



CEE review 09-001

DOES TRANSLOCATION OF INDIVIDUALS BETWEEN POPULATIONS RESULT IN NET COST OR BENEFIT TO FITNESS IN OFFSPRING OF MIXED POPULATIONS?

Systematic Review Protocol

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1 BACKGROUND

Genetic diversity is important for the sustainability of populations of living organisms. It provides the raw genetic material upon which natural selection can act in order to adapt them to changes in their environment (including current anthropogenic changes such as increased nitrogen deposition and climate change). For many taxa, genetic diversity is also important for the avoidance of inbreeding depression, which is the decline in fitness of offspring associated with sexual reproduction among related individuals. Inbreeding depression becomes more likely as populations become smaller in size and may contribute to the extinction of small populations (Reed and Frankham, 2003, O'Grady et al., 2006).

The need to equip natural populations with sufficient genetic variation for conservation purposes has been recognized for some time (Frankel, 1974, Frankel and Soulé, 1981), and a compelling body of evidence exists in the scientific literature documenting the detrimental effects of inbreeding depression in wild populations (reviewed in Crnokrak and Roff, 1999). A classic example is the case of the Florida panther, whose population had declined to a perilously small size by the latter part of the 20th century (Roelke et al., 1993). Individuals of this population suffered problems such as poor sperm quality, kinked tails and un-descended testicles, maladies that were attributed to the effects of inbreeding depression (Roelke et al., 1993) and reduced the chances of population survival and recovery. During 1995 individuals were translocated to the Florida population from a distinct population in Texas. Offspring descended from the mixed population had a much reduced occurrence of the detrimental phenotypes exhibited in the population prior to the translocation event (Land et al., 2001) and contributed to a subsequent recovery in numbers (Pimm et al., 2006). This effect of fitness recovery after translocation is termed “genetic rescue”, and has been observed in diverse taxa, for example, in fish (Vrijenhoek, 1994), birds (Westemeier et al., 1998) and plants (Willi et al., 2007) .

Despite the potential benefits (especially to small populations of naturally out-breeding species) of creating hybrid populations, the conservation movement remains cautious about applying population translocations widely as a tool to enhance population sustainability (Gregory et al., 2006). This caution is well founded, since it has been shown that in some circumstances, population hybridisation can lead to a reduction in fitness of the mixed population after translocation, called *outbreeding depression*. The basis of such a fitness reduction is that populations being mixed may have become adapted to the specific environments in which they exist, and/ or they may have diverged genetically such that genomic incompatibilities are exposed upon population mixing. A well-known example of this phenomenon is the reduction in fitness that can be observed in offspring derived from mating between individuals of different species (Arnold and Hodges, 1995). Within species it is expected that the extent and duration of outbreeding depression will depend on factors such as the physical, genetic and ecological distance amongst populations that become admixed (Edmands, 2002). For example, it is widely assumed that geographical distance between populations is a surrogate for the environmental (climatic, ecological) similarity among sites occupied by populations, and thus serves as a proxy for the potential adaptive differential between them. A further factor influencing the extent of local adaptation within and among populations is the degree of population connectivity, or gene-flow between them, related to the dispersal capacity and mating system of the species under consideration. Where migration among populations is high, the genetic composition of populations will become more homogeneous, potentially hindering the development of local adaptation (Storfer, 1999). The severity of outbreeding depression may also decline as natural selection begins to act upon the expanded pool of genetic variation that has been created by a hybridisation event (e.g. Carney et al., 2000), removing maladapted individuals and adapting the mixed population to its environment.

There are a number of reasons why conservation managers may choose to translocate or not to translocate individuals in order to reinforce a population that is in decline. These include, in addition to the scientific arguments outlined above, economic considerations and concerns that translocations may destroy the unique features of threatened populations that constitute biological heritage. There is also a risk that translocation might encourage the spread of parasites or disease. Finally, the conservation manager must take into account the possibility that in avoiding translocation, the threatened population may lack the genetic variability necessary for adaptation to future environmental changes. The scientific arguments for and against population reinforcement by translocation are currently of great interest to conservation practitioners (Gregory et al., 2006) and are also hotly debated, with different NGOs taking very different viewpoints about this problem. A growing body of evidence exists on outbreeding-depression, and it is in the interest of the conservation community that this evidence should be synthesized and disseminated so that conservation actions may be enhanced and guided by the best available evidence.

The aim of this review is to ask whether we can defend scientifically the position of avoiding population mixture by translocation because of possible differences in local adaptation between the source populations. Does outbreeding depression (as a *net* cost to fitness in the progeny arising from population mixtures) really exist? If it does exist, in what sorts of species can we expect to observe it? How does it relate to physical distance between populations and to species' intrinsic dispersal capabilities, and can we expect the initial effects of outbreeding depression to decline during the generations following a translocation event?

2 OBJECTIVE OF THE REVIEW

2.1 Primary question

Is there a net decline in fitness in the offspring of populations that have been reinforced with individuals from a different population?

3 METHODS

3.1 Search strategy

3.1.1 Scope of search

We will search the following computer databases for relevant literature and data:

BIOSIS previews
Copac
Dissertation Abstracts
Index to Theses online
ISI proceedings
ISI Web of Science
Science Direct
Scirus (first 50 results from journals-only searches)
Scopus

We will also use the following search engines to obtain internet-based material:

www.excite.com
www.google.co.uk

The first 50 hits (Word and/or PDF documents where this can be separated) from each internet search will be examined for appropriate data which will be retrieved.

The following GO and NGO libraries will be examined for grey literature:

NE
CCW
SNH
JNCC
Plantlife
BSBI

Bibliographies and citations of all relevant articles arising from the sources above will be searched for further material. Once relevant literature has been identified, we will contact the authors of this work to determine whether further unpublished data exist that are relevant to the problem.

The GO and NGO libraries specified above do not represent a complete list of all possible sources of grey literature from GO and NGO organisations worldwide. Preliminary assessment of articles from these sources indicated a very low yield of relevant material (and therefore a high cost to searching these data-sources). The subject of the question genetics/evolution also increases the likelihood that relevant material will be found in journal articles rather than in the grey literature. Therefore we will not at present expand the list of organisations through which we aim to search for grey literature. We will investigate the potential for publication bias that this might introduce (see below) and if necessary modify our search strategy at that point.

3.1.2 Search terms

We will use the search terms set out below to retrieve articles from the databases specified above. More specifically, we will conduct searches using compound search strings generated by combining the search terms in groups (i) and (ii) with each of the terms in group (iii) for a total of $9 + 15 = 24$ search strings. Search terms in group (iv) are catch-all phrases that will be used to carry out searches without combination in to more complex strings. The search may be modified to adjust specificity and sensitivity in relation to the functionality of the different sources. Records of search strategy used will be maintained to ensure repeatability and transparency.

i. Outbreeding-related terms

Outbreeding depression OR out-breeding depression OR outcrossing depression OR out-crossing depression OR out-mating depression OR outmating depression
(Hybridisation OR hybridization) AND (population OR interpopulation OR inter-population) NOT (interspecific OR inter-specific)
Heterosis AND population

- ii. Search terms related to the movement of individuals for conservation purposes

- Translocation AND conservation
- Reinforcement AND conservation
- Augmentation AND conservation
- Restoration AND conservation
- Genetic rescue AND conservation

- iii. Fitness-related terms

- Fitness
- Surviv* OR mortality OR longevity
- Fecundity OR reproduc*

- iv. Catch-all search phrases

- Distance-dependent fitness
- Distance-dependent crossing success
- Distance-dependent mating success

3.1.3 Secondary search to identify inbreeding extent and effects

The search terms described above exclude terms related to inbreeding depression. However, in terms of the central question of this review, inbreeding is relevant since a translocation may effect relief of inbreeding depression (gain in fitness), and because inbreeding is one end of a continuum that involves outbreeding depression. However, the literature on inbreeding is enormous and this may complicate delivery of the review. Therefore we will search for literature on inbreeding extent and effects for only those taxa that are subjects of relevant studies identified in the primary search. This approach will circumvent the confounding of outbreeding and inbreeding effects and will provide information on inbreeding with which to contextualise our findings. If sufficient information exists on inbreeding for taxa in which outbreeding has also been studied, we will include inbreeding as a source of heterogeneity (see below).

3.2 Study inclusion criteria

3.2.1 Relevant subject(s)

Individuals of any natural population of animal or plant (or experimental individuals derived directly therefrom), at any location globally, and their progeny. We define natural populations to include naturalised or (re-) introduced populations that persist in the absence of further human intervention. Studies that describe populations with alternative phraseology such “provenance”, “land-race” or “cultivar” will be retained until it can be ascertained from the article full-text whether they fit the other criteria stated in this section. For the purposes of this review, outbreeding effects on progeny are defined as occurring within post-zygotic portion of the life-cycle only. This condition excludes pre-zygotic mating incompatibilities from consideration, but may

include, for example, progeny traits such as germination success. We will not include studies involving the measurement of fitness for progeny arising exclusively from crosses amongst agricultural cultivars or strains, among micro-organisms or populations under captive management (e.g. zoo populations). These populations have been subjected to a different selective regime (e.g. directional selection for yield and or disease resistance, adaptation to captivity) than may prevail in natural populations. We will, however, include studies that observe the effects of hybridisation between natural populations and farmed individuals of a wild species (not strains or cultivars). Studies focussing on crosses or hybridisation among different recognised species (but not sub-species) will be excluded. Studies focussing on crosses or hybridisation among taxa with uncertain taxonomy at the species level will be excluded.

3.2.2 Types of intervention

We will consider the following interventions:

- i. Experimental translocation of individuals from a donor population to a recipient population within a single species.
- ii. Observation of natural migration between populations and the assessment of relative fitness of immigrants, native and hybrid offspring (for a single species).
- iii. Experimental crosses between individuals from within one population and between individuals from two populations (within a single species).

3.2.3 Types of comparator

Sexual progeny that is less outbred than progeny in the treatment group. This may include progeny arising from matings occurring over a lesser physical distance than those in the treatment group, or progeny whose parents originated from a common population versus treatment progeny whose parents came from distinct populations.

3.2.4 Types of outcome

- i. Survival, reproductive success, or other fitness related phenotype of progeny resulting from crosses or population admixture in F1 or subsequent generations
- ii. Increases or declines in traits that are components of fitness (fecundity, fertility, viability, longevity, survival, survival and reproduction of progeny) in F1 or later generations
- iii. Population-level measures of translocation success (changes in population size or density post-translocation), but only where appropriate comparator populations exist.

3.2.5 Types of study

Any study of fitness effects arising from out-crossing between populations where there is either a within-population cross as comparator, or data on the fitness of the parent population(s) preceding the out-crossing event. We will not include studies in which mean d-squared has been regressed against components of fitness within natural populations (1) because there is no comparator and (2) because doubt exists as to whether the measure faithfully reflects differences in the quantity of inbreeding

or outbreeding that occurred to create an individual's genome (Hedrick, 2001). Finally, we will not include studies that review or meta-analyse the existing literature or those studies whose results are based on theoretical modelling.

3.2.6 Potential sources of heterogeneity (effect modifiers)

We will investigate the effects of the following sources of heterogeneity (effect-size moderators) in influencing the outbreeding effect sizes in individual studies:

- i. Taxon-specific responses (e.g. insect, plant, mammal)
- ii. Physical and latitudinal distance among treatment and comparator groups
- iii. Time in generations since initial outbreeding/ hybridisation event
- iv. Life history component/ trait type (e.g. reproductive, morphological, viability/survival)
- v. Level of genetic distance or divergence between treatment and comparator groups
- vi. Mating system (e.g. inbreeding/ outbreeding/ mixed)
- vii. Environment in which fitness observed (e.g. experimental vs field)
- viii. Dispersal ability of study taxa (since this influences population connectivity and the expectation of outbreeding depression)
- ix. Inbreeding coefficient within control group (to account for possible confounding between inbreeding and outbreeding effects)
- x. Population history (large, small, expanding, contracting)

3.2.7 Assessment of study relevance

We will assess studies for inclusion in the meta-analysis based on a hierarchical assessment of relevance by scanning article titles, followed by reading the abstract of articles with relevant titles, followed by reading the full-text of articles with relevant titles and abstracts. Studies will be deemed relevant based on the presence of the desired subject and intervention, at least one of the comparators listed above, as follows:

- i. Pre-assessment removal of irrelevant medical literature

Trial searches using the original search terms (above) pick up a significant number of medical references that can be removed from the database by searching the endnote library with the terms specified below and deleting the results. The validity of this approach was confirmed by carrying out the searches on a randomly-selected 400 reference subset of the database and counting the number of relevant references that occur based on title assessment. No relevant references were found with these terms.

Searches in 'Journal'/ 'Secondary Title'

- Cancer

Searches in 'Any Field'

- In situ hybridisation
- In-situ hybridisation
- In-situ-hybridisation

- In situ hybridization
- In-situ hybridization
- In-situ-hybridization
- HPV
- Carcinoma
- Oncology

ii. Assessment of relevance by title

During assessment of the titles of the collected literature we will adhere to the following rules:

- Any title pertaining to quantified inbreeding or outbreeding effects should be retained,
- Any reports that describe habitat conservation or restoration projects will be excluded.
- Any studies reporting molecular markers linked to specific traits should be excluded.
- Any study reporting solely the description of a species mating system or the genetic characteristics of a species mating system should be excluded.
- Any study reporting between-species comparisons should be excluded.

Articles that are either meeting abstracts or book sections will be assessed for relevance as above, and all reasonable effort will be undertaken to recover original data (or summaries thereof) from the authors where it has not also been published in the primary literature.

Repeatability of the article selection process will be determined through the assessment of the same literature database (or subset) by two investigators working independently. The congruence of study selection by the investigators will be assessed by kappa analysis.

3.3 Study quality assessment

Once all relevant full-text articles have been gathered, we will assess the quality of the collected literature by determining, for each article, a score based on the presence or absence of attributes that indicate its quality and suitability for inclusion in the meta-analysis. The attributes to be investigated are summarised in Table 1 below. We will assign one point for each of the desired attributes that a study possesses.

Table 1 Attributes used to assess the quality of studies short-listed for inclusion in the systematic review

	Design feature	Study attribute
Internal validity of study	Correct comparator	Both progeny from inbred and intra-population crosses as comparator
	Temporal controls	Are outbred and more inbred individuals studied at the same time, or within the same generation?
	Accuracy of trait measurement	Are traits measured objectively?
		Are individual trait measurements repeatable, as judged by formal tests of repeatability?
	Sampling strategy	Were multiple sets of populations studied?
Was the study itself replicated on multiple occasions?		
Random sampling of study populations		
Random allocation of study individuals to crossing treatments OR random selection of study individuals in the wild		
External validity of study	Environment for trait observations	Traits of progeny observed within the environmental context of a natural population
	Observation window for inbred/outbred individuals	Fitness of progeny tracked until at least the F2 generation

3.4 Data extraction strategy

The review team will extract data from the short-listed studies, and also approach the authors of these studies to (1) request the raw data underpinning their study and (2) determine whether unpublished data exist that are suitable for inclusion in the review

3.5 Data synthesis

3.5.1 Effect size metric

We will summarise fitness trait data measured on continuous or counted scales (e.g. growth rate, seed production, lifespan) using Cohen's d , drawing upon a pooled standard deviation for outbred and control groups in each case study. Where trait data are binary in nature (e.g. survival) we will either:

- i. Conduct separate sub-group analyses

- ii. Convert the log odds ratio of the trait into a conventional effect size (Chinn, 2000)
- iii. Meta-analyse the outbreeding coefficient ($X_{\text{OUTBRED}}/X_{\text{CONTROL}} - 1$; Edmands, 2007), since this measure is appropriate for both binary and continuous outcomes. X_{OUTBRED} and X_{CONTROL} are mean trait values for outbred and control offspring groups, respectively

3.5.2 Model and hypothesis testing

Effect size estimates will be meta-analysed using a linear mixed-effects model implemented via the S-Plus/R-function MiMa (Viechtbauer, 2006). In this model, case study identity will be fitted as a random effect, and effect size moderators (sources of heterogeneity) will be fitted as fixed effects. The sampling variance of effect size estimates will enter the model as error variance terms. Residual heterogeneity in effect size will be estimated via restricted maximum-likelihood. Some studies will contain results for multiple traits for a single species. This situation will be handled by labelling the results from different traits with the same random effect label for case study identity

The meta effect-size and effect size moderator parameters will be extracted from the model. Significance of each of these parameters will be tested through the MiMa function via a z-test against the null hypothesis that the true parameter value is equal to 0.

An omnibus test that any of the effect-size moderators (as a group) have parameter estimates significantly different from 0 will also be carried out (Viechtbauer, 2006).

Presence of residual heterogeneity among effect size estimates will be tested using the standard Q statistic (Hedges and Olkin, 1985)

Results of the meta-analysis will be presented graphically using standard approaches such as forest plots for the effect- and meta effect-sizes and funnel plots as a graphical check for the presence of publication bias.

3.5.3 Sensitivity analysis

We will run sensitivity analyses to determine the effects of study quality on the outcomes of the meta-analysis. This will involve splitting the data into two groups based on the study quality score (Section 3.3). These will then be meta-analysed separately as above in order to investigate the role of study quality in modifying the results.

We will also run similar sensitivity analyses if we identify studies that contribute individual results that are outliers. In this case the outlier studies(s) will be excluded to investigate the dependence of the results on these extreme values.

The sensitivity of the results to the “file drawer problem” will be quantified by calculating a fail-safe n for any significant meta-effect-size detected. We will also attempt to identify temporal trends in the proportion of studies that report heterosis vs. outbreeding depression, since there may have been initial under-reporting of outbreeding depression due to doubts regarding its existence. This may indicate

whether there is a specific “file drawer problem” surrounding the outbreeding depression literature.

The sensitivity of the results to situations in which individual studies contribute more than one (potentially non-independent) effect size will be investigated by random truncation of such studies to a single effect size and reanalysis of the truncated data set.

3.6 Recognised limitations

The approach adopted in this review has several recognised limitations:

- i. The literature suitable for meta-analysis may be biased in taxonomic scope, either through low study number, or through bias towards those taxa in which the problem of outbreeding depression is most tractable
- ii. This review will admit studies where fitness has been investigated in an experimental environment (as well as in the wild). This raises the possibility that outbreeding depression is either over- or under-estimated in the experimental environment, or that traits relevant to survival in the field are not measured
- iii. Fitness is a combination of both reproductive output over the lifespan and survival (including the subsequent success of offspring). Studies synthesised in this review may fail to capture this broader definition of fitness, and so may contribute biased estimates of outbreeding depression
- iv. There may be relatively few studies that follow later generation effects of outbreeding. This may mean that any meta-effect size detected may rely too heavily on the initial outbreeding effect, while not taking into account the longer term benefits or costs to outbreeding

3.7 Knowledge transfer strategy

On completion of the review, we will produce a summary document written in accessible language that can be used by conservation practitioners. This document and the completed review will be made available on the internet, and distributed amongst organisations that are members of the Genetics in Conservation Working Group and the Plant Conservation Working Group.

In communicating the systematic review and its summary to practitioners we will endeavour to make clear the potential limits and pitfalls in interpreting the results. This is necessary to avoid over-simplification of the results and extrapolation of the findings to situations in which they are no longer relevant. For example we will make clear that if population mixture (outbreeding) has a net benefit, it is not also possible (from this study) to conclude that translocations will be beneficial with respect to mitigating the effects of climate change. We will, however, communicate the follow-on questions that can and need to be addressed as a result of the findings of our review.

4. POTENTIAL CONFLICTS OF INTEREST AND SOURCES OF SUPPORT

5. REFERENCES

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