Hatching failure increases with severity of population bottlenecks in birds

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Severe bottlenecks can reduce genetic diversity and increase inbreeding as individuals are forced to mate with close relatives, but it is unknown at what minimum population size the negative fitness consequences of bottlenecks are expressed. The New Zealand avifauna contains a large number of species that have gone through bottlenecks of varying severity, providing an exceptional opportunity to test this question by using the comparative method. Using decreased hatchability as a measure of fitness costs, we found that hatching failure was significantly greater among both native and introduced species that had passed through bottlenecks of <150 individuals. Comparisons between pre- and postbottleneck populations of introduced species suggest that hatchier7.3erozygosity, increased genetic load, and increased expression of deleterious alleles (1). Inbreeding may yield significant costs to fitness and decrease population survival (2), a process termed inbreeding depression, but its importance has been questioned (3–5), and examples of the negative fitness consequences due to inbreeding in small populations of wild animals are few (6, 7). Despite the potential importance of bottleneck size to conservation biology, the number of individuals required to avoid the fitness costs of small population size and maintain the viability of a population has been difficult to test in free-living animals (1).

Theoretical models suggest that minimum effective population sizes range from 50 to 5,000 individuals, depending on levels of acceptable loss of genetic variability and the timeframe of population persistence (8, 9). The exact number is not a trivial question because the survival of many endangered species depends on the reliability of such guidelines. It has even been suggested that severe bottlenecks may be advantageous because they reduce inbreeding depression by purging deleterious alleles (10) although whether such benefits are great enough to justify deliberate inbreeding have been questioned (11, 12). The problem for conservation biologists is to understand whether bottlenecks create fitness costs and at what population size these costs become so severe that they threaten the viability of a population.

The New Zealand avifauna provides an ideal opportunity to examine the potential fitness costs of small population size across a range of species that have experienced bottlenecks of varying severity. Human settlement brought drastic changes to the avifauna: >30% of endemic bird species became extinct and many surviving species are threatened (13). For example, the black robin (*Petroica traversi*) was reduced to a single breeding pair before conservation efforts increased the population to several hundred (14). A widespread program of building new populations by transferring birds to islands free of introduced predators has similarly increased numbers of other endangered species. Despite such promising recoveries, monitoring has revealed high levels of hatching failure in some species (15). Only about half the eggs that survive the incubation period successfully hatch in the highly endangered kakapo (*Strigops habroptilus*; refs. 15 and 16), and similar problems have been observed in the black robin (17).

Increased hatching failure is a common outcome of inbreeding in captive and wild populations of birds and is a useful measure of the effect of an inbred genome on embryological development (18, 19). Hatching failure in out-bred birds averages ≈10% (20); thus, lev TJT*[(indiv)-39.8(iduals.)-353.9(Endangered)-353.9(populations)-353.9(of)-353.9(i

species had passed through the bottleneck. A total of 1,241 nests were used for this analysis.

Population bottleneck size was compared with rate of hatching failure by linear regression, with more than one value of y per x (21). This test allowed us to partition variance within and between species and also to test for a relationship with bottleneck size. Bottleneck size was log transformed, and hatching failure rates were angular transformed. Independent contrasts were then used on the mean values to control for phylogeny (22) by using the computer program CAIC (Comparative Analysis by Independent Contrasts) (23). Such controls are required because the levels of hatching failure may be similar in closely related species through inheritance from a common ancestor rather than as a consequence of bottleneck size. We used a phylogeny constructed from Sibley and Ahlquist (24) and added body mass as a third variable in a multiple regression of hatching failure contrasts on bottleneck size contrasts. Body mass is often a confounding variable in comparative studies, and a multiple regression allowed us to control for body size while comparing hatching failure rates with bottleneck size. We then calculated a series of unique linear contrasts for each node in our phylogeny for which there was variation in the independent variable. To test for relationships between taxa, the linear contrasts of one variable were correlated with those of another. All correlations were forced through the origin as recommended (22). Data on levels of consanguious matings were unavailable most species
so we assumed that inbreeding frequency increased with decreasing bottlenecks. For comparison, we also used data on hatchings

Fig. 3. (axis labels: Hatching failure (%), Number introduced)
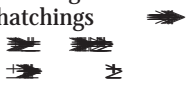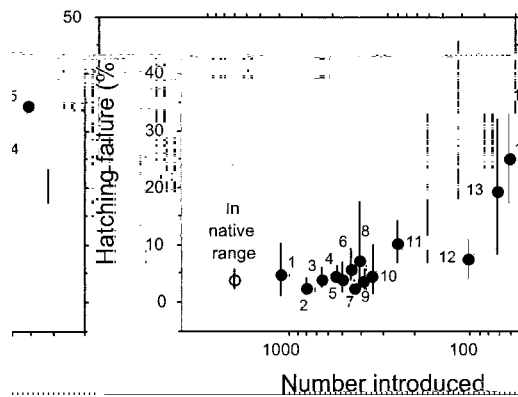
countries (primarily the United Kingdom). Thus, a comparison between introduced species in their native range ("before" a bottleneck) with that in New Zealand ("after" a bottleneck) provides a matched-pair experiment of bottleneck size on level of hatching failure. Consistent with the hypothesis that small population size increases fitness costs such as hatching failure, we found an increasing difference in hatching failure rates between populations in their native and introduced range with severity of bottleneck (Fig. 3). The greatest differences in hatching failure rates were observed in introduced species that passed through the most severe bottlenecks. The line in this regression intercepts the x axis at 606 individuals (95% confidence limit: 490–1,585 individuals). This is the number of founders in which hatching success in a postbottleneck population does not differ from that in its prebottleneck population.

Alternative explanations cannot account for our findings. For example, pesticide pollution has been implicated in hatching failure among endangered birds elsewhere (29). However, pollution-induced hatching failure cannot explain the results here because most native species in our sample occur in remote areas, and populations declined before the introduction of persistent pesticides. Levels of pesticides in species most susceptible to bioaccumulation (e.g., New Zealand falcon *Falco novaeseelandiae*) are also not high enough to affect hatching success (30). Instead, the primary cause threatening the survival of native New Zealand birds is predation by introduced mammals (31). The rapid recovery of many species after the removal of exotic predators also argues against pesticides as the cause of bottlenecks.

It is possible that species with high levels of hatching failure before a bottleneck (for reasons unrelated to inbreeding) were more likely to experience a bottleneck, and this bias could explain the pattern we found. In this case, high initial levels of hatching failure may have increased the severity of the bottleneck when such species were exposed to other negative demo-

graphic factors such as habitat fragmentation or the introduction of exotic predators. The lack of information on levels of hatching failure in native species before they declined precludes any test of this hypothesis. However, the levels of hatching failure in introduced species in their native range was not significantly correlated with the number of individuals subsequently released in New Zealand ($F = 1.65$, df $= 1,14$, $P = 0.65$). In other words, introduced species with a small number of founders did not by chance have high levels of hatching failure in their native range. This finding suggests that high levels of hatching failure were caused by the severe bottlenecks that these species passed through and were not the cause of the bottlenecks in the first place.

Another possible explanation for our results is that the most endangered species are now confined to marginal or degraded habitat, and this environment leads to greater hatching failure because of poor adult condition. For example, hatching failure of takahe (*Porphyrio hochstetteri*) translocated to offshore islands is higher than in their source population in mainland alpine habitats (17). However, this hypothesis cannot account for increased hatching failure in introduced species that also passed through severe bottlenecks (Fig. 2). Introduced species are common and widespread, and hatching failure should not therefore be the result of confinement to marginal environments. Differences in environmental conditions between the source and transplanted ranges in introduced species could still account for their increased hatching failure in New Zealand even if these differences do not limit population size. For example, dietary deficiencies in the introduced range could lead to increased hatching failure. Detailed studies of diet differences and other potentially stressful environmental factors are lacking to test this idea, but such a problem would have to disproportionately affect the most severely bottlenecked species to explain the pattern we found.

Our comparison of introduced species between their native and introduced ranges suggests that as many as 600 individuals