Title: Effect of sampling proportion of parents on parentage assignment
Authors: T.H. Dann, K. Shedd, C. Habicht, and W. Templin
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1 **Abstract**

2 Parentage analysis based upon few sampled parents can result in low effective sample sizes and a
3 lack of statistical power to address experimental hypotheses regarding fitness differences
4 between groups. Uncertainty about the utility of results of parentage and fitness analyses when
5 only a proportion of parents are sampled motivated us to conduct parentage simulations. We
6 used the popular parentage analysis program *CERVUS* to simulate parentage analysis to
7 determine how many offspring would be assigned to parent pairs for different sampling
8 proportions and two stray rate scenarios. We found an exponential increase in the number of
9 offspring assigned to parent pairs with an increase in the proportion of parents sampled. Very
10 few offspring were assigned to hatchery-hatchery parent pairs in the low stray rate scenario,
11 regardless of proportion of parents sampled. These results stress the need to prioritize sampling
12 as high a proportion of parents as practicable in order to obtain a large number of offspring
13 assigned to parent pairs when broken out by cross type. Future simulations will explore how
14 different sampling proportions affect statistical power to test for differences in fitness between
15 hatchery and natural stocks.

16 **Background of AHRP**

17 Extensive ocean-ranching salmon aquaculture is practiced in Alaska by private non-profit
18 corporations (PNP) to enhance common property fisheries. Most of the approximately 1.7B
19 juvenile salmon that PNP hatcheries release annually are pink salmon in Prince William Sound
20 (PWS) and chum salmon in Southeast Alaska (SEAK; Vercessi 2013). The large scale of these
21 hatchery programs has raised concerns among some that hatchery fish may have a detrimental
22 impact on the productivity and sustainability of natural stocks. Others maintain that the potential
23 for positive effects exists. ADF&G convened a Science Panel for the Alaska Hatchery Research
24 Program (AHRP) whose members have broad experience in salmon enhancement, management,
25 and natural and hatchery fish interactions. The AHRP was tasked with answering three priority
26 questions:

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[1] This document serves as a record of communication between the Alaska Department of Fish and Game Commercial Fisheries Division and other members of the Science Panel of the Alaska Hatchery Research Program. As such, these documents serve diverse ad hoc information purposes and may contain basic, uninterpreted data. The contents of this document have not been subjected to review and should not be cited or distributed without the permission of the authors or the Commercial Fisheries Division.

27   I.   *What is the genetic stock structure of pink and chum salmon in each region (PWS and*
28   *SEAK)?*
29   II.   *What is the extent and annual variability in straying of hatchery pink salmon in PWS and*
30   *chum salmon in PWS and SEAK*?
31   III.   *What is the impact on fitness (productivity) of natural pink and chum salmon stocks due*
32   *to straying of hatchery pink and chum salmon*?

Introduction

33

Measuring the Impact on Fitness

34

35   To answer the third question, we need to know the origin and pedigree of each fish captured in
36   select streams across multiple generations. **Origin** refers to the type of early life-history habitat
37   (hatchery or natural) that a fish experienced. **Pedigree** refers to the family relationship among
38   parents and offspring. **'Ancestral origin'** refers to the origin of an individual's ancestors (e.g.,
39   two parents of a single origin [hatchery/hatchery or natural/natural] or two parents of mixed
40   origin [hatchery/natural]). These ancestral origins can be determined by combining information
41   from three sources: identification of hatchery origin from otolith marks, pedigree from genetic
42   data, and age from scales (for chum salmon from SEAK). By pairing these data within fish and
43   across generations, we can estimate **reproductive success** (**RS**) among cross types (i.e. hatchery-
44   hatchery, hatchery-natural, and natural-natural origin crosses). The AHRP is using the **relative**
45   **reproductive success** (**RRS**) *of hatchery-origin fish to natural-origin fish as the measure of*
46   *fitness in this study* (Tech Doc 1 – Shedd et al. 2014).

Concerns about Sampling a Low Proportion of Parents

47

48   Pedigree construction for the AHRP will be based upon parentage assignments where offspring
49   are assigned to parents based upon molecular data.   The use of molecular techniques to
50   determine parentage and address ecological, evolutionary and quantitative genetic questions
51   grew rapidly with the availability of hypervariable microsatellite genetic markers (Jones and
52   Ardren 2003, Jones et al. 2010; Mobley 2011).   Similarly, the rapid development of single
53   nucleotide polymorphisms (SNPs) and their many positive laboratory qualities has made their
54   use for parentage analyses both accepted and widespread (Anderson and Garza 2006, Hauser et
55   al. 2011).

56   Parentage analysis utilizes the principle of Mendelian inheritance to assign offspring to one or
57   more parents. While there are six different methodological approaches to parentage analysis (see
58   Box 1 below, reproduced from Jones et al. 2010), all are built on the basic exclusion-based
59   method. According to Mendelian inheritance, diploid offspring receive one allele from the
60   mother and the other from the father for all loci. The exclusion method determines parent-
61   offspring relationships by screening offspring against all potential parents to exclude potential
62   parents that fail to share at least one allele at all loci. Offspring then may be assigned to non-
63   excluded potential parents (i.e. those that share at least one allele at all loci).   In practice,
64   assigning offspring to parents that mismatch at one or more loci may be permitted in order to

65 accommodate for genotyping errors or mutations (Kalinowski et al. 2007, Jones et al. 2010). Of
66 the six approaches to parentage analysis (Box 1; Jones et al. 2010), the GCL has explored and
67 will likely adopt a Bayesian or likelihood-based categorical allocation method, pending future
68 review. Other more complex techniques have significant drawbacks in terms of 1) computational
69 speed and 2) applicability to tests of fitness differences between hatchery and natural-origin
70 groups. Other researchers have found that sibship reconstruction with programs such as
71 *COLONY2* (Wang 2004) can be computationally prohibitive with large data sets of SNP markers
72 (Hauser et al. 2011). While full-probability parentage analysis appears to be a promising
73 approach given that it simultaneously models population-level parameters while performing
74 parentage analysis, it has not been widely used by researchers and may require additional
75 unknown assumptions to be made (Jones et al. 2010). The GCL will continue to explore other
76 parentage analysis methods as they become available.

Box 1: Six approaches to parentage analysis (reproduced from Jones et al. 2010)

Exclusion – The exclusion method takes advantage of the fact that in diploid, sexually reproducing organisms, each parent shares at least one allele per locus with each of its offspring. In this approach, the genotypes of candidate parents are compared with that of a focal offspring. Any candidate parent who fails to share at least one allele with the offspring at any locus is eliminated from consideration. In practice, most exclusion studies actually require at least two mismatching loci between the candidate and the offspring to account for typing errors or mutations.

Categorical Allocation – If complete exclusion is impossible, then a parentage allocation approach (also known as parentage assignment) can be used to choose among the remaining nonexcluded candidate parents. In categorical assignment, the entire offspring is assigned to the candidate parent with the highest likelihood or posterior probability of being the true parent. Categorical assignment approaches can handle scoring errors or mutations and can include methods for determining confidence in parentage assignment.

Fractional Allocation – In the fractional allocation approach, likelihoods or posterior probabilities are determined in the same way as in the categorical assignment methods. Each offspring is then assigned partially to each of the nonexcluded candidate parents on the basis of their relative likelihoods of parentage. Even though a fractional assignment has no biological meaning, from a statistical standpoint, this approach may have better properties than categorical allocation.

Full Probability Parentage Analysis – The full probability approach estimates patterns of parentage in a modelling framework. Many different models are possible, but this approach has the potential to estimate simultaneously patterns of parentage and other population-level variables of interest. This approach makes better use of the data by incorporating any uncertainty in the parentage analysis into the estimation of the variables of interest.

Parental Reconstruction – The parental reconstruction technique uses the genotypes of offspring in full- or half-sib families to reconstruct parental genotypes. For full- or half-sib progeny arrays, all of the offspring will share at least one parent. The genotype of the shared parent may be available from the sampling scheme or can be reconstructed by identifying a pair of alleles, for which every offspring inherited at least one of the members of the pair. The genotypes of the unknown parents can be determined by examining associations of alleles originating from the unknown parents across loci. Available techniques are based on parsimony (i.e. assuming the minimum number of parents), maximum likelihood or Bayesian approaches. Once the genotypes are reconstructed, they can be compared with the genotypes of candidate parents to assign parentage.

Sibship Reconstruction – If no parents are available and known groups of full- or half-sibs cannot be sampled, then sibship reconstruction is the last resort in the realm of parentage analysis. This technique requires a sample of individuals, some of which are full- or half-sibs. The algorithms use patterns of relatedness or maximum likelihood techniques to group individuals into different classes of relationship, often full-siblings, half-siblings and unrelated individuals. Once half-sib or full-sib groups are identified by these approaches, the parental genotypes can be reconstructed and used for parentage analysis.

Source: Jones et al. 2010

77

78 Regardless of the method of parentage analysis, sampling only a portion of parents is
79 problematic as offspring cannot be assigned to parents that are not sampled (Nielsen et al. 2001).
80 While this issue has been noted in the literature and modeled by some researchers (Hinrichsen
81 2003, Anderson et al. 2011), it remains uncertain how missing parents and/or offspring may
82 affect rates of parentage assignments, estimates of fitness, and tests of differences in fitness
83 among cross types (Araki and Blouin 2005).

84 Many likelihood-based parentage programs that produce categorical assignments (i.e. offspring
85 are fully assigned to parent[s], as opposed to fractional assignments) rely on an accurate estimate
86 of the sampled proportion of parents. This is required to put bounds on the space to analyze for
87 making parentage assignments based upon the estimate of the proportion of potential parents that
88 have been sampled (e.g., *CERVUS*; Kalinowski et al. 2007). Newer Bayesian approaches do not
89 require precise estimation of demographic parameters that may influence parentage assignments
90 (Christie 2010) and have been implemented in *SOLOMON* (Christie et al. 2013).

91 In order to better understand how sampling only a proportion of parents will affect parentage
92 analyses and the sample sizes available for subsequent tests of fitness differences between cross
93 types, we used *CERVUS* to simulate data for different sampling proportions and conduct
94 parentage analysis.

95 These initial analyses are not comprehensive. Nonetheless, we feel the take-home messages will
96 hold true after more in-depth analyses. We felt it was important to provide the Science Panel
97 some timely analyses to help inform decisions regarding field sampling methods and to get
98 direction from the Panel on future simulations.

99   **Methods**

100 Allele frequencies were simulated by sampling 96 SNPs from 1,000 randomly generated SNPs
101 from a normal distribution of minor allele frequencies (MAF; $\mu=0.3$, SD=0.05). A mean minor
102 allele frequency of 0.3 was chosen given that 1) SNPs with a MAF of < 0.2 have limited utility
103 in parentage analysis (Anderson and Garza 2006), and 2) we expect that our SNP discovery
104 process will produce SNPs with a similar range of minor allele frequencies given previous
105 experiences with SNP discovery in other salmonids (DeCovich et al. 2012). We assumed that
106 the allele frequencies were the same among groups of different ancestral origins (hatchery vs.
107 natural).

108 We conducted simulations in the widely used parentage analysis program *CERVUS* (Kalinowski
109 et al. 2007). In order to generate simulated genetic data for parentage analysis, *CERVUS*
110 requests input for many parameters, including numbers of female and male parents, the
111 proportion of parents sampled, information about the genetic markers used for parentage,
112 proportion of loci mistyped and others. The simulation parameters we used are summarized in
113 Table 1. In the interest of time, we did not include replicates for each simulation scenario.
114 Hatchery and natural origin was assigned randomly. We assumed random mating among

individuals of different ancestral origins (hatchery vs. natural). Thus, the proportions of offspring by cross type follow Hardy-Weinberg expectations.

We summarized results from *CERVUS* output files in *R* (R Core Team 2014) to determine the number of individuals with parental pair assignments for different scenarios under both relaxed (80% confidence) and strict (95% confidence) assignment criteria by cross type. Different scenarios assumed 1) the two hatchery:natural fish ratio scenarios described in the RFP [low stray rate – 15% of individuals (or 450 of 3,000) in the stream are of hatchery origin, and high stray rate – 50% of individuals (or 1,500 of 3,000) in the stream are of hatchery origin], and 2) sampling proportions of parents ranging from 1/6 to 6/6 in increments of 1/6.

Results

The sample of 96 SNPs had MAF that ranged from 0.17 to 0.40 (mean=0.31, SD=0.05). This sample is representative of what we expect from the pink salmon SNP development that has been contracted to the Seeb Laboratory at the University of Washington.

There was an exponential increase in proportion of offspring assigned to a parent pair with an increase in the proportion of parents sampled (Figure 1). For a given proportion of parents sampled, the relaxed assignment threshold in *CERVUS* provided on average a 20% increase in the proportion of assignments relative to the strict assignment threshold (Figure 1). The number of offspring assigned to a cross type was lower for natural x natural and hatchery x hatchery pairs than for natural x hatchery hybrid pairs in the high stray rate (50% hatchery-origin) scenario (Table 2; Figure 2). The low stray rate scenario (15% hatchery-origin scenario) resulted in very few hatchery x hatchery pairs, regardless of the proportion of parents sampled in the previous generation (Table 2; Figure 3).

Discussion

The *CERVUS* analysis reported here was an initial step to evaluate potential success expected for parent-pair assignments for given proportions of parents sampled. Note that these simulations assumed random mating between the ancestral-origin groups (hatchery and natural), equal reproductive success among all cross types, and that all (100%) of the returning adult offspring were sampled. Given these assumptions, these simulations represent a "best-case" scenario, as all returning offspring will not be sampled. Regardless, the major findings are that:

1. the number of offspring assigned to parent pairs increases exponentially with the number for parents sampled (Figure 1);
2. the relaxation in confidence in assignment thresholds from 95% to 80% confidence increased the proportion of offspring assigned to parent pairs by about 20% (Figure 1); and
3. both hatchery cross types are better represented for assigned parent pairs in the higher stray rate streams (Table 2; Figure 2 and 3).

151   These results mean that sampling a low proportion of parents will result in a high number of
152   unassigned offspring which represent "lost" effort (i.e. fish that were sampled in the field, but are
153   not available for the statistical analysis of fitness differences between hatchery and natural
154   groups). The statistical power to test for differences in reproductive success will largely be
155   governed by the sample size of the smallest group (cross type), keeping in mind that sample size
156   refers to the number of families assigned to that group, not the number of individual offspring.
157   The distribution of RS in salmonids had been shown to be highly variable and best approximated
158   by the negative binomial distribution (Williamson et al. 2010; Anderson et al. 2011; Anderson et
159   al. 2013; Christie et al. 2014). Given the high variability in RS, it is important to be able to
160   assign a large number of offspring to a given cross type in order to accurately characterize the
161   distribution of RS for that cross type. Other simulation work has shown that sampling only a
162   portion of parents or offspring will result in a truncated distribution of RS, as it is unlikely to
163   adequately represent very large families (Hinrichsen 2003, Araki and Blouin 2005).

164   The increased number of assignment with relaxation of confidence in assignment thresholds
165   suggests that further analysis of the costs of this relaxation is warranted. Finally, although both
166   hatchery cross types are best represented in the high stray rate stream scenario, the low stray rate
167   steams provided high proportions of NxN and NxH cross type progeny. These low stray rate
168   streams might offer the most insight into genetic effects of hatchery-natural interactions because
169   the natural fish are less likely to have been as heavily influenced by introgression of hatchery
170   fish from previous generations.

Proposed Guidelines for Future Simulation Work

172   Future simulations, to be completed by November 1, 2014 will expand upon these results to
173   manipulate not only the stray rate and the proportion of parents sampled, but also:

174   1. parameterize the proportion of offspring sampled;
175   2. parameterize the effect size (difference in reproductive success between ancestral
176   groups);
177   3. examine the trade-offs between relaxation of confidence in assignment thresholds and
178   errors in assignment; and
179   4. determine the statistical power to detect known differences given the proportion of
180   parental sampling, proportion of offspring sampling, stray rate, and difference in
181   reproductive success of hatchery and natural-origin fish.

Questions for the Science Panel

183   1. Do the guidelines for future simulations seem appropriate? Are there additional
184   parameters to consider?

185 **Science Panel Review and Comments**

186 *This technical document was discussed at the December 12, 2014 meeting of the AHRG. In*
187 *addition it was reviewed by email exchange prior to the meeting.*

188 The AHRG found the guidelines appropriate and did not offer any additional parameters.

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Tables

246 Table 1.–Parameter values used in simulations in the program *CERVUS*. Parameters were the
247 same for all simulations with the exception that the proportions of female and male parents
248 sampled ranged from $1/6^{th}$ to $6/6^{ths}$ in increments of $1/6^{th}$.

Parameter	Value
Number of offspring	3,000
Number of candidate mothers	1,500
Number of candidate fathers	1,500
Proportion of candidate mothers/fathers sampled	1/6 – 6/6
Number of potential parent pairs	2,250,000
Proportion of loci genotyped	0.95
Proportion of loci mistyped	0.01
Error rate in likelihood calculations	0.01
Minimum number of genotyped loci per individual	77 (80% of markers)

249 Table 2.– Number of offspring out of the 3,000 offspring produced in the simulations assigned to
250 a parent pair among cross types for different sampling proportions of parents for a strict
251 assignment threshold (95% confidence) in *CERVUS* under both a low stray rate (15% hatchery-
252 origin fish) and high stray rate (50% hatchery-origin fish) scenario. Note that these simulations
253 assume random mating with respect to ancestral origin (hatchery and natural) and 100%
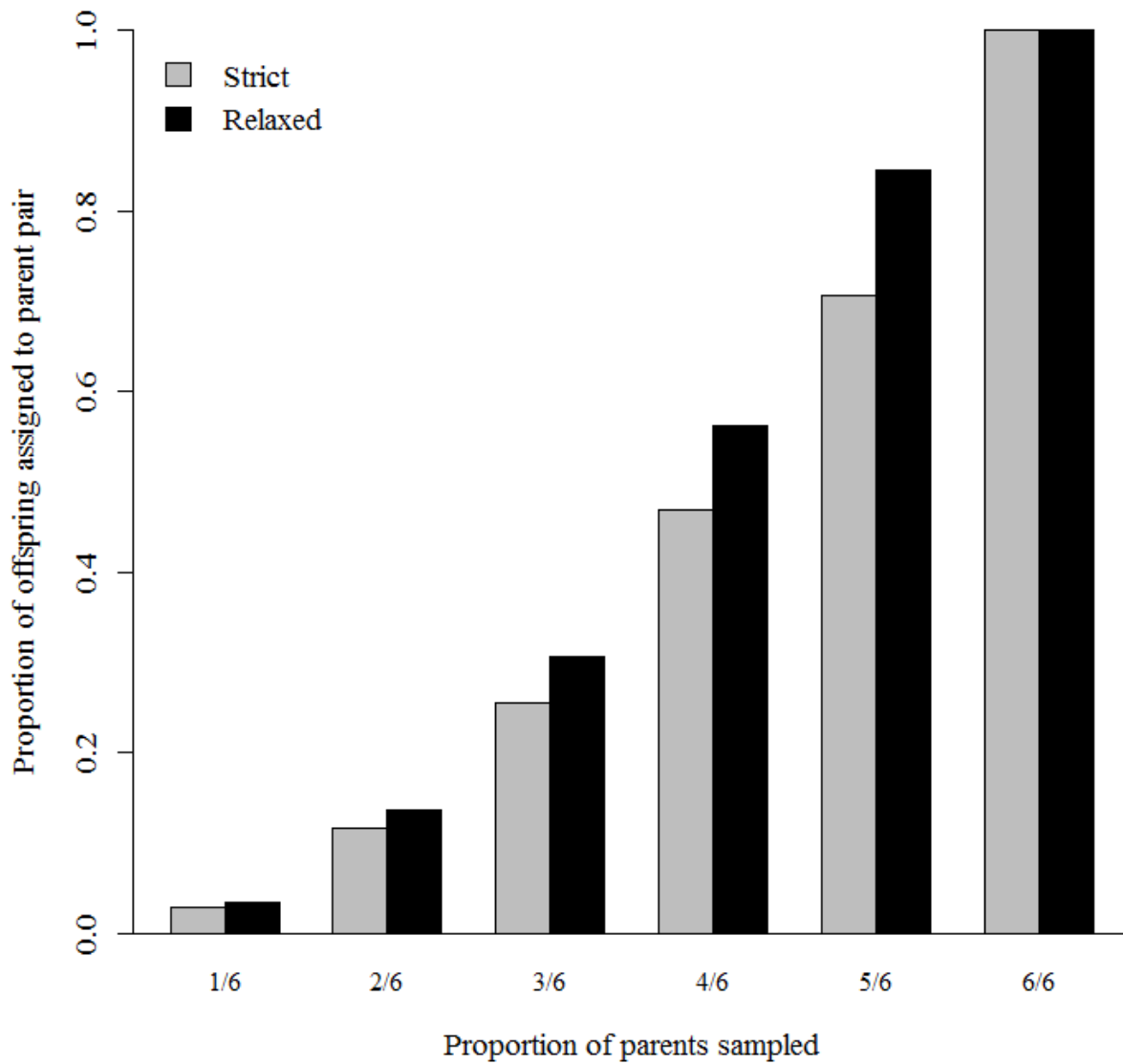254 sampling of offspring.

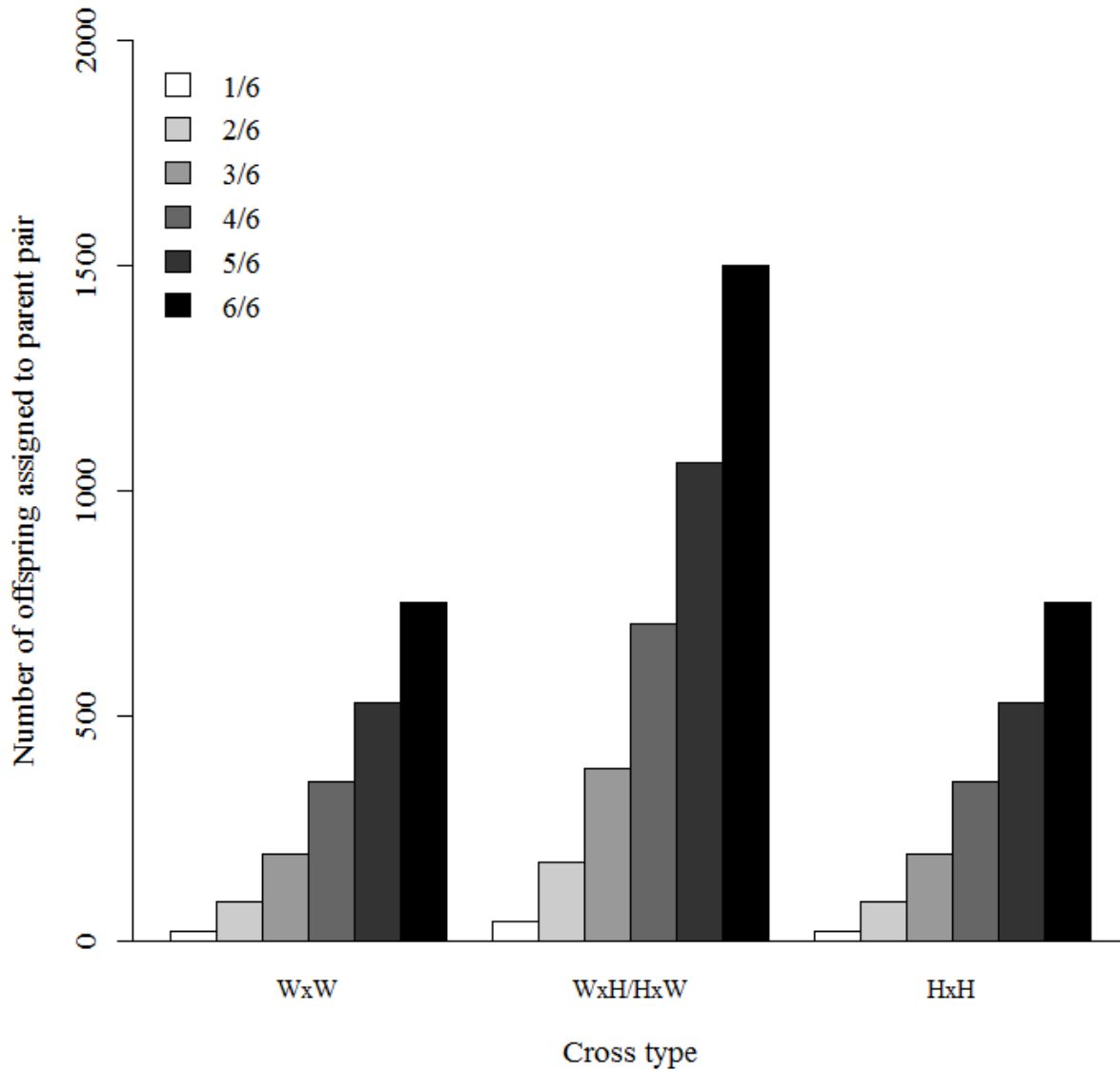| Stray rate	Cross type	Number of offspring assigned to a parent pair for a given proportion of parents sampled					
		1/6	2/6	3/6	4/6	5/6	6/6
Low (15%)	NxN	60	251	551	1017	1532	2168
	NxH / HxN	21	88	195	359	540	765
	HxH	2	8	17	32	48	67
	Total	83	347	763	1408	2120	3000
High (50%)	NxN	21	87	191	352	530	750
	NxH / HxN	41	173	381	704	1060	1500
	HxH	21	87	191	352	530	750
	Total	83	347	763	1408	2120	3000

255 **Figures**

256 Figure 1.–Proportion of offspring assigned to a parent pair for different sampling proportions of
257 parents for both strict (95% confidence) and relaxed (80% confidence) assignment thresholds in
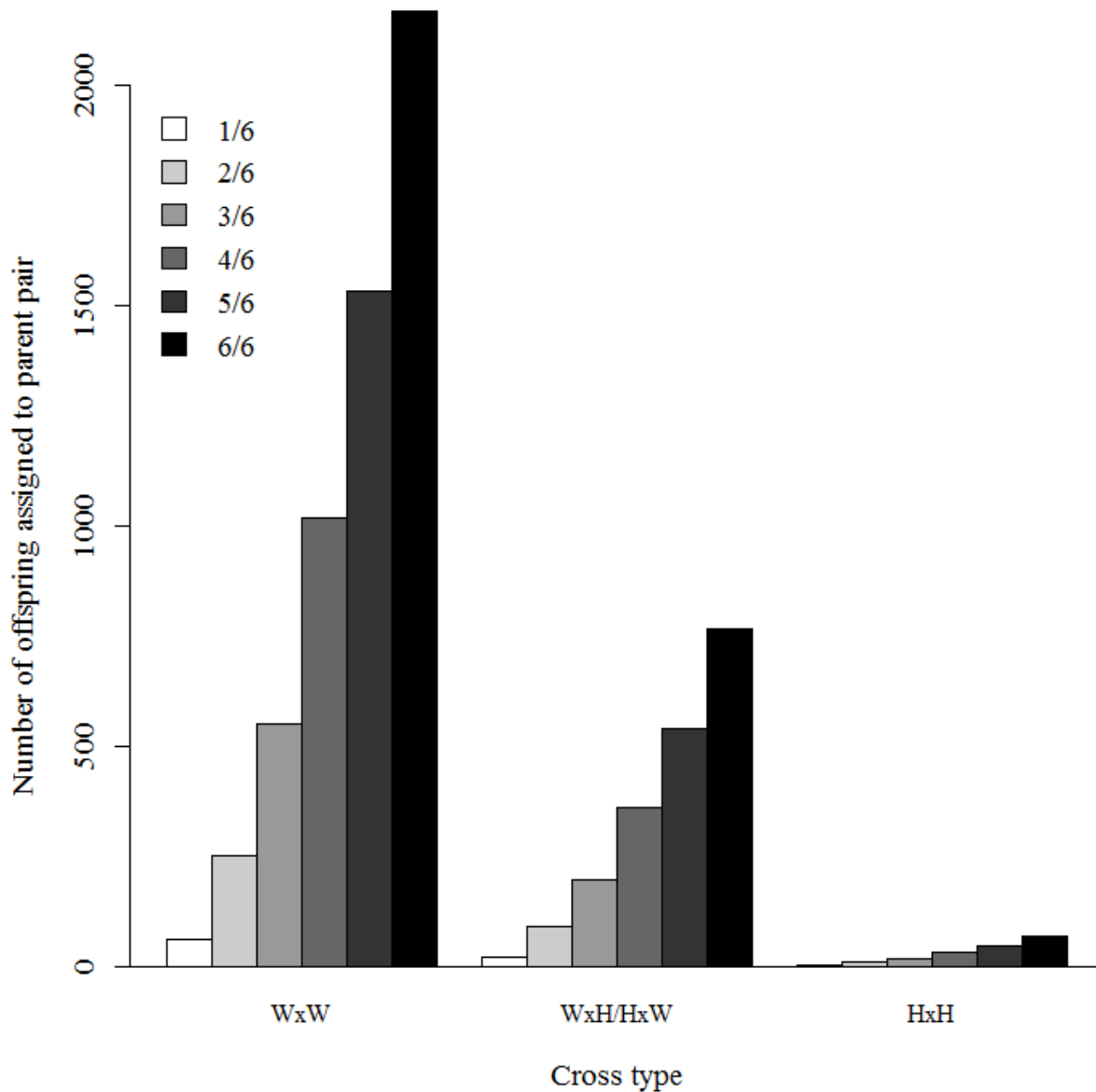258 *CERVUS*. Sampling of offspring was 100%.

259

260

261 Figure 2.–Number of offspring assigned to a parent pair among cross types for different
262 sampling proportions of parents for a strict assignment threshold (95% confidence) in *CERVUS*
263 under a high stray-rate scenario (50% hatchery-origin fish). Note that these simulations assume
264 random mating without respect to ancestral origin (hatchery [H] and natural [N]). Cross types
265 are indicated by an "x", e.g., HxH indicates mating of two hatchery-origin parents. Sampling of
266 offspring was 100%.

267

268 Figure 3.–Number of offspring assigned to a parent pair among cross types for different
269 sampling proportions of parents for both strict and relaxed assignment thresholds (95%
270 confidence) in *CERVUS* under a low stray-rate scenario (15% hatchery-origin fish). Note that
271 these simulations assume random mating without respect to ancestral origin (hatchery [H] and
272 natural [N]). Cross types are indicated by an "x", e.g., HxH indicates mating of two hatchery-
273 origin parents. Sampling of offspring was 100%.

274