

## FROM THE COVER

# Genetic rescue of small inbred populations: meta-analysis reveals large and consistent benefits of gene flow

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## Abstract

Many species have fragmented distribution with small isolated populations suffering inbreeding depression and/or reduced ability to evolve. Without gene flow from another population within the species (genetic rescue), these populations are likely to be extirpated. However, there have been only ~ 20 published cases of such outcrossing for conservation purposes, probably a very low proportion of populations that would potentially benefit. As one impediment to genetic rescues is the lack of an overview of the magnitude and consistency of genetic rescue effects in wild species, I carried out a meta-analysis. Outcrossing of inbred populations resulted in beneficial effects in 92.9% of 156 cases screened as having a low risk of outbreeding depression. The median increase in composite fitness (combined fecundity and survival) following outcrossing was 148% in stressful environments and 45% in benign ones. Fitness benefits also increased significantly with maternal  $\Delta F$  (reduction in inbreeding coefficient due to gene flow) and for naturally outbreeding versus inbreeding species. However, benefits did not differ significantly among invertebrates, vertebrates and plants. Evolutionary potential for fitness characters in inbred populations also benefited from gene flow. There are no scientific impediments to the widespread use of outcrossing to genetically rescue inbred populations of naturally outbreeding species, provided potential crosses have a low risk of outbreeding depression. I provide revised guidelines for the management of genetic rescue attempts.

*Keywords:* evolutionary potential, genetic rescue guidelines, heterosis, inbreeding depression, outbreeding depression, outcrossing

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## Introduction

Many species have isolated populations that are subject to inbreeding, loss of genetic diversity and elevated extinction risks (Frankham *et al.* 2014). Genetic theory indicates that it should often be possible to genetically rescue such populations by outcrossing within species (augmenting gene flow: Tallmon *et al.* 2004; Whiteley *et al.* 2015). However, outcrossing can also be deleterious (outbreeding depression: Edmands 2007; Frankham *et al.* 2011).

Crossing of populations has frequently been used to successfully reverse inbreeding depression in

laboratory and agricultural species (heterosis: Sinha & Khanna 1975; Falconer & Mackay 1996; Frankham *et al.* 2010). For example, heterosis has been fundamental to the green revolution that has substantially increased crop plant yields (Borlaug 2000). Despite this, only ~ 10–20 genetic rescues have been attempted for conservation purposes (Adams *et al.* 2011; Frankham *et al.* 2011), probably a miniscule proportion of the populations that might benefit from outcrossing (Frankham *et al.* 2014). The limited use of genetic rescue is probably due to concerns about the following:

- 1 outbreeding depression/upsetting local genetic adaptation/local purity and provenance,

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- 2 limited quantitative information on the expected consequences of outcrossing,
- 3 lack of clear guidelines,
- 4 costs,
- 5 risks of disease, pest and parasite spread,
- 6 disrupting social systems in some animals,
- 7 moving biological material across political jurisdictions,
- 8 regulatory barriers.

The first three are primarily genetic issues, while the remainder require consideration, but are rarely insuperable barriers to gene flow (see Discussion). Frankham *et al.* (2011) developed a decision tree for predicting the risk of outbreeding depression and provided preliminary evidence that it was effective. This contribution focuses on the second and third issues.

Information on the magnitude and consistency of genetic rescue effects is critical for cost/benefit analyses of alternative conservation management options for fragmented populations. Here, I address this issue using a meta-analysis, a tool widely used to overcome low statistical power in individual studies and to obtain comprehensive quantitative overviews (Frankham *et al.* 2010; Koricheva *et al.* 2013). In practice, genetic rescues are only contemplated for populations with elevated inbreeding and/or reduced genetic diversity, so my study was restricted to them. As both beneficial and deleterious effects can occur simultaneously, the practical issue is whether the net effects of outcrossing on fitness are beneficial or deleterious.

As genetic rescue for fitness in the absence of outbreeding depression involves the reversal of inbreeding depression (Wright 1977; Vrijenhoek 1994; Falconer & Mackay 1996), the variables affecting it are expected to be the same as those that predict inbreeding depression, but with effects in the opposite direction (see Appendix S1, Supporting information). Thus, genetic rescue is expected to depend upon the difference in inbreeding coefficients between the inbred and outcrossed populations in mothers ( $\Delta F_m$ ) and in zygotes ( $\Delta F_z$ ), on the environment where the effects are measured (stressful > benign), to be greater for naturally outbreeding than inbreeding species and for outbred than inbred immigrants. Genetic rescue effects should be greater in the F1 than the F2 and later generations for traits determined by the zygotic genotype, but show the opposite pattern for traits determined by maternal genotype (see Appendix S2, Supporting information).

The aims of this study were to determine the following: (i) the consistency and magnitude of effects of outcrossing small inbred populations to another population within species (where the cross had a low risk of outbreeding depression); (ii) variables affecting the

magnitude of the effect and (iii) whether the screen for outbreeding depression proposed by Frankham *et al.* (2011) is effective in such circumstances. Genetic rescue effects were evaluated for both fitness and ability to evolve (especially in changing environments).

## Materials and methods

### Data selection criteria

Studies were screened to identify relevant data sets that encompassed the following:

- 1 a parental (target) population known or presumed to be inbred and/or having low genetic diversity,
- 2 gene flow into the inbred population from one or more other (inbred or outbred) populations genetically isolated from the target population, but belonging to the same species,
- 3 fitness and/or evolutionary potential data on the inbred and outcrossed populations,
- 4 sexually reproducing species, as strictly asexual species do not experience inbreeding depression, heterosis or outbreeding depression,
- 5 populations where crosses were assessed as having a low risk of outbreeding depression, using the Frankham *et al.* (2011) decision tree (same species, no fixed chromosomal differences, adapted to similar environments and gene flow within the last 500 years),
- 6 wild species, not ones used in plant or animal agriculture, forestry or aquaculture and subjected to strong artificial selection (in case domestic species have different characteristics to wild ones: Frankham 2009).

Further details of data selection criteria are given in Appendix S3 (Supporting information).

### Sources of data

Data were obtained from (i) studies already known to me; (ii) references in 35 reviews on genetic rescue, outbreeding depression and related issues; (iii) 40 advanced level textbooks, research monographs and scientific meeting proceedings; (iv) keyword searches for both genetic rescue/heterosis, outbreeding depression and related terms in Google Scholar and Web of Science and (v) further references cited within > 400 studies identified by all the above means.

The data search revealed 156 comparisons of outbred/inbred fitness from 77 taxa (18 invertebrates, 15 vertebrates and 44 plants) (Table S1, Supporting information) with references from Darwin (1876) to January 2014. Only fourteen of the taxa were described by the

authors as belonging to a threatened category, and only a small proportion of crosses were likely performed for conservation purposes. Gene flow into inbred populations ranged from a single immigrant to crosses with several other isolated populations (immigration levels of 0.025–0.94). There were only six comparisons found for evolutionary potential for fitness traits, all for invertebrates (Table S2, Supporting information).

### *Effect sizes*

The ratio of mean fitness in outcrossed/inbred populations [genetic rescue (GR) ratio] was used as the effect size for fitness, as it is intuitive to conservation audiences, relevant to management, and response ratios are well-recognized measures in meta-analyses (Borenstein *et al.* 2009). The ratio of selection response per generation ( $GR_{EvP}/Gen$ ) in the outcrossed/inbred populations was used as the effect size for evolutionary potential (see Appendix S4, Supporting information).

### *Data analyses for fitness*

As GR ratios are not normally distributed, central tendencies were reported as medians (more conservative) and both nonparametric analyses on untransformed data and parametric analyses on transformed data performed using MINITAB 16 or 17 software (Minitab Inc., State College, PA, USA). First, nonparametric tests were performed to evaluate the effects of potential predictor variables individually and rank correlations used to test for the effects of the continuous variables  $\Delta F_m$  and  $\Delta F_z$ .

Second, parametric multivariate regressions were performed on natural logarithms of GR ( $\ln GR$ ) ratios (closer to, but still not normal) as recommended by Borenstein *et al.* (2009) to evaluate the combinations of predictor variables, and best models delineated using AICc model selection (Akaike 1992; Anderson 2008). There were too few comparisons to include breeding system or differences among major taxa in model selection analyses, and the F1–F2 differences are statistically confounded with  $\Delta F_z$  and  $\Delta F_m$ . The order of inclusion and exclusion of variable in the model selection analyses was informed by the probabilities associated with each variable in multiple regressions. Analyses weighted by sample size were not used, as this would have exaggerated taxonomic weighting towards invertebrates (especially *Drosophila*).

As AICc model selection requires data sets with no missing values (Anderson 2008), and information on potential predictor variables was not always available, different subsets of my fitness data were used to evaluate different questions, as follows: (1) all fitness data points, (2) all data reduced to minimize pseudoreplication

(see below), (3) data points of (2) that had information on all predictor variables, (4) composite fitness data with pseudoreplication minimized and (5) data points from (4) that had complete information on all predictor variables and restricted to outbreeders (as the single entry for an inbreeding species was insufficient to allow testing of mating system effects). The entries used in each data set are identified in Table S1 (Supporting information).

Pseudoreplication was minimized by reducing the data set to a single entry per inbred/outbred population comparison [usually the one with the greatest statistical power and the most complete measure of fitness (see Appendix S3, Supporting information)], but with the retention of F1 versus F2 and later assessments, evaluations in benign and stressful environments and those with different  $\Delta F_s$ .

### *Consistency of genetic rescue effects*

The consistency of effects were evaluated using sign tests to compare the number of beneficial versus deleterious outcrossed/inbred comparisons both in fitness (data set 1) and in the evolutionary potential data sets.

### *Magnitude of genetic rescue effects on composite fitness*

The analyses of magnitude of effects of outcrossing concentrated on composite fitness (encompassing both reproduction and survival), as this is an approximation to total fitness, the ultimate measure of interest. Median GR ratios for composite fitness were computed for data set 4. These were carried out for all breeding systems and separately for naturally outbreeding species for both benign and stressful environments.

### *Tests for publication bias in the fitness data set*

I used a funnel plot approach (Møller & Jennions 2001) to assess the relationship between  $\ln GR$  ratio and log sample size weighting factor on fitness data set 1. However, I objectively assessed potential bias using linear regression and rank correlation, rather than relying on visual inspection, as researchers show poor ability to correctly interpret the plots (Terrin *et al.* 2005). The sample size weighting factor combined the number of inbred ( $n_I$ ) and crossed populations ( $n_C$ ), as done in *t*-tests ( $n_I n_C / [n_I + n_C]$ ; Sokal & Rohlf 2012). As both beneficial and deleterious effects on fitness in crosses are published (genetic rescue versus outbreeding depression), directional bias is unlikely.

## Results

### Consistency of fitness effects

The effects on fitness of gene flow into the inbred populations were overwhelmingly beneficial, with 145 beneficial, 2 equal and 9 deleterious ( $\chi^2 = 120.1$ , d.f. = 1,  $P < 0.0001$  for deviation from equality). Genetic rescue effects were exhibited for diverse fitness measures [e.g. composite fitness, population persistence, population growth rate, fecundity, survival, proportion of normal offspring, sperm quality and fertilization success (in plants)].

GR ratios ranged from 0.86 (mildly deleterious) in *Lymnea stagnalis* snails to infinity in *Ziziphus celata* plants (where the inbred self-incompatible populations only set seed after outcrossing; Weekley *et al.* 2002; Gitzendanner *et al.* 2012). Of the 10 highest ratios, half were self-incompatible plants, yet only 22 cross/inbred comparisons were for self-incompatible species.

The nine data points that showed deleterious effects of outcrossing on fitness (Table S3, Supporting information) involved combinations of low statistical power (8/9), measurement of fitness in benign environments (8/9), measurement of a single fitness component, rather than composite/total fitness (5/9), or were for species that naturally inbred, or derived from an inbred base populations of a naturally outbreeding species (6/9). Seven cases were for F1 data and only two for generations beyond F2, whereas outbreeding depression is more likely in the F2 and later generations. Convincing evidence for outbreeding depression was restricted to self-fertilizing *Caenorhabditis elegans* nematodes and was only mild (Dolgin *et al.* 2007).

### Magnitude of benefits for composite fitness

On average, there was a 57.5% improvement in composite fitness (data set 4) due to outcrossing across all mating systems (i.e. median GR = 1.575), but the benefits of outcrossing were much greater in stressful environments than in benign ones (148% versus 44.6%: Kruskal–Wallis  $H = 9.85$ ,  $P = 0.001$ ,  $n = 74$ ) as illustrated in Fig. S1 (Supporting information). For outbreeding taxa, the corresponding improvements were 162.5% and 50.9% (Kruskal–Wallis  $H = 9.73$ ,  $df = 1$ ,  $P = 0.001$ ,  $n = 64$ ).

### Variables affecting the magnitude of genetic rescue for fitness

Nonparametric tests of the effects of individual variables on fitness (Table 1) identified breeding system (outbreeding > inbreeding; see also Appendix S5,

Supporting information]), environment (stressful > benign) and source of immigrants (outbred > inbred) as significant comparisons. Benefits of outcrossing were 52% greater in outbreeding taxa than in inbreeding ones. Genetic rescue effects on fitness were not significantly different among invertebrates, vertebrate and plant taxa.

Somewhat surprisingly fitness in the F1 was less than that in F2 and later generations. However, the usual expectation of  $F1 > F2$  applies to traits determined by the zygotic genotype, while  $F2 > F1$  is expected for maternally determined traits (Appendix S2). Thus,  $\Delta F_m$  should be a predictor of genetic rescue, and this proved to be the case (Fig. 1). The rank correlation between GR and  $\Delta F_m$  for outbreeding species was significant (0.266,  $P = 0.014$ ,  $n = 69$ ), but that with  $\Delta F_z$  was not ( $-0.009$ ,  $P = 0.973$ ,  $n = 68$ ), and the simple linear regressions on  $\ln$  GR yielded similar conclusions (Appendix S5, Supporting information).

Akaike model selection on data set 3 (Table 2) identified environment as a predictor of the magnitude of genetic rescue in the best supported model (see Appendix S5, Supporting information for details of the best model). The adjusted  $r^2$  values for the best fitting model was 25.9%, higher than the averages of 2.5–5.4% for meta-analyses for ecology and evolutionary biology (Møller & Jennions 2002).  $\Delta F_m$  was also included as a predictor in the second best fitting model. Similar conclusions applied to AICc model selection on composite fitness with data set 5 (Table S4, Supporting information), and the  $r^2$  was 36.5%.

### Tests for publication bias in fitness data

Analyses on the all fitness data set 1 did not reveal statistical support for publication bias, as the regression of  $\ln$  GR on log weighting factor was nonsignificant ( $b = -0.203 \pm 0.152$ ,  $P = 0.18$ , adjusted  $r^2 = 0.53\%$ ,  $n = 150$ ) (funnel plot in Fig. S2, Supporting information), as was the rank correlation ( $r_s = -0.059$ ,  $P = 0.47$ ).

### Genetic rescue effects on evolutionary potential

Outcrossing within species had significant beneficial effects on evolutionary potential for fitness traits (6 beneficial: 0 deleterious comparisons:  $P = 0.016$  for deviation from equality) (Table S2, Supporting information). The median improvement in response per generation due to outcrossing was 22.4%. Further, there was a significant positive regression of  $\ln$  GR<sub>EVP</sub> per generation on  $GD_x/GD_I$ , the ratio of genetic diversity in the cross to that in the inbred population (Fig. 2), as predicted (see Appendix S4, Supporting information).

**Table 1** Median genetic rescue (GR) ratios and tests for effects of different variables on fitness based on data set 2\*

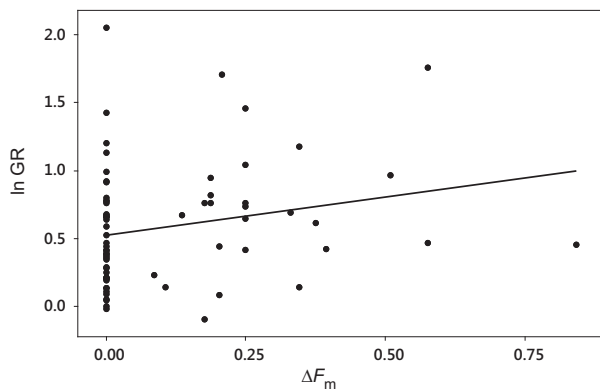
Variable	Median GR <sup>†</sup>	P-value <sup>‡</sup>	n
Mating system	Outbreeding > inbreeding		133
Outbreeding	1.788	<0.001	112
Selfing or mixed mating	1.165		21
Major taxa			133
Invertebrates	1.584	0.297	62
Vertebrates	1.942		22
Plants	1.591		49
Environment	Stressful > benign		114
Stressful/wild	2.139	<0.001	39
Benign	1.480		75
Generation			122
F1	1.509	0.032	85
F2 and later	1.964		37
Immigrants	Outbred > Inbred		120
Outbred	2.136	<0.001	33
Inbred	1.519		87
Trait	Composite > component		133
Composite	1.591	0.861	73
Component	1.637		60

\*See, Materials and Methods (Data analyses) for details of the different data sets.

<sup>†</sup>Medians vary for different data sets.

<sup>‡</sup>Probabilities based on nonparametric Kruskal–Wallis tests (one-tailed for directional hypotheses and two-tailed for major taxa and generation [F1 versus F2 and later generations]).

n = sample size.



**Fig. 1** Relationship between genetic rescue and decrement in maternal inbreeding. Plot of natural logarithm of genetic rescue ratio for fitness ( $\ln GR$ ) against difference in maternal inbreeding coefficient between crossed and inbred populations ( $\Delta F_m$ ) for outbreeding species in data set 2, with line of best fit shown ( $b = 0.563 \pm 0.298$ ,  $P = 0.32$ ).

## Discussion

The effects of outcrossing on fitness were consistently beneficial, resulting in a 148% higher composite fitness in stressful conditions and a 45% one in benign ones, and even greater benefits for outbreeding species (see also Appendix S6, Supporting information). These are likely to be underestimates of benefits, as most composite

fitness values were not for total fitness, and effects are likely to be greater as more of the life cycle is included (Frankham *et al.* 2010). Further, missing information (especially on chromosomes) increases the risk of outbreeding depression. Large fitness benefits of outcrossing inbred populations of domestic animals and plants have also been reported (Table S5, Supporting information).

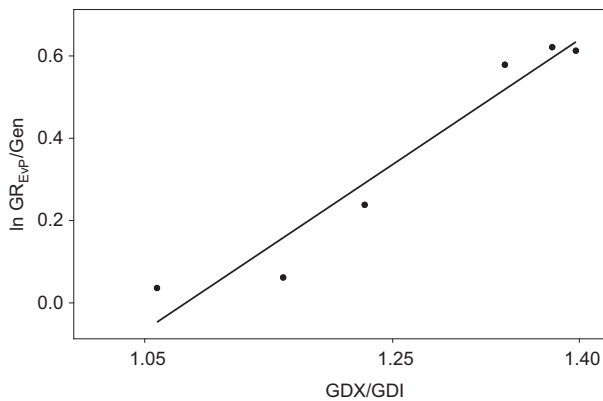
Detailed examination of the nine cases where gene flow mildly reduced fitness revealed only one convincing case of outbreeding depression and that was in a selfing nematode where the benefits of outcrossing are expected to be less than in natural outbreeders, and the risks of outbreeding depression are higher (Frankham *et al.* 2011). Even if there is modest outbreeding depression in a few cases, natural selection typically improves fitness over subsequent generations (Frankham *et al.* 2011), and crosses typically result in improved evolutionary potential.

The variables affecting the magnitude of fitness benefits from outcrossing were similar to those that affect inbreeding depression ( $\Delta F_m$ , stressfulness of the environment and mating system). Further, genetic rescue effects were similar for invertebrates, vertebrates and plants, concordant with observations that inbreeding depression is similar across major taxa (Crnokrak & Roff 1999; Frankham *et al.* 2010, 2014).



**Table 2** Model selection statistics for genetic rescue ratios for fitness based upon the Akaike AICc procedure for data set 3 ( $n = 53$ ). The best fitting model is bolded.  $K_i$  = number of parameters estimated, AICc = Akaike’s information criterion adjusted for small sample size,  $\Delta_i$  = deviation of the model from the best fitting model,  $w_i$  = Akaike weights (approximate probability that the model is the best information theoretical one), C is the constant (intercept),  $\Delta F_z$ , and  $\Delta F_m$  the difference in maternal and zygotic inbreeding coefficient between the outcrossed and inbred populations, and trait the difference between composite and component fitnesses

Model	$K_i$	AICc	$\Delta_i$	$w_i$
<b>C, environment, <math>\sigma^2</math></b>	<b>3</b>	<b>-90.401</b>	<b>0</b>	<b>0.5213</b>
C, $\Delta F_m$ , environment, $\sigma^2$	4	-89.405	0.995	0.3169
C, $\Delta F_z$ , $\Delta F_m$ , environment, $\sigma^2$	5	-87.440	2.960	0.1186
C, $\Delta F_z$ , $\Delta F_m$ , environment, trait, $\sigma^2$	6	-85.159	5.241	0.0326
C, $\Delta F_z$ , $\Delta F_m$ , environment, trait, inbred vs outbred immigrants, $\sigma^2$	7	-82.516	7.885	0.0101
C, $\sigma^2$	2	-75.759	14.639	0.0003



**Fig. 2** Relationship between genetic rescue for evolutionary potential per generation ( $GR_{EVP}/Gen$ ) and the ratio of genetic diversities in the outcrossed to that in the inbred parent populations ( $GD_X/GD_I$ ) ( $b = 2.015 \pm 0.255$ ,  $P = 0.0005$ ).

The finding that F2 genetic rescue was greater than for F1 may surprise many readers, as it conflicts with predictions based on zygotic  $\Delta F$  (Whiteley *et al.* 2015). This was likely due to fitness consisting of a combination of maternal and zygotic influences that have contrasting predictions for the sign of this difference. There was support for  $\Delta F_m$  as a predictor of GR in my analyses (rank correlation, single factor regression and the second best Akaike models for all fitness and composite fitness), despite its tests having relatively low statistical power.

The lack of statistical support for  $\Delta F_z$  as a predictor of genetic rescue for fitness is surprising (see Appendix S5, Supporting information for additional information). However, most fitness measures were truncated to less than the end of life, reducing the power of tests for zygotic inbreeding effects. However,  $\Delta F_z$  was still not supported by the model selection for composite fitness (with intercept fitted) (Table S4, Supporting information). Further, if the base populations from which the recipient and donor populations derived were already inbred, this will also reduce potential benefits of out-

crossing (but this will also reduce  $\Delta F_z$  effects). There can be no doubt that  $\Delta F_z$  effects exist, as they have been documented for grain yield in maize (Lindstrom 1941; Stringfield 1950; Moll *et al.* 1965), litter size in mice (Roberts 1960) and fitness in *Drosophila* (Barnes 1968), the latter two in addition to  $\Delta F_m$  effects.

The meta-analysis of fish crosses by McClelland & Naish (2007) also did not find F1 > F2, while that of Whitlock *et al.* (2013) found F1 > F2 for fitness traits across diverse animal and plant taxa. Such contradictory results likely arise from different mixtures of traits with predominantly maternal versus predominantly zygotic determination in different studies. Notably, Whitlock *et al.* (2013) avoided maternally influenced traits, but still found evidence for maternal influences, as outbreeding responses were lower for early acting than mid- and late-acting traits. Clearly, maternal inbreeding needs to be routinely included in considerations of genetic rescue.

There were also highly consistent and substantial benefits of outcrossing on evolutionary adaptation for fitness traits. All data came from invertebrates, but similar results are expected for vertebrates and plants. The effects of outcrossing are also overwhelmingly beneficial for traits only peripherally related to fitness in mice and *Drosophila* (see Appendix S7, Supporting information).

The screen against outbreeding depression developed by Frankham *et al.* (2011) was highly effective and justifies its use in assessing risk in crosses of inbred populations. However, the current results do not establish whether the screen for outbreeding depression is over zealous (Frankham *et al.* 2011).

My results signify that the current reluctance to use outcrossing to genetically rescue isolated inbred populations is not justified on genetic grounds, given the large and consistent benefits of genetic rescue and the effectiveness of the screen for outbreeding depression revealed herein. What of the other concerns listed in the Introduction that may be impeding genetic rescue attempts?

Lack of clear guidelines for genetic rescue (Point 3) may have discouraged managers of wild populations from attempting it. In Table 3, I provide updated guidelines, based on this study and Frankham *et al.* (2011, 2012). Hedrick & Fredrickson (2010) and Edmands (2007) previously provided guidelines, but mine are less stringent for reasons detailed in Appendix S8 (Supporting information).

The financial costs of augmenting gene flow (Point 4) may also impede its use. However, improvements in fitness averaging ~ 148% in stressful/wild conditions and 45% in benign/captive ones are very worthwhile in comparison with many other procedures that might be applied in the management of threatened populations, especially in plants. Even in high-cost cases, such as African lions, cost did not impede its use (Trinkel *et al.* 2008). High-cost cases will often correspond to charismatic species where it is easier to obtain funds for conservation actions.

There are legitimate concerns about spreading diseases, pest and parasites if these differ between the populations to be crossed (Point 5). However, populations that have experienced gene flow in the recent past (my focus) are likely to already share these organisms (or will in the future). Regardless, assessment by veterinarians (as is already routine for movement of zoo animals and often for translocations) or plant pathologists is recommended.

Mixing of animals for genetic rescue purposes may disrupt social systems (Point 6). However, means for

minimizing such problems have already been devised for zoo augmentations. For example, Taronga Zoo in Sydney, Australia, successfully introduced a young male into their chimpanzee population to reduce inbreeding, albeit over an extended period. Artificial insemination avoids disruption of social systems where it can be performed (e.g. black-footed ferrets, cheetahs, giant pandas, whooping cranes and elephants: Frankham *et al.* 2010). Alternatively, a resident male with a harem can be replaced with one that will augment gene flow, often with minimal social disruption.

Movement of individuals across political jurisdictions (countries, states and provinces) may be required for genetic rescues (Point 7). However, zoos and botanic gardens regularly move taxa across state and country boundaries (Fa *et al.* 2011). Several genetic rescues have involved such movements, including for Florida panthers (Johnson *et al.* 2010), greater prairie chickens in Illinois (Westemeier *et al.* 1998) and Lakeside daisies in Illinois (Demauro 1993). Recruiting community and NGO support is typically desirable in such cases. Regulatory barriers (Point 8) will typically slow genetic rescue attempts, but should rarely preclude outcrosses with species. Even an outcross between different subspecies was approved in the Florida panther case (Johnson *et al.* 2010).

None of the nongenetic issues impeding genetic rescue attempts is insuperable, especially given the large and consistent benefits typically revealed by genetic rescue attempts.

**Table 3** Guidelines for management of genetic rescues (see also Appendix S8, Supporting information)

When should we contemplate genetic rescues?

- 1 When there is a (recipient) population that is inbred and/or has low genetic diversity for fitness (or evolutionary potential), especially when it is known or suspected to be suffering from inbreeding depression for fitness
- 2 When there is another isolated population(s) of the same species (donor) to which it can be outcrossed to reverse inbreeding and loss of genetic diversity
- 3 When the risk of outbreeding depression in crosses between the donor and recipient populations is low through to the F3 generations or beyond, as determined for example using the decision tree of Frankham *et al.* (2011)
- 4 When the potential benefits of outcrossing are sufficiently large to justify the financial costs and any risk of outbreeding depression  
The benefits are expected to depend upon the following:
  - a The magnitude of  $\Delta F_z$  and  $\Delta F_m$
  - b The mode of reproduction in the species (sexually reproducing > asexual)
  - c The mating system in the species (self-incompatible > other naturally outbreeding > mixed mating > selfing)
  - d The ploidy in the species (diploid  $\geq$  polyploid > haplodiploid > haploid)
  - e The intended environment (stressful/wild > benign/captive)
  - f Inbreeding level in immigrants (outbred > inbred)
  - g Demographic history of, and genetic diversity in the base population from which the recipient and donor populations were derived (numerically large  $N_e$  with high genetic diversity > numerically small with low genetic diversity)
- 5 How many immigrants should be used? Any are better than none when the risk of outbreeding depression is low. At the upper end, there is the risk of genetically swamping the recipient population, so immigrant alleles should generally be  $\leq 50\%$  of the crossed population (see Appendix S8, Supporting information)
- 6 Will more than one augmentation of gene flow be required? The need for additional rounds of outcrossing will depend upon the proportion of immigrants (low > high), their inbreeding level (high > low) and the  $N_e$  in the crossed population (low > high)
- 7 Should the programme be monitored? Yes, essentially as suggested by Hedrick & Fredrickson (2010)

### Conservation implications

Large improvements in fitness and evolutionary potential can be made by augmenting gene flow into small inbred populations, provided the crosses have a low risk of outbreeding depression according to the screen of Frankham *et al.* (2011). The limited use of augmented gene flow in conservation settings is not justified scientifically, given the result of this study.

I recommend a much broader use of augmentation of gene flow to genetically rescue small inbred populations and to reduce species extinctions. If isolated populations continue to be small in size, outcrossing/augmentation of gene flow will need to be performed at regular intervals (Bouzat *et al.* 2009; Hedrick *et al.* 2014).

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R.F. conceived the study, collected and analysed the data and wrote the manuscript.

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### Data accessibility

Genetic rescue data sets for fitness and evolutionary potential are given in Tables S1 and S2 (Supporting information), respectively, as detailed below.

### Supporting information

Additional supporting information may be found in the online version of this article.

**Table S1** Genetic rescue (GR) data set 1 for fitness.

**Table S2** Genetic rescue data for evolutionary potential ( $GR_{EVP}$ ) for fitness traits.

**Table S3** Characteristics of the nine studies that reported deleterious effects of outcrossing.

**Table S4** Variables affecting the magnitude of genetic rescue for composite fitness based on model selection statistics using the Akaike AICc procedure on data set 5 ( $n = 29$ ).

**Table S5** Mean genetic rescue ratios (GR: F1/inbred parents) for outcrosses of inbred parental populations for fitness traits in several domestic plant and animal species.

**Appendix S1** Factors expected to affect the magnitude of genetic rescues for fitness.

**Appendix S2** Inbreeding depression and genetic rescue for maternally and zygotically determined traits.

**Appendix S3** Additional details of data selection criteria

**Appendix S4** Response ratio for evolutionary potential and variables expected to affect it.

**Appendix S5** Additional considerations of results.

**Appendix S6** Additional Discussion.

**Appendix S7** Beneficial effects of outcrossing on evolutionary potential for traits peripherally related to fitness.

**Appendix S8** Additional discussion of genetic rescue guidelines.

**Fig. S1** Histograms of natural logarithm of genetic rescue ratio (ln GR) for composite fitness in outbreeding species in benign versus stressful environments.

**Fig. S2** Funnel plot for all genetic rescue data for fitness (ln GR) from data set 1 against logarithm of sample size weighting factor (log  $w$ ).