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SWINGER: a user-friendly computer program to establish captive breeding groups that minimize relatedness without pedigree information

JONATHAN SANDOVAL-CASTILLO,* CATHERINE R. M. ATTARD,*† SHASHIKANTH MARRI,‡ CHRIS J. BRAUER,* LUCIANA M. MÖLLER*† and LUCIANO B. BEHEREGARAY*

*Molecular Ecology Lab, School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia, †Cetacean Ecology, Behaviour and Evolution Lab, School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia, ‡Flinders Genomics Facility, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia

Abstract

Captive breeding programmes are often a necessity for the continued persistence of a population or species. They typically have the goal of maintaining genetic diversity and minimizing inbreeding. However, most captive breeding programmes have been based on the assumption that the founding breeders are unrelated and outbred, even though in situ anthropogenic impacts often mean these founders may have high relatedness and substantial inbreeding. In addition, polygamous group-breeding species in captivity often have uncertain pedigrees, making it difficult to select the group composition for subsequent breeding. Molecular-based estimates of relatedness and inbreeding may instead be used to select breeding groups (≥two individuals) that minimize relatedness and filter out inbred individuals. SWINGER **constructs breeding groups based on molecular estimates of relatedness and inbreeding. The number of possible combinations of breeding groups quickly becomes intractable by hand.** SWINGER **was designed to overcome this major issue in ex situ conservation biology. The user can specify parameters within** SWINGER **to reach breeding solutions that suit the mating system of the target species and available resources. We provide evidence of the efficiency of the software with an empirical example and using simulations. The only data required are a typical molecular marker data set, such as a microsatellite or SNP data set, from which estimates of inbreeding and pairwise relatedness may be obtained. Such molecular data sets are becoming easier to gather from non-model organisms with next-generation sequencing technology.** SWINGER **is an open-source software with a user-friendly interface and is available at http://www.molecularecology.flinders.edu.au/molecular-ecology-lab/software/swinger/swinger/ and https://github.com/Yuma248/Swinger.**

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Introduction

The unescapable influence of anthropogenic activities has led to the need for captive breeding of populations and species to preserve their unique evolutionary composition, which is inherently genetic. This requires captive breeding programmes to minimize inbreeding and the loss of genetic diversity (Frankham 2010), which is often accomplished through pedigree records and associated decisions about the breeder composition of the following generation (Ballou & Lacy 1995). However, kinship can be difficult or impossible to determine directly in species that live in groups or have a promiscuous mating system (Griffith *et al.* 2002). Also, in captivity, the populations are often closed and small, so fail to be sustainable as they will inevitably lose genetic diversity over time (Lacy 2013). There is a need to rectify such issues to advance ex situ conservation programmes.

Molecular markers have been used to inform and improve captive breeding programmes, such as by filling incomplete pedigrees (Ivy *et al.* 2009) and assessing genetic diversity (Witzenberger & Hochkirch 2011). They have, however, rarely been used to estimate pairwise relatedness in wild individuals brought to captivity to start a breeding programme (reviewed in Attard *et al.* 2016b). Such individuals are instead assumed to be unrelated and not inbred (Rudnick & Lacy 2008). Molecular inferences of relatedness are also rarely used in the captive management of polygamous group-breeding species

Correspondence: Jonathan Sandoval-Castillo, Fax: +61 08 8201 3015; E-mail: jonathan.sandoval-castillo@flinders.edu.au

that typically have uncertain pedigrees (e.g. Wang 2004). The under-utilization of molecular markers since their maturity is a major oversight in many ex situ conservation programmes. Of much concern is that the assumptions made about the founding individuals disregard that small population sizes, which are typical of the threatened populations from which founders are sourced, often result in increased genetic drift, loss of genetic diversity, high relatedness and inbreeding (Frankham 2005).

Relatedness estimates of the founders can be used to help implement the most popular captive breeding strategy for conservation, the mean kinship (MK) strategy. This strategy is breeding in pairs the individuals that have the lowest MK, where MK is the average kinship of an individual to itself and to every other individual (Ballou & Lacy 1995). Kinship (*f*) refers to the probability that two alleles at a locus, one randomly chosen from each of two individuals, are copies of one ancestral allele [identical by descent (IBD)]. As all alleles eventually coalesce at some point to a common ancestor, kinship is calculated relative to a reference generation or population where all individuals are assumed to be unrelated (Ivy & Lacy 2010). Relatedness (*r*) derived from molecular markers is an estimate of the expected proportion of alleles that are IBD between two individuals, and so can be converted to kinship by dividing by two. In addition, individuals with a high level of inbreeding could be removed from consideration as breeders. There are various molecular estimates of inbreeding, such as internal relatedness, which is an estimate of the relatedness between the parents of an individual (Amos *et al.* 2001). While not directly equivalent, this is similar to the coefficient of inbreeding, which is the probability that two alleles in an individual at a locus are IBD as calculated from a known pedigree (Wright 1922).

The MK strategy and any other pairing strategy can be difficult to implement in the many species that live in groups of more than two individuals or are polygamous. Group-breeding strategies must instead be implemented (e.g. Wang 2004). When founding these groups or rearranging groups already in captivity, the possible combinations are a factorial function of the number of breeders that therefore quickly increase with the number of potential breeders to millions of possibilities. It is intractable to find the best solution by hand that minimizes relatedness between potential parents within groups, as well as takes into account other factors like the level of inbreeding permitted within breeders, and the average relatedness permitted within each group. It is surprising that, to our knowledge, no programme has been developed for this purpose. The lack of such a programme can severely hinder the implementation of ex situ conservation genetics programmes for group-breeding species.

The development of the computer program presented here was motivated by a captive breeding programme of two endangered fish species of no economic importance that required breeding groups of more than two individuals (Attard *et al.* 2016b). One of the species, the southern pygmy perch (*N. australis*), is a small (<10 cm length) freshwater fish endemic to south-eastern Australia and is used here as the empirical example for the computer program. This species has a locally adapted lineage recognized as a management unit (MU) (Hammer 2001; Cole *et al.* 2016) in the lower Murray–Darling Basin. This lineage lost its habitat and presumably became extinct in the wild by 2010 due to a decade-long drought exasperated by irrigation for agriculture (Kingsford *et al.* 2011; Van Dijk *et al.* 2013). In a monumental, collaborative effort by government and nongovernment agencies and other stakeholders, the southern pygmy perch was rescued from the wild before local extinction, captively bred, and released when suitable habitat returned after the drought (Hammer *et al.* 2013). We at Flinders University, South Australia, conducted a genetic-based captive breeding programme to maximize the maintenance of genetic diversity and minimize inbreeding (Attard *et al.* 2016b). In brief, we developed species-specific microsatellites using NGS technology (Carvalho *et al.* 2012). The potential founders were analysed at 14 microsatellites for southern pygmy perch to estimate the internal relatedness of each individual and pairwise relatedness between individuals. We disregarded potential founders that were positive outliers for internal relatedness and, due to no available computer program, created breeding groups by hand that had low pairwise relatedness estimates within breeding groups. Due to the ~9.5E+67 possible combinations of breeding groups, while we tried to minimize relatedness, we undoubtedly did not have the best possible suite of breeding groups. We subsequently developed the computer program SWINGER, our solution to automating breeding group selection for founding breeding programmes and potentially subsequent generations in breeding strategies requiring groups of two or more individuals. It is designed to be highly flexible, with the user able to decide the number and sex composition of breeding groups and the allowable level of internal and pairwise relatedness given the available resources for captive breeding and the biology of the species. We present it here and showcase its potential using the empirical data from the founders for the pygmy perch breeding programme.

Program

SWINGER implements an algorithm to determine the best possible combination of breeding groups based on user-supplied estimates of internal relatedness and pairwise relatedness, and user-defined maximum thresholds of internal relatedness, pairwise relatedness, and average pairwise relatedness in breeding groups and across breeding groups. It is an open-source program freely available from http://www.molecularecology.flinders.edu.au/molecular-ecology-lab/software/swinger/swinger/ or https://github.com/Yuma248/Swinger with a user manual and example input files. It can be run in Windows, Linux or Unix operating systems. The algorithm is written in Perl, making it straightforward to alter for those with a limited amount of programming experience and has a user-friendly Java graphic interface (Fig. 1).

The user-supplied pairwise relatedness estimates can be derived from genotype data using any of the already available relatedness estimators and user-friendly programs, such as GENALEX (Peakall & Smouse 2006, 2012) or COANCESTRY (Wang 2011), the latter also being available as an R package RELATED (Pew *et al.* 2015). The estimates are supplied to SWINGER as a square matrix in a tab-delimited text file. As some programs that estimate relatedness may only output a table, such as COANCESTRY, there is an option within SWINGER to convert a table format to the required square matrix format. The input file also requires information about the sex and level of inbreeding for each individual. Inbreeding for each individual can be estimated as internal relatedness using STORM (Frasier 2008) and is hereafter referred to as internal relatedness to not confuse it with producing inbred offspring from the founders. Alternative measures that can be used are standardized heterozygosity (Charlesworth & Charlesworth 1999) and homozygosity by loci (Aparicio *et al.* 2006). These, as well as internal relatedness, can be calculated using the R package RHH (Alho *et al.* 2010) or the Excel macro 'IRmacroN4' (www.zoo.cam.ac.uk/directory/william-amos). If sex is unknown (e.g. hermaphrodites) or the user does not wish to consider internal relatedness (e.g. not enough individuals), then values in the input file and parameters

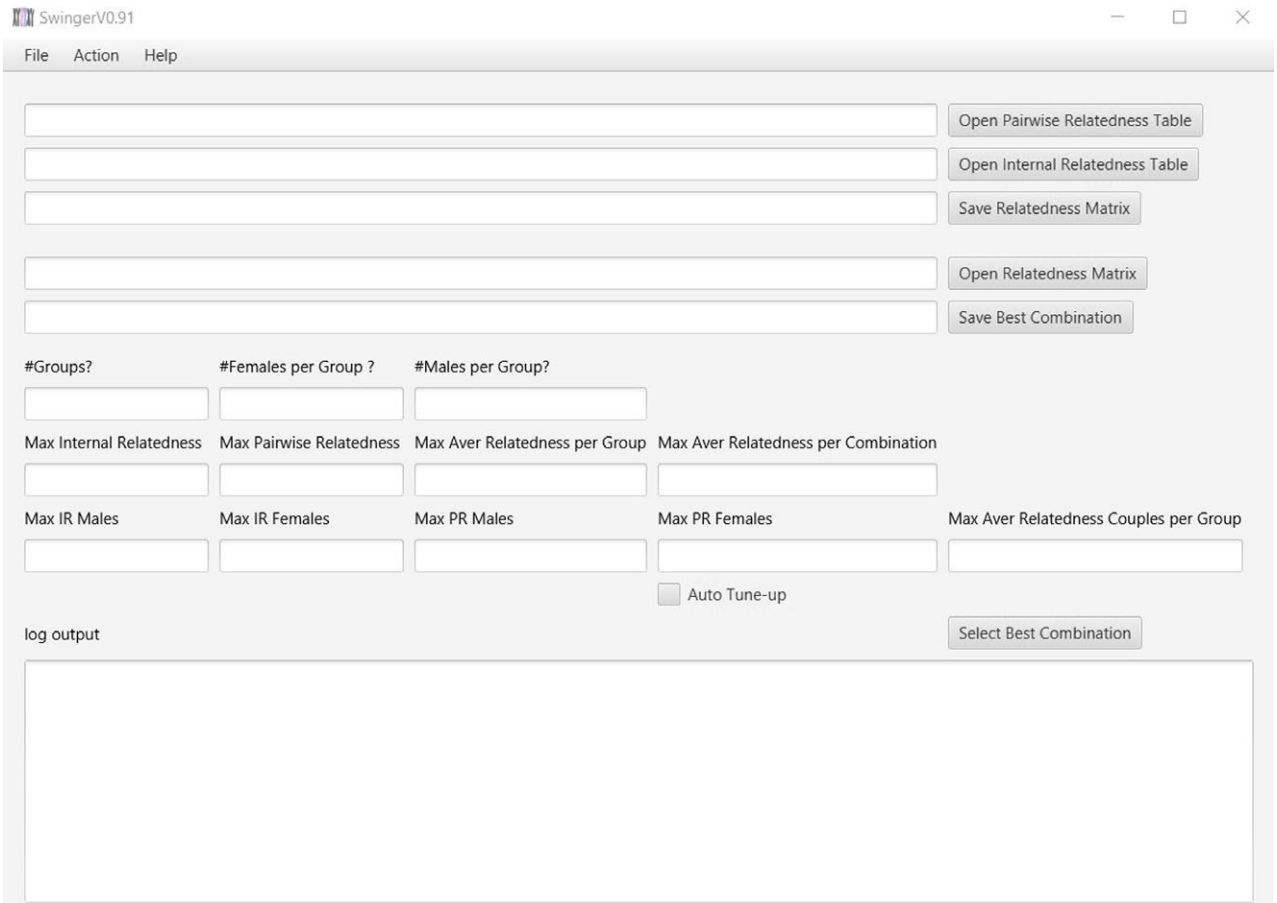

Fig. 1 The user-friendly SWINGER graphic interface. [Colour figure can be viewed at wileyonlinelibrary.com].

described below can be chosen in such a way that sex and internal relatedness are not considered in breeding group allocations.

Parameter values for the set-up the user desires for the breeding programme are entered directly into the graphic interface and are highly flexible. The structure of the breeding programme is determined by setting the number of breeding groups and how many females and males are in each group. The remaining parameters are maximum thresholds permitted for internal relatedness, pairwise relatedness, and average pairwise relatedness in breeding groups and across breeding groups. There are options for different thresholds of internal relatedness depending on sex, pairwise relatedness in groups depending on whether the pair is female–female, male–male or female–male, and for average pairwise relatedness in or across groups depending on whether to base it on all pairs regardless of sex or only on female–male pairs.

The algorithm (Fig. 2) first excludes from consideration as breeders the individuals that have an internal relatedness above the set value. It then excludes from consideration the pairs of individuals that have a pairwise relatedness above the corresponding threshold, taking into account whether there are different settings for male–male, female–female and female–male pairs. If the breeding programme is based on breeding groups of more than two individuals, the algorithm then forms breeding groups using the pairs that passed the previous pairwise relatedness filter and excludes any groups formed that are above the average relatedness threshold. This takes into account whether the average relatedness is based on all pairs regardless of sex or only female–male pairs. It then creates combinations of breeding groups that passed the previous thresholds to form the user-defined number of breeding groups. An individual is only permitted to be used in one of the groups within each combination. If desired, high value breeders can be used in multiple breeding groups by replicating them in the program input. The algorithm then excludes combinations that do not pass the threshold for pairwise relatedness averaged across all breeding groups.

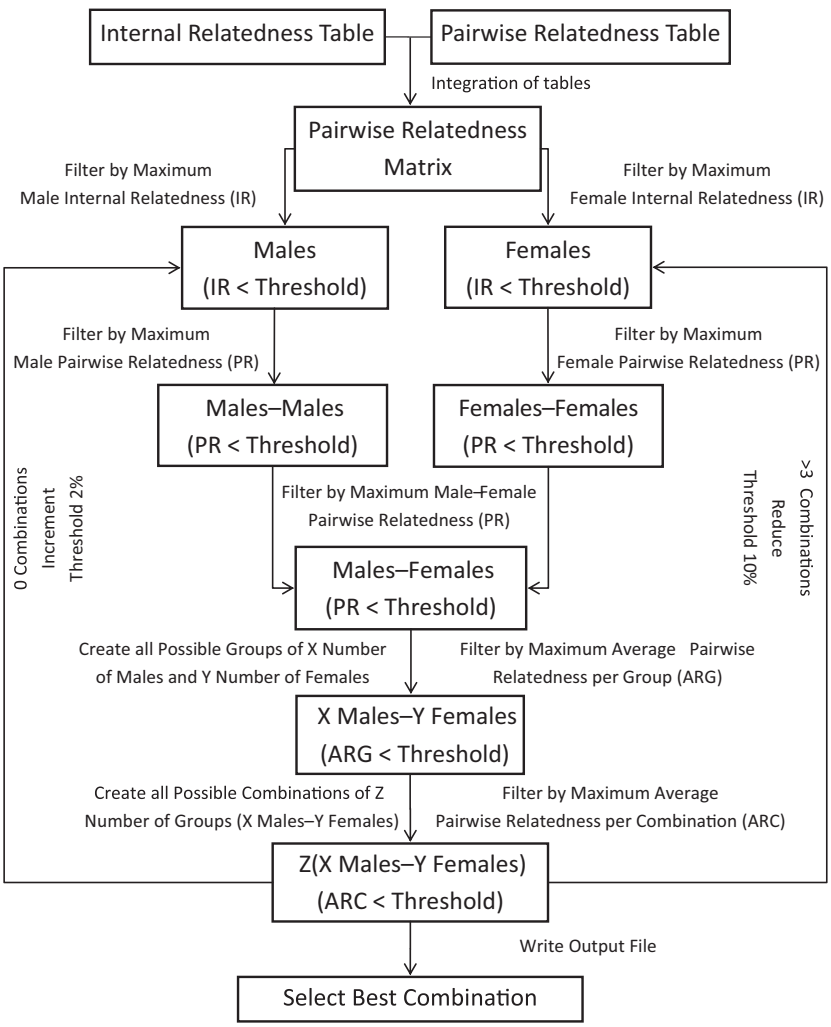

Fig. 2 Flow-chart representation of the algorithm implemented in SWINGER.

The algorithm will be unnecessarily computationally demanding when the user chooses relatively high values for parameters. Specifically, it will continue to search and report the tens, hundreds or orders of magnitude more combinations of breeding groups that pass the thresholds when only one or a few optimal solutions are typically desired. To prevent this, it will cease and produce an explanatory message when it finds a fourth solution, even if more as-yet-unknown solutions exist. The thresholds need to be decreased and the algorithm rerun until no more than three solutions are found. If a parameter or parameters are deemed by the user to be particularly important in improving the success of the breeding programme, these parameters may be made more stringent. If the user has little idea of what thresholds to initially try, we recommend setting the thresholds as follows: pairwise relatedness to the average pairwise relatedness of the data set, average pairwise relatedness in groups to 10%–50% less than the pairwise relatedness and average pairwise relatedness across all groups to 10%–50% less than the average pairwise relatedness in groups. When no solutions are reported, at least one of the parameters is too stringent and must be relaxed to reach a solution. The algorithm also has an option to automatically tune some parameters until one to three solutions are reached. It reduces by 10% the initial user-supplied values for pairwise relatedness and average relatedness within- and among-groups when there are more than three solutions, or increases by 2% these thresholds when there are no solutions. Although this function is useful, the final result is still dependent on the user-supplied values, and the function is computationally time-consuming if these are numerically far from the final threshold values. So, the user-supplied values should still be case specific when using this function.

Empirical example

The captive breeding of southern pygmy perch is provided as an empirical example. This breeding programme is described in detail by Attard *et al.* (2016b). We created founding breeding groups using SWINGER and compared these with those created by hand in the original breeding programme. The input files are available as example files on the webpage for SWINGER. The data set consists of 63 potential founders, with Queller & Goodnight (1989) pairwise relatedness estimates calculated using GENALEX, internal relatedness calculated using STORM, and sexes determined by visual inspection.

The number of breeding groups was 11, each consisting of two females and two males. These numbers were those used by Attard *et al.* (2016b) based on the mating system of the species and the number and composition of breeders available. The maximum threshold for internal relatedness was the value used by Attard *et al.* (2016b) to exclude outlier individuals: 0.424. This resulted in one southern pygmy perch being excluded as a founder from the original breeding programme. This individual was therefore not included in the input files for SWINGER.

Values for the maximum threshold for pairwise relatedness, and average relatedness in groups and across groups, were varied based on the empirical distribution of pairwise relatedness and trial runs of different parameters without the automatic tuning option. Then, the automatic tuning option was used on a final decided set of values. These were −0.05 for pairwise relatedness, −0.1 for average relatedness in groups and −0.15 for average pairwise relatedness across groups. Note that the average relatedness in groups and across groups was not considered in the original breeding programme due to the difficulty in accounting for this by hand. SWINGER output two solutions of breeding group combinations (Table S1, Supporting information). The final thresholds after tuning were −0.0605 for pairwise relatedness, −0.121 for average relatedness in groups and −0.166535 for average relatedness across groups. The groups had lower average relatedness and variance [solution 1 = −0.172 (0.024 SD), solution 2 = −0.175 (0.026 SD)] than the breeding groups that had been determined by hand by Attard *et al.* (2016b) [−0.095 (0.064 SD)].

Simulation analysis

The performance of SWINGER was tested by simulations in SIMUPOP v1.1.6 (Peng & Kimmel 2005). We used the empirical genotype data set and relatedness estimates from the captive breeding programme of southern pygmy perch (Attard *et al.* 2016b) to form four different breeding group data sets for simulation: (i) the 11 breeding groups selected by hand (Attard *et al.* 2016b); (ii) 11 breeding groups selected randomly from the 63 potential founders; (iii) the first combination of 11 breeding groups selected by SWINGER; and (iv) the second combination of 11 breeding groups selected by SWINGER. For each of these, we ran two models that differed in mating scheme: one model with homogeneous contribution of breeders to the next generation, and the second model with skewed contribution of breeders. To simulate the second model, one male and one female per breeding group were selected randomly to produce between 60% and 95% of the offspring of that particular breeding group following a binomial distribution probability. The skewed contribution percentages were selected to imitate the results observed during captive breeding of southern pygmy perch (Attard *et al.* 2016a,b). Sixty offspring were simulated for each breeding group so the genetic diversity of offspring in simulations could be directly

compared to the empirical diversity estimates of Attard *et al.* (2016b), which was based on genotyping approximately 60 offspring per breeding group. One thousand replicates were run for each data set under each model, which means 660 individuals were simulated for each replicate. Offspring genotypes were analysed using MSA 4.05 (Dieringer & Schlötterer 2003) to calculate expected heterozygosity, observed heterozygosity, allelic richness (as measured by the total number of alleles) and Shannon index of allelic diversity. Whether there were significant differences in diversity between data sets was examined using pairwise Student's *t*-tests in the R package STATS (R Core Team 2015).

The results of the simulations showed that offspring from breeding groups created using SWINGER have significantly higher diversity for all indices, except allelic richness, which is either lower or equal to the other data sets (Fig. 3; Tables S2 and S3, Supporting information). These differences between SWINGER and randomly selected breeders were found in just one generation; as there is no migration into most captive breeding populations, these differences are likely to become larger if SWINGER is used to select breeding groups in subsequent generations. It is important in captive breeding programmes to maximize effective population size and minimize genetic drift, which can be accomplished by reducing variation in genotype contribution to the next generation (Frankham *et al.* 2000; Allendorf *et al.* 2012). While allelic richness is determined just for the number of alleles, Shannon index and heterozygosity are affected by the evenness in frequency of alleles. Heterozygosity and Shannon index therefore better quantify the effective number of alleles (Allendorf *et al.* 2012; Greenbaum *et al.* 2014) and so may be better predictors of effective population size and how much genetic diversity will be lost. If nevertheless the user wishes to avoid decreases in allelic richness, internal relatedness may be more stringently filtered in SWINGER to remove from consideration individuals with higher homozygosity, which increases the likelihood of transmitting rare alleles to the next generation.

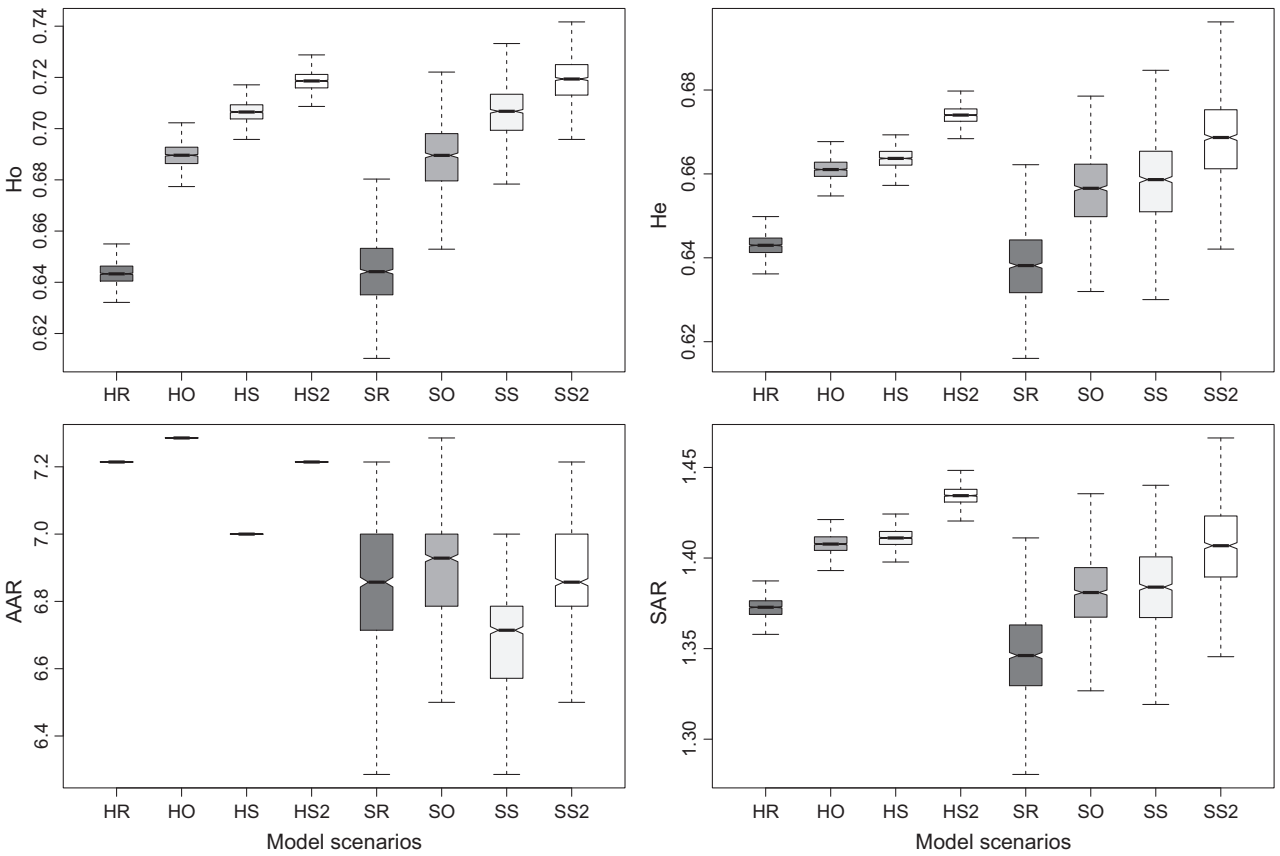

Fig. 3 Boxplot comparing four measures of genetic diversity from offspring simulated under eight different scenarios. Genetic diversity was measured as observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (AAR) and Shannon index of allelic diversity (SAR). The scenarios are combinations of the contribution of breeders to the next generation (first letter: H = homogeneous contribution, S = skewed contribution) and the data set (second letter: R = 11 breeder groups randomly selected, O = 11 breeder groups manually selected, S = best 11 breeder groups selected by SWINGER, S2 = second best 11 breeder groups selected by SWINGER).

There is a possible overestimation of genetic diversity in the offspring when using the same markers for estimating relatedness in the breeders. However, reducing average relatedness in breeding groups has been theoretically and empirically demonstrated as an effective method to retain genetic diversity in captive breeding programmes without pedigree information (Sonesson 2001; Allendorf *et al.* 2010; Ivy & Lacy 2012; Giglio *et al.* 2016). Moreover, the use of genomic data (ddRAD, GBS, etc.) is increasing the accuracy for relatedness estimations and, with this, the representation of the genomic diversity (Allendorf *et al.* 2010).

Discussion

SWINGER fills a major gap in ex situ conservation programmes: the optimization of breeding group composition in founders or subsequent generations. It is an innovative intermediate between two widespread applications of genetic theory: the use of observed pedigrees to minimize the loss of genetic diversity and to inhibit inbreeding in breeding programmes (Ballou & Lacy 1995), and the use of molecular data sets to investigate relatedness and inbreeding in wild populations (Jones & Wang 2010). Attard *et al.* (2016b) designed by hand the founding breeding groups for southern pygmy perch using molecular information, but with the knowledge that their solution would almost inevitably be suboptimal as there are millions of possibilities. As shown here, SWINGER can be used to successfully reach an optimal solution, which more effectively retains genetic diversity compared to random or manually selected breeding groups.

SWINGER was designed to be used across a wide range of situations. The parameters may be changed to suit the reproductive system of the species, the level of internal and pairwise relatedness in the potential founders, the available resources (e.g. breeders, enclosures, funding) for captive breeding and the priorities of the user. The best set of parameters and therefore the best solution or solutions needs to be judged on a case-by-case basis. For example, in the pygmy perch breeding system, there is no parental care of offspring and fertilization is external, so genetic-based parentage analyses need to be conducted to monitor the contribution of each breeder to the next generation. As such, the maximum allowed pairwise relatedness between female–female, male–male and female–male pairs was kept equal to both minimize inbred offspring and maximize the power of subsequent parentage analyses. Minimizing relatedness between pairs of the same sex is likely beneficial in other systems where parentage is not observable or social pairs do not always reflect mating pairs.

In contrast, the pairwise relatedness threshold may need to be greater in male–male or female–female pairs when there is a skewed sex ratio, a social system that involves same-sex kinship cooperation or competition between unrelated individuals, or sex-biased dispersal. For example, mammals typically have male-biased dispersal and therefore higher average pairwise relatedness between females in a population, whereas birds typically have female-biased dispersal and therefore higher average pairwise relatedness between males in a population (Prugnolle & de Meeus 2002). In addition, the allowable level of internal and pairwise relatedness may need to be relaxed if anthropogenic impacts that caused the need for captive breeding have resulted in unnaturally high inbreeding and relatedness levels (Spielman *et al.* 2004). Decisions could be made about whether low relatedness between potential pairs is more or less important than low internal relatedness. If there are very few individuals available for breeding, as is common in many endangered species, even inbred individuals may need to be used in the breeding programme.

All breeding programmes have an element of stochasticity or uncertainty and so require monitoring and adaptive management in addition to the solutions found by SWINGER or any other method. Some pairs of individuals may not breed and so need to be excluded from further consideration, paired with other individuals or possibly undergo in vitro fertilization. Group breeding is usually more complex as there are many possible breeding systems and mating results, and these are often influenced by sexual selection (Reynolds 1996). Skewed breeding is a frequent outcome that will always decrease the effective size of captive populations and make it more problematic to maintain genetic diversity (Hedrick 2005). When the parentage of the offspring is uncertain, which is common, parentage analyses can be conducted to maintain an accurate pedigree record and potentially help decide the breeding groups for the next generation. Attard *et al.* (2016b) provide an example of parentage analyses in captive breeding programmes, and Jones *et al.* (2010) provide an overview of parentage analyses and available programmes.

A main concern of molecular-based calculations of pairwise and internal relatedness is that they are estimates. The accuracy and precision of relatedness estimates vary depending on the number, polymorphism and allele frequency distribution of loci, and the level of relatedness and inbreeding in the individuals being assessed (e.g. Blouin *et al.* 1996; Van de Casteele *et al.* 2001). We recommend choosing an estimator for a particular data set as well as assessing its power by simulating individuals of known relatedness and comparing their true relatedness to that estimated by different estimators (Taylor 2015). This can be

conducted using COANCESTRY or the corresponding R package RELATED. Despite concerns with using estimators, in some circumstances, molecular-based estimates can prove superior to those from observed pedigrees (Hammerly *et al.* 2016), and estimators still provide an indication of which individuals are likely to be less inbred and less related. If a data set is found to have extremely low power, such as an overlap in the relatedness estimates of simulated unrelated and simulated first order relatives, data at more loci will be needed to produce reliable enough estimates for use in SWINGER. Similar to what we recommend for relatedness estimates, individuals with known internal relatedness can be simulated in COANCESTRY to assess their accuracy and precision (Taylor 2015). The accuracy and precision found for the best relatedness estimator and internal relatedness, along with the empirical distribution of pairwise relatedness and internal relatedness, can be used as a guide for determining parameter thresholds in SWINGER.

We expect that SWINGER will grow in applicability. The concerns of estimate reliability based on microsatellite data sets may soon become irrelevant due to the development of genomic data sets of thousands of SNPs (e.g. Leighton *et al.* 2015). There is also pressure towards zoos to move their breeding programmes from focusing on exhibiting animals in captivity to conservation-orientated maintenance in captivity and restoration to the wild (Conde *et al.* 2011; Conway 2011; Lacy 2013). This would be most successful if captive breeding programmes are short term as this minimizes adaptation to captivity (Williams & Hoffman 2009), as was performed for the southern pygmy perch (Attard *et al.* 2016b). Such programmes need to place a greater emphasis on choosing founding breeders based on molecular data sets as observed pedigrees are often unavailable. SWINGER can also be used for similar but alternative aims than that presented here, such as to aid in choosing individuals to found re-introduced populations while still keeping enough valuable, unrelated individuals for ex situ breeding programmes.

Conclusion

SWINGER implements an algorithm to form groups for breeding based on pairwise relatedness and, if desired, internal relatedness and sex. We know of no other programme designed to form breeding groups using molecular information. Most captive breeding programmes have instead assumed that founder individuals are unrelated and not inbred. Neither have they used molecular information to reallocate captive breeding group composition in already established breeding programmes when the pedigree is poorly known or unknown. SWINGER has a user-friendly graphic interface and input parameters that are highly flexible to the reproductive system of the target system and the biotic and abiotic resources available for captive breeding. We envision that this programme will improve the success of captive breeding and reintroduction programmes.

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J.S-C. developed the SWINGER program with input from C.R.M.A., S.M., C.J.B., L.M.M. and L.B.B.. C.R.M.A. wrote the manuscript with input from J.S-C., L.M.M. and L.B.B..

Data accessibility

The program, user manual and example input files are available from the Molecular Ecology Lab at Flinders University (MELFU) website (http://www.molecularecology.flinders.edu.au/molecular-ecology-lab/software/swinger/swinger/ or https://github.com/Yuma248/Swinger).

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pairwise relatedness in southern pygmy perch for the two combinations of breeding groups created using SWINGER.

Table S2 Diversity from simulated offspring results from eight different modelling scenarios.

Table S3 P values of pairwise *t*-tests adjusted using Bonferroni correction.