

## The panda and the phage: compensatory mutations and the persistence of small populations

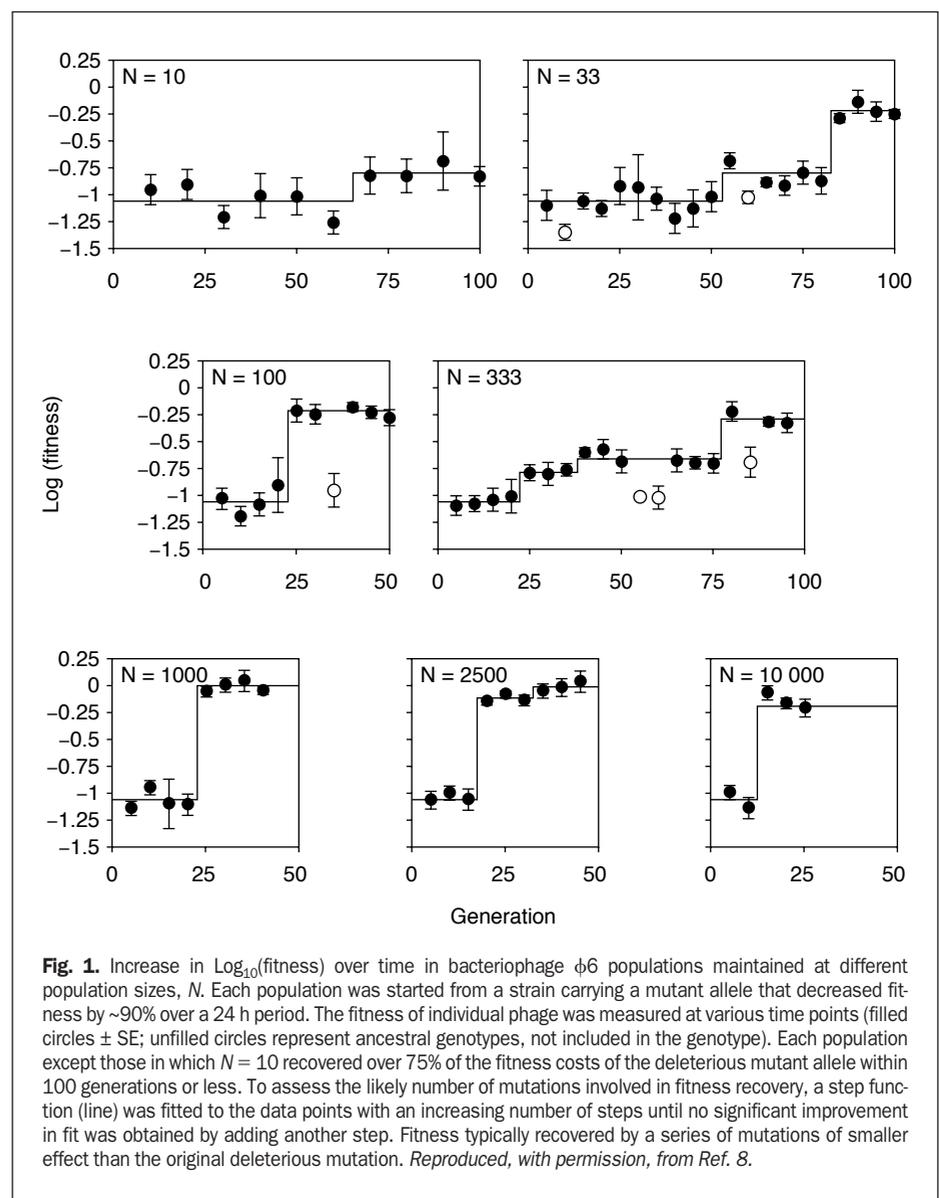
Mutation is the ultimate source of all the genetic variation necessary for evolution by natural selection; without mutation evolution would soon cease. Unfortunately, this comes at a cost: most mutations that affect fitness are deleterious<sup>1</sup>. For most large sexual populations, these less fit alleles are eventually eliminated from the population by natural selection. In small populations, however, new deleterious mutations can sometimes increase in frequency and even fix within the population. If harmful mutations fix repeatedly, the fitness of a population might eventually reach such a low level that the population is not capable of sustaining itself and may go extinct, the so-called 'mutational meltdown'<sup>2-4</sup>. The most important genetic threat to small endangered populations is thought to be this accumulation of new deleterious mutations by genetic drift<sup>5</sup>.

Even in a large population, mildly deleterious alleles can drift to fixation. So why aren't we all dead? If populations are large enough, the fixation of new beneficial mutations can balance the loss of fitness caused by fixation of bad alleles. This rescue effect is limited, however, by the apparently low rate at which beneficial mutations appear in most populations<sup>6</sup>. Two recent papers, from very different perspectives, shine new light on this problem<sup>7,8</sup>.

Lande<sup>7</sup> has recently shown that the rate of mutational decline toward extinction is strongly affected by the possibility of back mutation regenerating the original functional allele. Although in Lande's model back mutation alone is not capable of eliminating the risk of extinction from deleterious alleles, he has shown that the expected time to extinction can be increased as much as tenfold. Two factors constrain the benefit of back mutations: (1) a back mutation only helps at those sites that have previously fixed for a deleterious allele, so that the number of potentially useful back mutations at any given time is far fewer than the number of possible deleterious mutations and (2) some deleterious mutations, such as deletions, are nearly irreversible. Thus, while the expected time to extinction may be increased, reversible mutation does not solve the problem of the mutation meltdown<sup>7</sup>.

But there is hope. Back mutations are only one type of beneficial mutation, and they are a limited subset of the variety of ways in which a genome can mutate to repair the effects of a new deleterious mutation. A fascinating new paper<sup>8</sup> by Christina Burch and Lin Chao has demonstrated, using the bacteriophage  $\phi 6$  as a model system, the ready availability of novel mutations that are capable of compensating for the fitness effects of a fixed deleterious allele rather than simply reverting to the original genotype. They isolated a phage carrying a spontaneous mutation with a large negative effect on

fitness and used it to found several experimental lines. Each line was kept at a different population size, ranging from 10 to 10 000. Although population size was controlled only every fifth generation and grew rapidly between these time points, the average that matters is the harmonic mean population size, which is at most five times the census size during the generation when size was controlled. They then followed the change in fitness over the next 100 generations. In all but the smallest populations, the fitness of the populations returned to a value very close to the original fitness in a matter of tens of generations (Fig. 1). Furthermore, this increase in fitness occurred in a series of steps, evidence that the recovery was not simply the result of back mutations but of a series of other *compensatory* mutations. In contrast, control populations with the original genotype did not increase significantly in fitness over the same time, which indicates that there is much more potential for mutations to



**Fig. 1.** Increase in  $\text{Log}_{10}(\text{fitness})$  over time in bacteriophage  $\phi 6$  populations maintained at different population sizes,  $N$ . Each population was started from a strain carrying a mutant allele that decreased fitness by  $\sim 90\%$  over a 24 h period. The fitness of individual phage was measured at various time points (filled circles  $\pm$  SE; unfilled circles represent ancestral genotypes, not included in the genotype). Each population except those in which  $N = 10$  recovered over 75% of the fitness costs of the deleterious mutant allele within 100 generations or less. To assess the likely number of mutations involved in fitness recovery, a step function (line) was fitted to the data points with an increasing number of steps until no significant improvement in fit was obtained by adding another step. Fitness typically recovered by a series of mutations of smaller effect than the original deleterious mutation. *Reproduced, with permission, from Ref. 8.*

increase fitness in the context of fixed deleterious mutations. Burch and Chao's experiment therefore also provides strong evidence that the fitness effects of alleles depend on their genetic background.

In the context of mutational meltdown, these results are extremely encouraging. Although it has long been known that compensatory mutations could, for example, rescue some of the negative fitness consequences of visible mutations maintained in stock populations<sup>9,10</sup>, this is the first demonstration that this compensation can be so fast and so complete, even at small population sizes. Lande's results demonstrate that back mutations alone can slow mutational meltdown substantially; Burch and Chao's results show that mean fitness can increase, even at quite small population sizes, as a result of the potentially large number of ways in which evolution can achieve the same

fitness. Future experiments with other taxa and with other deleterious mutations would greatly increase our state of knowledge of the ways in which evolution can cope with the deleterious effects of mutation.

**Michael C. Whitlock**  
**Sarah P. Otto**

*Department of Zoology, University of British Columbia, Vancouver, BC, Canada V6T 1Z4 (whitlock@zoology.ubc.ca; otto@zoology.ubc.ca)*

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## The cuckoo chick tricks their reed warbler foster parents, but what about other host species?

The picture of a small passerine perching on the shoulder of a monstrous European cuckoo (*Cuculus canorus*) chick and feeding it remains a fascinating evolutionary enigma<sup>1–3</sup>. Young cuckoos are very successful in eliciting food from their foster parents. A single cuckoo chick receives an amount of food equivalent to that of a brood of host young<sup>4</sup>, and its growth rate is also the same as that of the entire brood<sup>2</sup>. The puzzle of why chicks of parasitic cuckoos are so successful in obtaining food from their foster parents has been explained in different ways<sup>1,5–7</sup>. In a new paper, Kilner *et al.*<sup>8</sup> provide novel insights into how the cuckoo chick tricks its reed warbler (*Acrocephalus scirpaceus*) hosts, with findings that raise intriguing questions\*.

Begging behaviour by nestlings involves directional stretching of the neck, wing shivering, gaping and calling, usually combined with gapes of exaggerated design and bright colouration. Such striking begging behaviour has recently received substantial attention from evolutionary biologists<sup>9–11</sup>, especially the

problem of how parasitic chicks deceive their foster parents<sup>6,7,12</sup>.

Parasitic cuckoos lay their eggs in the nests of other species, relying entirely on the foster parents to incubate the eggs and feed the chicks until independence. Thus, nestlings of brood parasites are attended by genetically unrelated individuals. Hence, the comparison of the begging behaviour of nestlings of parasitic and nonparasitic species can provide insight into the ways in which evolutionary forces shape begging behaviour. Kilner *et al.*<sup>8</sup> studied the begging behaviour by which reed warbler chicks signal need to parents, and analysed cuckoo exploitation of this parent–offspring communication system.

An initial analysis of the begging signals showed that both total gape area displayed by all chicks in the nest and begging call rate of broods of four reed warblers varied significantly with the duration of food deprivation. After the level of food deprivation was standardized by feeding chicks until satiation, nestlings were stimulated to beg every ten minutes. After 110 minutes the authors recorded the begging behaviour on video and audiotape, and quantified the amount of food consumed until begging ceased. This

amount was used as an indicator of the nestlings' need. They found significant independent effects of each begging signal (gape area and begging rate) on the amount of food consumed, and an interaction effect of the two signals combined: both signals together provided parents with more accurate information about offspring need than either signal alone. Thus, the authors predicted that parents adjust the provisioning rate to both begging signals.

Kilner *et al.*<sup>8</sup> then tested this prediction by experimentally manipulating vocal signals (by broadcasting playbacks of begging calls near the nest or by manipulating brood size) and visual signals (by manipulating brood size). In support of their prediction, they found that the feed-delivery rate by both parents was independently affected by the maximum number of gapes displayed per nest and the maximum number of chicks calling per nest (the number of chicks in the brood plus the number of chicks calling on the playback tape). Thus, parents appear to regulate their provisioning rate according to multiple signals: the rate at which the brood calls and the total gape area displayed.

Finally, the authors<sup>8</sup> analysed the way in which the cuckoo chick exploits the parent–offspring communication system, according to how chicks signal their level of need and how parents adjust their provisioning rate by integrating visual and vocal nestling displays.

Although the gape of the cuckoo chick is enormous compared with that of one reed warbler chick, Kilner *et al.* found that a single nestling cuckoo displays less gape area than a brood of four reed

\*See Correspondence (p. 320) for comments on this article by R.M. Kilner, D.G. Noble and N.B. Davies.