction term was also nonsignificant ($F_{2,24} = 0.028$, $P = 0.973$). Bars with different letters are significantly different from each other.

The results could be explained by the adaptation of seeds to conditions in the area from which they were collected, with seeds failing to germinate in fragments because of a change in location, rather than as a direct result of fragmentation. However, this mechanism seems unlikely unless seeds are adapted to very broad conditions, such as 'continuous forest'. Seeds were collected from an area of more than 3.5 km^2, and one site of continuous forest was about 20 km from the collection area. When compared with other continuous forest sites, this site never had the lowest germination frequency for any treatment. Even if local adaptation were responsible for the observed pattern, the demographic consequences for *Heliconia* populations in these fragments would be the same. Populations in fragments have few flowering individuals (E.M.B. and W. J. Kress, unpublished data), so most seeds for these populations probably come from outside the fragments.

Seeds from tropical rainforest plants rarely survive in seed banks for more than a year because they are highly susceptible to fungal pathogens, seed predation and burial under leaf litter[10]. My results therefore represent the outcome of a population's entire reproductive effort for a year. Although germination success is likely to vary from year to year, my results indicate that seeds dispersed into rainforest fragments are less likely to germinate than those in continuous forest.

Furthermore, my estimates of the effect of fragmentation on seed germination are probably conservative, as plants reproducing in forest fragments may be inbred and suffer from reduced heterozygosity[11]. If inbred seeds are less likely to germinate, these genetic effects could further reduce the recruitment of seedlings into forest fragment populations, helping to explain why plant populations in habitat fragments often fail to persist in the long term.

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Vision

Dichromatism in macaque monkeys

Old World primates have trichromatic vision because they have three types of cone photoreceptor, each of which is maximally sensitive to short, middle or long wavelengths of light[1]. Although a proportion of human males (about 8% of caucasians, for example) have X-chromosome-linked colour-vision abnormalities[2], no non-human Old World primates have been found to be colour-vision defective[3,4]. We have tested 3,153 macaque monkeys but found only three dichromats, a frequency that is much lower than in humans.

Most defects in colour vision in humans arise from the loss of the gene that encodes

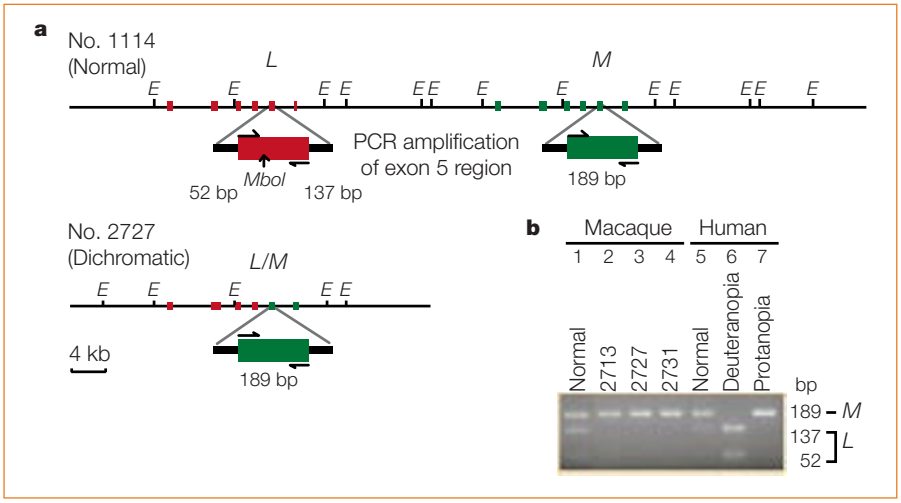

Figure 1 Physical maps of the *L* and the *M* genes. **a,** Top, the 77-kilobase (kb) region of a wild-type macaque (no. 1114), showing exons of the *L* (red) and *M* (green) genes. Enlarged exon-5 regions are shown underneath. The PCR primers and products for exon 5 of the *L* and *M* genes are shown in red (137 and 52 base pairs (bp), cleaved by *Mbo*I digestion) and green boxes (189 bp, uncleaved), respectively. An *Mbo*I site specific for exon 5 of the *L* gene is conserved among Old Word monkeys[8]. Bottom, the 36-kb region of a dichromatic candidate (no. 2727). The enlarged PCR-amplified fragment is shown underneath. **b,** Gel electrophoresis analysis. The bands of 189, 137 and 52 bp shown in **a** are indicated as *M* (green) or *L* (red). Lane 1, macaque wild type; lanes 2–4, subjects 2713, 2727 and 2731, respectively, showing only the exon 5 fragment of the *M* gene; lane 5, human wild type; lanes 6 and 7, deuteranopic (*M* cone absent) and protanopic (*L* cone absent) humans, respectively.

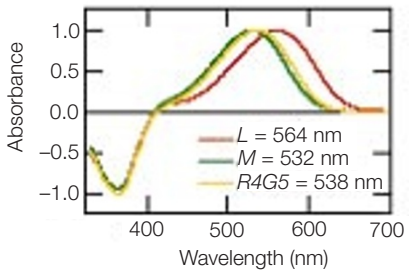

Figure 2 Absorbance spectra of *L*, *M* and hybrid (*R4G5*) pigments. Pigments expressed in 293T cells were extracted from the membrane fractions after 11-*cis*-retinal was added. Difference spectra were calculated from the spectra before and after photobleaching with light of wavelength ⩾590 nm for 1 min in the presence of 10 mM NH_2OH (ref. 13). The λ_{max} values of the *L* and *M* pigments are in good agreement with those previously measured by microscopic photometry[14].

pigments sensitive to either middle (*M*) or long (*L*) wavelengths of light. These genes are located on the X chromosome at Xq28 in a head-to-tail tandem array, and the repetitive units are gained or lost, and hybrid pigment genes generated, by unequal chromosomal recombination[5–7].

In our attempt to find dichromatic monkeys, we co-amplified the exon-5 region of the *L* and *M* genes with a set of common primers and discriminated between them with an *Mbo*I restriction site specific for the exon-5 fragment of the *L* gene[8] (Fig. 1a). We studied DNA from 744 male and 1,301 female crab-eating monkeys and 455 males and 653 females from 18 other macaque species, including samples from the Tsukuba Primate Center and the Nihon Monkey Center, and found only three DNA samples from males that lacked the exon-5 fragments of the *L* gene (Fig. 1b). These were from groups of crab-eating monkeys (26 males and 31 females) whose blood was sampled in the Pangandaran National Park, Indonesia.

The frequency of the dichromat males in this species (3 out of 744) is 0.4%, a value that is significantly lower (chi-squared test, $P < 0.002$) than in human males, for which a survey of 30,000 caucasian males found that 2% were dichromats[2]. No dichromat candidates were found in other macaque species.

We then constructed physical maps of the *L* and *M* genes from a wild-type (no. 1114) and a dichromatic (no. 2727) monkey from two genomic DNA libraries. The *L* and *M* genes were both present in the wild type but the dichromatic monkey had only a single pigment gene (Fig. 1a). Nucleotide sequence analysis then revealed that this single pigment gene was an *L*/*M* hybrid (called *R4G5*) consisting of exons 1 to 4 from *L* and exons 5 and 6 from *M*, indicating that the recombination point was located between exon 4 of *L* and exon 5 of *M*. Southern hybridization analysis revealed the presence of the *L*/*M* hybrid gene but no other *L* and *M* genes in this monkey. We found by polymerase chain reaction (PCR) using primers that specifically detect the *R4G5* hybrid gene that these three males and two females carried the hybrid gene.

We then used transient transfection into 293T cells to express complementary DNAs encoding the *M*, *L* and hybrid (*R4G5*) pigments (Fig. 2). Analysis of photobleaching difference absorption spectra showed that the absorbance maxima (λ_{max}) of reconstituted *L*, *M* and hybrid pigments are 564, 532 and 538 nm, respectively. The λ_{max} for the hybrid was red-shifted by 6 nm from that of the *M* pigment, indicating that these monkeys are almost protanopic (insensitive to red)[9,10].

In humans, between 58% and 78% of individuals possess several *M* genes[11,12], perhaps as a result of unequal recombination. In contrast, examination of 129 male crab-eating monkeys by quantitative Southern hybridization revealed that only six monkeys (5%) have more than one *M* gene, up to a maximum of four. All the other monkeys, apart from the dichromats, have just one *L* and one *M* gene. The variation in the number of *L* and *M* genes may be rare in macaques, but the sequence of the visual pigment genes is similar to that in humans[5,6].

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