

Unbiased estimation of relative reproductive success of different groups: evaluation and correction of bias caused by parentage assignment errors

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Abstract

Parentage assignment is widely applied to studies on mating systems, population dynamics and natural selection. However, little is known about the consequence of assignment errors, especially when some parents are not sampled. We investigated the effects of two types of error in parentage assignment, failing to assign a true parent (type A) and assigning an untrue parent (type B), on an estimate of the relative reproductive success (RRS) of two groups of parents. Employing a mathematical approach, we found that (i) when all parents are sampled, minimizing either type A or type B error insures the minimum bias on RRS, and (ii) when a large number of parents is not sampled, type B error substantially biases the estimated RRS towards one. Interestingly, however, (iii) when all parents were sampled and both error rates were moderately high, type A error biased the estimated RRS even more than type B error. We propose new methods to obtain an unbiased estimate of RRS and the number of offspring whose parents are not sampled (zW_z), by correcting the error effects. Applying them to genotypic data from steelhead trout (*Oncorhynchus mykiss*), we illustrated how to estimate and control the assignment errors. In the data, we observed up to a 30% assignment error and a strong trade-off between the two types of error, depending on the stringency of the assignment decision criterion. We show that our methods can efficiently estimate an unbiased RRS and zW_z regardless of assignment method, and how to maximize the statistical power to detect a difference in reproductive success between groups.

Keywords: assignment error, incomplete samples, microsatellite, parentage, relative reproductive success, salmonid

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Introduction

Parentage assignment using molecular markers is one of the most effective approaches to analyse mating systems, population dynamics and natural selection in recent molecular ecology (Richardson *et al.* 2001; Avise 2004; Bishop *et al.* 2004). Many methods can be used for the assignment of parents to offspring. These include categorical or fractional allocation, with exclusion, likelihood and /or Bayesian methods (Thompson 1975; Devlin *et al.* 1988; Marshall *et al.* 1998; Neff *et al.* 2001; reviewed in Jones & Ardren 2003). However, all methods are subject to assignment errors that can include failing to identify the

true parent when it is present, and assigning offspring to an untrue parent (Oddou-Muratorio *et al.* 2003). Incorrect assignment decisions result from sampling a finite number of loci and from genotyping errors, mutation or null alleles (SanCristobal & Chevalet 1997; Bernatchez & Duchesne 2000; Hoffman & Amos 2005). Failing to sample all potential parents can also create very high rates of assignment error that can only be accounted for if one has an accurate estimate of the number of missing adults (Marshall *et al.* 1998; Nielsen *et al.* 2001). There has been little discussion of how best to estimate confidence in individual assignment decisions (Marshall *et al.* 1998; Neff *et al.* 2001; Nielsen *et al.* 2001; Oddou-Muratorio *et al.* 2003), and even less discussion of the consequences of assignment error for different types of studies. For example, assignment error could strongly affect the estimation of quantitative genetic parameters, of

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variance in reproductive success among individuals, or of selection gradients in wild populations (Morgan & Conner 2001; Kruuk *et al.* 2002). One interesting application is the estimation of relative reproductive success (RRS) of different groups. Such groups can be geographical populations, behavioural groups or groups of different life histories. For example, Nielsen *et al.* (2001) showed that male whales (*Megaptera novaeangliae*) that escort females dominantly during the mating season (dominant males) tend to have higher reproductive success than subdominant males. They also showed that failing to account for nonsampled males caused a substantial underestimation of RRS of dominant males relative to that of subdominant males.

In this study, we investigated the effects of assignment errors on an estimate of RRS of different groups. A primary goal of this study is to explicitly lay out the different types of errors possible when doing parentage assignments, and to evaluate the effects of assignment errors on an estimate of the absolute fitness and of RRS of two groups. We employed a simple mathematical approach that takes nonsampled parents into consideration. Based on the relationship between the estimated RRS and assignment error rates, we propose new methods to obtain an unbiased estimate of RRS and the number of offspring whose parents are not sampled. Our approach can be applied regardless of assignment method, and to RRS estimations for different individuals or groups. Finally, our methods are applied to a genotype data set from steelhead trout (*Oncorhynchus mykiss*) to show how one can empirically estimate the assignment error rates. This application illustrates how high the assignment error rates can be in real data, and how strongly different criteria for assignment decisions influence the trade-off between the two assignment error rates. We discuss how broadly our approach can be applied, how best one can estimate an unbiased RRS and zW_z and how to maximize the statistical power to detect a difference in reproductive success between groups.

Materials and methods

Definitions of assignment errors in a parentage assignment

For the general case in which there are assignment errors and not all potential parents have been sampled, there are

five ways to make a correct or incorrect assignment decision (Table 1). Correct decisions include assigning the true parent when it is present, and assigning no parent when the true parent is absent. Assignment errors fall into two categories. First, one can fail to assign the correct parent when it is present in the sample. Second, one can assign the offspring to an untrue parent, which can occur when the true parent is absent or when the true parent is present but failed to be assigned. We refer to the first type of error as 'type A' error (fail to assign a true parent), and the second as 'type B' error (assign an untrue parent). Note that these errors are different from type I (α) and type II errors (β) in parentage assignment tests (see Results and Appendix I for details).

Biological materials and genetic markers

Scale samples from steelhead trout were collected at Powerdale Dam in the Hood River, Oregon, in 1993–2000. A total of 502 offspring samples that was born in 1994 and 381 parental samples that returned to the dam in 1993–1994 were used in this study. Genotypes in eight microsatellite loci (Omy1001, Omy1011, Omy1191, Omy77, One108, One2, Ssa407 and Str2, Morris *et al.* 1996; Cairney *et al.* 2000; Nichols *et al.* 2003; Spies *et al.* 2005) were identified for these samples. We followed a standard Chelex protocol for DNA extraction and amplification (see Nelson *et al.* 1998), with minor modifications (50–55 °C of annealing temperature). The genotype scoring was done on the ABI 3100 Capillary Electrophoresis system (Applied Biosystems). Ambiguous scorings for one mismatch between parental and offspring samples were checked twice and either corrected or re-analysed to minimize scoring errors. The average number of alleles and the average heterozygosity among the loci were 32.5 and 0.915, respectively.

We employed the exclusion method for simplicity. Parent-offspring pairs with the minimum of six loci comparisons were accepted, and the total exclusion probability was > 0.9999 . The parentage assignments were done using the CERVUS program version 2.0 (Marshall *et al.* 1998) allowing 0–2 mismatch loci. In the few cases that more than one candidate parent shared the same minimum number of mismatch to an offspring, a parent with the highest LOD score was assigned. All the samples above were used to estimate the allele frequencies in the population.

Table 1 Types of correct and incorrect decisions in a parentage assignment

Parent in a data set	Assignment	Decision	Type of error	Rate
Present	to a true parent	correct	—	$(1 - a)$
	failed	incorrect	A	$a(1 - b)$
Absent	to an untrue parent	incorrect	$A \cap B$	ab
	failed	correct	—	$(1 - b)$
	to an untrue parent	incorrect	B	b

Results

Observed RRS when all parents are sampled

Consider two categories (groups) of samples, X and Y. We wish to know the RRS of the two groups, W_x/W_y , where W_x and W_y are the mean (absolute) fitness of individuals in each group. Thus, RRS = 1.0 means equal fitness for the two groups. Assume that we have x and y potential parents in each group, and that all parents of the sampled offspring are sampled. The total number of offspring produced by group X and Y are xW_x and yW_y , respectively. If there is no type A or type B error, we will correctly assign all the xW_x and yW_y offspring to each group. If we have type A error at the rate of a ($0 = a = 1$) but no type B error, we will assign offspring only at a rate of $(1 - a)$ from each group and a total of $a(xW_x + yW_y)$ offspring will remain unassigned. The observed RRS of the two groups, \hat{W}_x/\hat{W}_y , holds $\hat{W}_x/\hat{W}_y = W_x(1 - a)/W_y(1 - a) = W_x/W_y$. Thus, although type A error reduces the expected absolute fitness of each group, it does not bias the observed RRS unless type A error rate differs between the groups.

Now consider type B error at a rate of b ($0 = b = 1$). Here, b is the rate that an offspring, which is not assigned to its true parent, is assigned to an untrue parent. All through the current study, we assume $(x + y) \gg 1$ and the same genetic composition for different groups. The former assumption is for a randomly chosen parent being an untrue parent, and for the ratio of the estimates (\hat{W}_x/\hat{W}_y) approximately being the expectation of RRS. The latter is for an untrue parent being chosen in proportion to the size of each group [$x/(x + y)$ in group X and $y/(x + y)$ in group Y]. Because we have $a(xW_x + yW_y)$ unassigned offspring now, the total numbers of offspring assigned to group X and Y are given by

$$xW_x(1 - a) + x\left(\frac{xW_x + yW_y}{x + y}\right)ab \tag{eqn 1}$$

and

$$yW_y(1 - a) + y\left(\frac{xW_x + yW_y}{x + y}\right)ab, \tag{eqn 2}$$

respectively, and the expectation of RRS, $E\{\hat{W}_x/\hat{W}_y\}$, becomes

$$E\{\hat{W}_x/\hat{W}_y\} = \frac{W_x(1 - a) + \left(\frac{xW_x + yW_y}{x + y}\right)ab}{W_y(1 - a) + \left(\frac{xW_x + yW_y}{x + y}\right)ab} \tag{eqn 3}$$

Because $(xW_x + yW_y)$ is the total number of offspring, $N_{\text{offspring}}$, and $(x + y)$ is the number of parents, N_{parent} , and because the average fitness of the two groups, \bar{W} , is given by $(N_{\text{offspring}}/N_{\text{parent}})$, equation 3 can also be written as

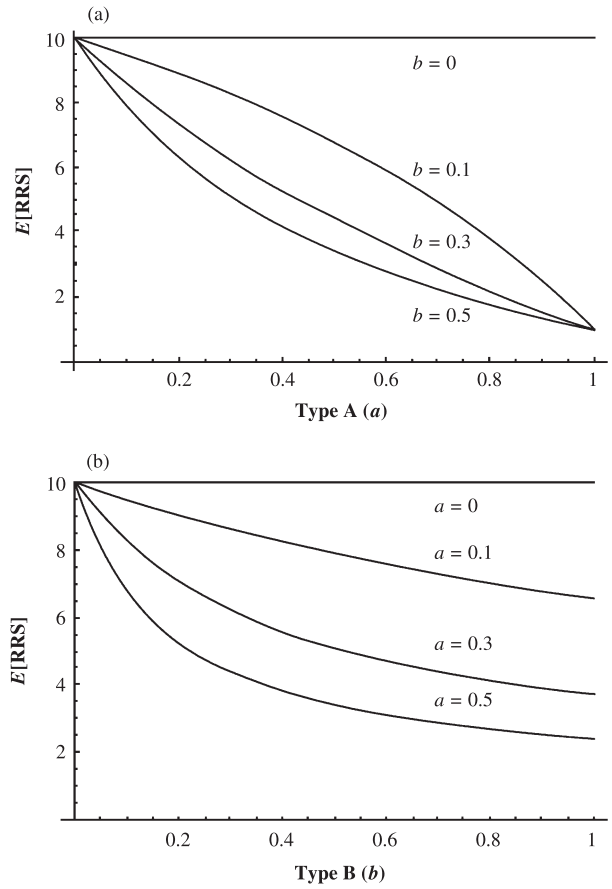


Fig. 1 Relationship between expected RRS and assignment error rates when all parents are sampled ($W_x/W_y = 10.0$). All the plots are calculated from equation 4, assuming the same number of parents for two groups ($x = y$). (a) Expected RRS as a function of type A error rate (b) that of type B error rate. Type B and type A error rates were fixed as 0, 0.1, 0.3 and 0.5 in (a) and (b), respectively.

$$E\{\hat{W}_x/\hat{W}_y\} = \frac{W_x(1 - a) + \bar{W}ab}{W_y(1 - a) + \bar{W}ab} \tag{eqn 4}$$

This equation shows that the observed RRS is biased towards one only when both types of error rate are high. However, type A error affects the observed RRS more than type B error because type A error not only increases the contribution of the error term ($\bar{W}ab$) in equation 4, but because it also decreases the contribution of the actual absolute fitness via the term $(1 - a)$. Figure 1 represents how the expectation of RRS is skewed by the two types of error when $W_x/W_y = 10.0$ and $x = y$. For example, RRS becomes 6.27 when $a = 0.3$ and $b = 0.3$, but becomes 9.85 and 9.79 when a or b is reduced to 1%, respectively [$(a = 0.01, b = 0.3)$ and $(a = 0.3, b = 0.01)$]. Thus, minimizing either type A or type B error insures the minimum bias on an estimation of RRS.

Observed RRS when some parents are not sampled

In the above example, all parents were sampled. Now consider a third group, Z, of nonsampled parents. Individuals in this group can be nonsampled members of groups X and Y, or belong to a completely nonsampled third group that shares the same genetic composition. The z individuals in this group contribute zW_z offspring to the total sample of offspring, where W_z is the mean fitness in group Z. Now, x and y are the numbers of sampled potential parents in groups X and Y, and N_{parent} (= x + y) is the number of sampled parents. The total number of offspring is given by

$$N_{\text{offspring}} = xW_x + yW_y + zW_z. \tag{eqn 5}$$

Again, assuming group Z has the same genetic composition to the others, b of all offspring in group Z will be randomly assigned to untrue parents in groups X and Y. The estimates of the absolute fitness of groups X and Y are

$$E\{\hat{W}_x\} = W_x(1 - a) + \left(\frac{xW_x a + yW_y a + zW_z}{x + y} \right) b \tag{eqn 6}$$

and

$$E\{\hat{W}_y\} = W_y(1 - a) + \left(\frac{xW_x a + yW_y a + zW_z}{x + y} \right) b, \tag{eqn 7}$$

respectively, and the expectation of the observed RRS becomes

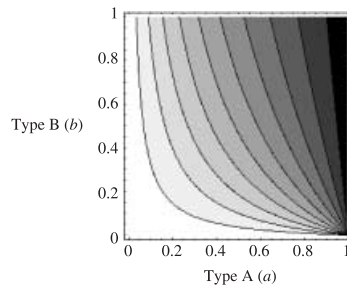
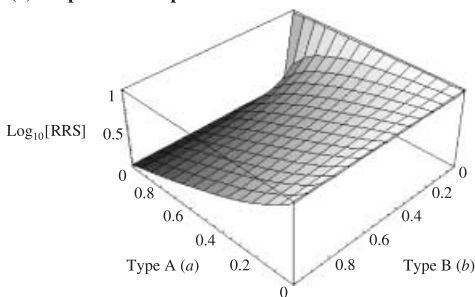
$$E\{\hat{W}_x/\hat{W}_y\} = \frac{W_x(1 - a) + \left(\frac{xW_x a + yW_y a + zW_z}{x + y} \right) b}{W_y(1 - a) + \left(\frac{xW_x a + yW_y a + zW_z}{x + y} \right) b} \tag{eqn 8}$$

Note that equation 3 is simply a case of zW_z = 0 in equation 8. As in equation 3, equation 8 shows that the observed RRS is biased towards one by these errors, and that the impact of type A error on the RRS is limited when the type B error rate is small. However, unlike before, the effect of type B error cannot be ignored when zW_z is large, even if the type A error rate is small. When a = 0, equation 8 becomes

$$E\{\hat{W}_x/\hat{W}_y\} = \frac{W_x + \left(\frac{zW_z}{x + y} \right) b}{W_y + \left(\frac{zW_z}{x + y} \right) b}. \tag{eqn 9}$$

This equation shows that the bias cannot be controlled by reducing type A error, and that the observed RRS will be substantially biased when the number of offspring from group Z is large, and when a high portion of them are incorrectly assigned to untrue parents relative to the total number of sampled parents [i.e. zW_zb/(x + y) ~ W_x or ~ W_y] owing to type B error. Figure 2 illustrates the effects of the assignment errors on the estimation of RRS. A case that all parents are sampled (Fig. 2a) was compared with a case that only half of the parents are sampled (Fig. 2b), assuming x = y, W_x = 1.0 and W_y = 0.1 (W_x/W_y = 10.0). The general

(a) All parents sampled



(b) Half of the parents sampled

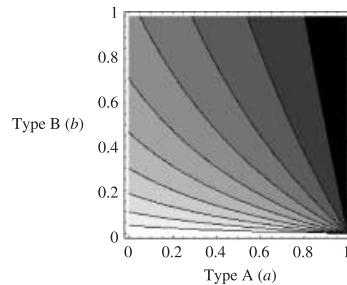
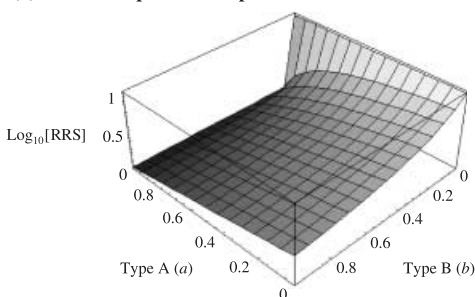


Fig. 2 Expected RRS with assignment errors when all parents are sampled (a) and when only half of the parents are sampled (b). The logarithm of the expected RRS was drawn with type A and type B error rates in 3D plot (left) and its contour plot (right). These values were calculated from equation 8, assuming W_x/W_y = 10.0 (W_x = 1.0, W_y = 0.1) and x = y. zW_z = 0 and zW_z = (xW_x + yW_y) were assumed for (a) and (b), respectively. Lighter colour in the contour plots indicates smaller deviation of the estimate from the true RRS (= 10.0) and the darker colour indicates the larger deviation towards one. Note the effects of the assignment errors in the contour plots are exactly the same between W_x/W_y = 10.0 and its reciprocal (W_x/W_y = 0.1). MATHEMATICA version 4.1 program was used for these calculations.

pattern of the effects of assignment errors on RRS is similar between these cases, but the missing parents from the data strongly influence the estimation of RRS when the type A error rate is small. This influence became stronger as the proportion of missing parents increased (data not shown). Note that the estimates of RRS for $W_x/W_y < 1.0$ can be obtained simply by the reciprocal of those for $W_x/W_y > 1.0$ (W_y/W_x), and that the effects of assignment errors on RRS for W_x/W_y (the contour plots in Fig. 2) are exactly the same as for W_y/W_x .

Because the number of offspring assigned, N_{assigned} , is

$$N_{\text{assigned}} = N_{\text{offspring}} - (1 - b)(xW_x a + yW_y a + zW_z) \quad (\text{eqn 10})$$

and because $N_{\text{parent}} = x + y$, equation 8 can also be written as

$$E(\hat{W}_x/\hat{W}_y) = \frac{W_x(1 - a) + \left[\frac{N_{\text{offspring}} - N_{\text{assigned}}}{N_{\text{parent}}} \right] \frac{b}{1 - b}}{W_y(1 - a) + \left[\frac{N_{\text{offspring}} - N_{\text{assigned}}}{N_{\text{parent}}} \right] \frac{b}{1 - b}} \quad (\text{eqn 11})$$

$(N_{\text{offspring}} - N_{\text{assigned}})/N_{\text{parent}}$ is the ratio of the number of offspring that could not be assigned to the number of parents sampled. $(N_{\text{offspring}} - N_{\text{assigned}})$ simply reflects type A error when all the parents are sampled, while it reflects both type A error and offspring from nonsampled parents when some parents are missing from the dataset.

Table 2 represents examples of the estimate of RRS by equation 11, in the case of $W_x/W_y = 10.0$. We used parameters as follows; $\{W_x, W_y\} = \{1.0, 0.1\}$ (Case A-) or $\{10.0, 1.0\}$ (Case B-), $(N_{\text{offspring}} - N_{\text{assigned}})/N_{\text{parent}} = \{0.1, 1.0, 3.0\}$, $a = \{0, 0.1, 0.2, 0.3, 0.5, 0.9\}$, $b = \{0, 0.1, 0.2, 0.3, 0.5, 0.9\}$. The parameter sets $(N_{\text{offspring}} - N_{\text{assigned}})/N_{\text{parent}} = \{0.1, 1.0, 3.0\}$ are corresponding to $N_{\text{assigned}} = \{490, 400, 200\}$, respectively, when $N_{\text{offspring}} = 500$ and $N_{\text{parent}} = 100$, but the absolute values do not influence the results as long as $(N_{\text{offspring}} - N_{\text{assigned}})/N_{\text{parent}}$ is constant. Again, Table 2 shows that type B error influences the estimate of RRS more than type A error when some parents are not sampled. In addition, Table 2 illustrates the strong dependencies of the estimated RRS on the ratio of nonassigned offspring to sampled parents $(N_{\text{offspring}} - N_{\text{assigned}})/N_{\text{parent}}$ and on the absolute fitness of each group (Case A- and B-).

Unbiased estimation of RRS by correcting the error effects

Based on equations 6 and 7, an unbiased RRS can be obtained by

$$RRS_{[\text{unbiased}]} = \frac{\hat{W}_x - \left(\frac{xW_x \hat{a} + yW_y \hat{a} + zW_z}{x + y} \right) \hat{b}}{\hat{W}_y - \left(\frac{xW_x \hat{a} + yW_y \hat{a} + zW_z}{x + y} \right) \hat{b}} \quad (\text{eqn 12})$$

In many cases, we do not know the number of missing parents (z) and the number of offspring produced by them (zW_z). However, as we did for equation 11 above, equation 6 can also be written as

$$W_x = \left(\frac{1}{1 - a} \right) \left[\hat{W}_x - \left(\frac{N_{\text{offspring}} - N_{\text{assigned}}}{N_{\text{parent}}} \right) \left(\frac{b}{1 - b} \right) \right] \quad (\text{eqn 13})$$

Note that $N_{\text{offspring}}$, N_{assigned} and N_{parent} are number of offspring sampled, number of offspring assigned to a parent, and number of parents sampled, respectively, and that all these values should be available when a parentage assignment has been done. We can use equation 13 to obtain an unbiased estimate of absolute fitness of one group (or of an individual when $x = 1$) if estimates of type A and type B error rates are available. In the same way, the unbiased estimation of RRS is given by

$$RRS_{[\text{unbiased}]} = \frac{\hat{W}_x - \left(\frac{N_{\text{offspring}} - N_{\text{assigned}}}{N_{\text{parent}}} \right) \left(\frac{\hat{b}}{1 - \hat{b}} \right)}{\hat{W}_y - \left(\frac{N_{\text{offspring}} - N_{\text{assigned}}}{N_{\text{parent}}} \right) \left(\frac{\hat{b}}{1 - \hat{b}} \right)} \quad (\text{eqn 14})$$

Thus, we can estimate an unbiased RRS by equation 14 if an estimate of the type B error rate is available. How to obtain the estimate of the error rates is discussed below.

Number of offspring whose parents are not sampled

In some cases, the proportion of missing parents or of offspring whose parent is missing is of particular interest. Based on estimates of a and b , we can estimate the number of offspring whose parents are not sampled (zW_z) by equation

$$z\hat{W}_z = \frac{(1 - \hat{a} + \hat{a}\hat{b})N_{\text{offspring}} - N_{\text{assigned}}}{(1 - \hat{a})(1 - \hat{b})} \quad (\text{eqn 15})$$

which was obtained from equations 5 and 10. The number of missing parents (z), on the other hand, cannot be obtained unless absolute fitness of them (W_z) is known.

Estimating type A and type B error rates

To estimate an unbiased RRS and zW_z , one needs to obtain the type A and type B error rates. The simplest way to obtain

Table 2 Expected RRS when some parents are not sampled

A-1: $W_x = 1.0, W_y = 0.1, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 0.1$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	9.10	9.01	8.90	8.77	8.36	5.26	5.26
0.2	8.20	8.04	7.86	7.63	7.00	3.57	3.57
0.3	7.30	7.10	6.86	6.58	5.85	2.70	2.70
0.5	5.50	5.26	5.00	4.71	4.00	1.82	1.82
0.9	1.90	1.82	1.73	1.65	1.47	1.10	1.10
A-2: $W_x = 1.0, W_y = 0.1, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 1.0$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	5.26	5.03	4.77	4.48	3.79	1.74	1.74
0.2	3.57	3.38	3.18	2.97	2.50	1.35	1.35
0.3	2.70	2.56	2.42	2.26	1.94	1.21	1.21
0.5	1.82	1.74	1.67	1.59	1.43	1.09	1.09
0.9	1.10	1.09	1.08	1.07	1.05	1.01	1.01
A-3: $W_x = 1.0, W_y = 0.1, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 3.0$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	3.08	2.91	2.74	2.56	2.17	1.26	1.26
0.2	2.06	1.96	1.87	1.77	1.56	1.12	1.12
0.3	1.65	1.59	1.53	1.46	1.34	1.07	1.07
0.5	1.29	1.26	1.23	1.21	1.15	1.03	1.03
0.9	1.03	1.03	1.03	1.02	1.02	1.00	1.00
B-1: $W_x = 10.0, W_y = 1.0, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 0.1$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	9.90	9.89	9.88	9.86	9.80	9.10	9.10
0.2	9.78	9.76	9.73	9.69	9.57	8.20	8.20
0.3	9.63	9.59	9.54	9.48	9.29	7.30	7.30
0.5	9.18	9.10	9.00	8.88	8.50	5.50	5.50
0.9	5.74	5.50	5.24	4.94	4.21	1.90	1.90
B-2: $W_x = 10.0, W_y = 1.0, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 1.0$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	9.10	9.01	8.90	8.77	8.36	5.26	5.26
0.2	8.20	8.04	7.86	7.63	7.00	3.57	3.57
0.3	7.30	7.10	6.86	6.58	5.85	2.70	2.70
0.5	5.50	5.26	5.00	4.71	4.00	1.82	1.82
0.9	1.90	1.81	1.73	1.65	1.47	1.10	1.10
B-3: $W_x = 10.0, W_y = 1.0, [N_{\text{offspring}} - N_{\text{assigned}}]/N_{\text{parent}} = 3.0$							
$b \backslash a$	0	0.1	0.2	0.3	0.5	0.9	
0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
0.1	7.75	7.57	7.35	7.10	6.40	3.08	3.08
0.2	6.14	5.91	5.65	5.34	4.60	2.06	2.06
0.3	4.94	4.71	4.45	4.17	3.52	1.65	1.65
0.5	3.25	3.08	2.89	2.70	2.29	1.29	1.29
0.9	1.32	1.29	1.26	1.23	1.16	1.03	1.03

In all the cases above, $W_x/W_y = 10.0$ was assumed and the expected RRS was calculated by equation 11.

them is an empirical method, if independent information on parentage is available. In that case, error rate a can be estimated by matching offspring to a sample that contains true parents, and b can be estimated by matching offspring to a sample that contains no true parents. We demonstrate it in an application to the data set of steelhead trout below. If independent parentage information is not available, one can use computer simulations to estimate a and b . For example, one of the most common parentage assignment programs, CERVUS (Marshall *et al.* 1998), provides a simulation function that can be used for the estimation of a and b . Their simulation chooses the value of a test statistic that controls the rate of 'type I' error, which is the proportion of incorrectly assigned offspring among all the assigned offspring. Thus, they test the null hypothesis that a candidate parent is *not* the parent of an offspring, and make an assignment when the null hypothesis is rejected. Type II error rate would be the rate of incorrect decisions among all unassigned offspring. Type I and II errors are not the same as type A and type B errors, but we can obtain the latter error rates from the former rates by simple equations (Appendix I). This conversion can be used with any other parentage assignment method for which type I and II errors can be estimated (e.g. Nielsen *et al.* 2001). Because the CERVUS program provides so called 'success rate', $P_{\text{assigned}} = (N_{\text{assigned}}/N_{\text{offspring}})$, instead of type II error rate, we also obtained an equation to estimate type A and type B error rates from the type I error rates and P_{assigned} in Appendix I.

Application to the sample of steelhead trout

Here we use samples of wild and hatchery steelhead trout to illustrate how to estimate the type A and type B error rates empirically, and how two types of error rates change depending on the decision criterion used in a parentage assignment. We also demonstrate the estimation of the number of offspring whose parents were not sampled, based on the different criteria.

Parentage assignments were made using exclusion. In order to demonstrate the effects of the stringency of the assignment decision criterion on type A and B error rates, we used three levels of stringency: 0, 1 or 2 mismatching loci allowed (see Materials and methods for details).

To estimate the type A error rate, a total of 279 (clipped) steelhead, which were born in a hatchery in 1994, were matched against 381 potential parents returned in 1993–1994. Because we knew that we had all the true parents (27 male and 26 female broodstocks) of these offspring in the samples, and because we knew all the mating crosses of the broodstocks made in the hatchery in 1994, we could obtain the proportion of incorrect assignment when all true parents are present in the data (type A error rate). To estimate the type B error rate, the same offspring were matched against the same parent data set excluding either male or female brood stocks, so that all the assignments made were incorrect (type B error). Note that hatchery and wild parents would share similar genetic compositions because hatchery broodstocks were randomly selected from the wild population in the same year. Table 3 shows the results of these estimations. Type A and type B errors were estimated as 21.1% and 1.4%, respectively, when no mismatch was allowed, but were reversed to 1.4% and 30.5%, respectively, when up to two mismatches were allowed. These results show the trade-off between type A and type B errors, obviously controlled by the stringency of the assignment decision criterion used to make a match.

Next, we matched a total of 502 (clipped and unclipped) offspring, which were either hatchery or wild-born steelhead born in 1994, against all the available parental samples. We then used the estimates of a and b obtained above to estimate the number of offspring of missing parents in this sample (zW_z). Table 3 shows that the number of offspring assigned to parents increased from 284 to 370 as the decision criterion was relaxed from 0 to 2 mismatches. However, the estimate of zW_z was relatively stable (ranging from 145.1 to 185.4, or 29–37% of offspring). Because we obtained these estimates by assigning both parents (mother

Table 3 Estimates of the type A, type B errors and number of offspring from missing parents in samples of steelhead trout

Offspring Parents	Hatchery ($N_{\text{offspring}} = 279$) true and untrue parents		Hatchery ($N_{\text{offspring}} = 279$) untrue parents only			Wild and Hatchery ($N_{\text{offspring}} = 502$) all available	
	N_{assigned}	\hat{a}	N_{assigned}	\hat{b}	$\hat{a}\hat{b}$	N_{assigned}	$z\hat{W}_z$
0 mismatch	220	0.211	3	0.014	0.00296	284	145.1
≤ 1 mismatch	270	0.032	18	0.065	0.00208	347	154.5
≤ 2 mismatches	275	0.014	85	0.305	0.00427	370	185.4

Type A error rate was estimated by matching the hatchery-born offspring to all the potential parents, including all the true parents (broodstocks). Type B error rate was estimated by matching the same hatchery-born offspring to the wild samples that cannot be their parents. The estimated number of offspring from missing parents ($z\hat{W}_z$) was calculated from equation 15. Incorrect assignments between one wild-born mother (#1994–0435) and one hatchery-born mother (#1994–0246) were ignored in this analysis, because these fishes shared identical genotypes.

and father) to offspring, this result indicates that only 63–71% of offspring had both parents sampled in our data set, and that the rest of the offspring had either one or both parents that were not sampled.

The estimation of zW_z provides a basis for choosing an appropriate criterion for estimating RRS. If zW_z is close to zero, a criterion with the smallest a and ab will provide the best estimate of RRS (equation 3). However, in the case of steelhead trout, the large values for the estimate of zW_z suggest that we should choose more stringent criterion to reduce the type B error rate for a less biased estimate of RRS.

Discussion

Relationship between observed RRS and assignment errors

We showed that minimizing either type A or type B error will insure the minimum bias on an estimate of RRS when all parents are sampled. It may be surprising that type A error has a larger effect on the observed RRS than type B error in this case (Fig. 1). This results because type B error can be effective only when assignment to the true parents failed, which corresponds to type A error. On the other hand, when a large portion of parents is not sampled, the impact of type B error on the observed RRS becomes large because all the offspring who have no parent in the data set are subject to the type B error (Fig. 2 and Table 2). Nielsen *et al.* (2001) noted the same phenomenon by showing how ignoring missing parents causes a high proportion of incorrect assignments. Our study provided an explicit explanation for this phenomenon (i.e. high type B error caused by missing parents).

All through the examples in this study, we assumed equal number of parental samples ($x = y$) and the same genetic compositions between different groups for simplicity. However, equations in this study are also applicable to investigate the cases of unequal parental sample sizes between groups. Because the error terms in equations 3 and 8 are proportional to $(xW_x + yW_y)$, the errors influence the estimate of RRS more when $W_x > W_y$ and $x > y$, than when $W_x > W_y$ and $x < y$. In other words, if total number of parents and the absolute fitness of two groups are constant, the RRS is more biased when the proportion of parental samples from a group with *higher* fitness is high, while the bias is limited when that with *lower* fitness is high. On the other hand, in a case that two groups have different genetic compositions, like in different geographical populations or strays, we need to introduce different assignment error rates for each group and to formulate another equation, because assignment errors will not occur proportionally to the size of groups, and because offspring will not be matched randomly when error occurs. However, the effects of assignment errors on an estimate of RRS

may be limited in this case, because offspring from a parent in one group tend to be assigned to a parent in the same group if two groups are genetically differentiated. In other words, we expect better resolution of each genetic marker for distinguishing two groups in this case.

Efficacy and applicability of the correction methods

In this study, we proposed a new method for unbiased estimation of RRS. The efficacy of our method can be illustrated by a categorization of high (group X) and low (group Y) fecundity groups in our data of steelhead trout. For example, we could intentionally create a group of 61 male parents with $\hat{W}_x = 1.13$ and that of 62 male parents with $\hat{W}_y = 0.161$, using the one-mismatch criterion. The observed RRS was 7.01. However, using equation 14 and the estimated type B error rate (6.50%, Table 3), the estimate of RRS was corrected to 12.26, which was 75% higher than the observed. This example suggests that our method can efficiently estimate an unbiased RRS, even when assignment error rates are relatively high. This fact is extremely important, because it indicates that we can maximize the number of samples used, as long as accurate assignment error rates are obtained. Our correction method must be beneficial especially for studies based on categorical parentage assignment methods, because they often suffer from small number of samples available, to eliminate a large portion of ambiguous matches in parentage assignment.

If one cannot obtain accurate estimates of assignment errors, it is still worth remembering that there will be a trade-off between a and b that is controlled by the stringency of the assignment decision criterion (Table 3). Our results from the genotypic data of steelhead trout is well consistent with Oddou-Muratorio *et al.* (2003), in which computer simulations and categorical likelihood method (Marshall *et al.* 1998) were employed to estimate the assignment error rates in genotypic data from plants. This agreement suggests the generality of our results regardless of assignment method and of organisms studied. The results of these studies should dictate whether one should err on the side of minimizing type A or type B error, depending on the goals of a study.

Other methods for obtaining unbiased estimates of RRS

The fractional assignment is an alternative method to the categorical assignment (Smouse & Meagher 1994; Devlin *et al.* 1988; Nielsen *et al.* 2001). In this approach, reproductive contribution to one offspring is fractionally allocated to parents based on their likelihood of being the parent (Devlin *et al.* 1988). This method was further developed to estimate RRS of different groups when some of the parents are not sampled (Nielsen *et al.* 2001). Note, however, that the fractional assignment is still subject to

assignment errors. Assume that one offspring is assigned to two potential fathers with equal probability. In this case, we can make only a 50% of correct decision even if one of the fathers is the true parent. Eventually, 50% of type A error and 50% of type B error are introduced in the analysis of this offspring. Assuming genetic similarity between two groups, the second half will be proportionally allocated to untrue parents in the two groups on average, and the net effect of these errors will be the same as that in categorical methods. In a case that two groups have different genetic compositions, assignment errors can be minimized if one directly applies it to groups (not to individuals in the groups) as Nielsen *et al.* (2001) did, but further study is required to formulate and evaluate the error effects in that case. It is also noteworthy that these methods require different kinds of estimates in advance, i.e. proportion of parents sampled in Nielsen *et al.* (2001) and type B error rate in our method, to obtain an accurate estimate of RRS. Obtaining accurate estimates of them will be the key for the good estimation of RRS for these methods.

The ultimate solution to assignment errors will be reducing both type A and type B errors at the same time, so that we can make correct decisions in parentage analyses without reducing the number of samples assigned. For this goal, the number of loci and alleles to be analysed, and the test statistic for the parentage assignment have been extensively discussed (SanCristoval & Chevalet 1997; Marshall *et al.* 1998; Davies *et al.* 1999; Bernatchez & Duchesne 2000). However, recent studies suggest that a few genotyping errors can influence the accuracy of parentage assignment quite strongly (Hoffman & Amos 2005). In addition, mutation rate on microsatellite loci is reported to be quite high in some cases (0.57–1.56%, Brohede *et al.* 2002), which will also affect the accuracy of parentage assignment. These studies indicate that any method for parentage assignment is still subject to assignment errors at this point, even though large number of loci with large number of allele can be analysed.

Estimating assignment error rates

Because our methods require an estimate of the assignment error rate, we obtained it by the simplest empirical method, as demonstrated with the data from steelhead trout (Table 3). An alternative method for estimating the assignment error rates is also provided based on computer simulations (Appendix I). However, it is noteworthy that the accuracy of the estimated RRS depends on the accuracy of error rate estimations, and the values of a and b obtained via simulation are only as good as the input parameters and underlying assumptions (e.g. the proportion of parents sampled, the number of loci genotyped accurately on each individual, and the rate of genotyping error and mutation). Thus, empirical estimates of a and b may be preferable if independent parentage information is available. Indeed,

the good agreement in the estimations of $z\hat{W}_z$, based on the estimates of a and b by different stringency criteria (Table 3), suggests that the empirical estimation of a and b is reasonably accurate. In mammals, these estimates may be obtained by mother–offspring relationships observed, and they can be applied to the estimate of RRS between different groups of fathers. Developing a more sophisticated method to obtain an accurate error rate will contribute to obtaining a better estimate of RRS, and an application of the maximum-likelihood method (Burczyk *et al.* 2002 for example) may be one of the potential approaches to this issue.

Testing the difference in fitness

In this study, we focused on a point estimate of RRS. Along with obtaining an estimate of RRS, one will often want to test whether the difference in fitness between groups is statistically significant. Because distributions of individual fitness are likely to be highly skewed, it will be necessary to use permutation tests of significance. Note that a permutation test of whether the difference in fitness between groups differs from zero is equivalent to a test of whether the ratio differs from one (Appendix II). The null hypothesis of the test is $H_0: \hat{W}_x - \hat{W}_y = 0$. From equations 6 and 7, we obtain

$$\hat{W}_x - \hat{W}_y = (W_x - W_y)(1 - a) \quad (\text{eqn 16})$$

Therefore, although type B error biases the point estimate of RRS, it has no effect on the difference in fitness, unless the rate of type B error differs between groups. On the other hand, the difference in absolute fitness is linearly decreased and biased towards zero by type A error. This occurs because the average number of offspring assigned to each group is reduced by failure to assign. Note that in this sense, type A error has the same effect as failing to sample all the offspring produced by the sampled parents.

Further study is required to address how assignment errors bias the test of difference in fitness, but the discussion above leads us to predict that the type A error plays an important role in the power of the test. We can see this effect by a simple example (Table 4). In this example, we mimicked the effect of errors by reducing $(W_x - W_y)$ without changing W_x/W_y for type A error, and by reducing W_x/W_y without changing $(W_x - W_y)$ for type B error. When we applied them to a permutation test, statistical power was decreased only by the type A error, but no effect was observed by the type B error. Note, however, that this example for type B error is an extreme case of a uniform distribution of the prior probability for being parent (Neff *et al.* 2001), and that the effect of the assignment errors on the power of test will also depend on this distribution. As we pointed out, the effect of type A error on significance testing is the same as that of failing to sample all the offspring. The fact that missing parents and missing offspring

Table 4 An example for the test of the difference in fitness between groups

Error type	$X_{\text{offspring}}$	$Y_{\text{offspring}}$	$W_x - W_y$	W_x/W_y	P
No error	{3, 2, 2, 1, 1, 1, 0, 0, 0, 0}	{1, 1, 0, 0, 0, 0, 0, 0, 0, 0}	0.8	5.0	0.037*
Type A error	{2, 1, 1, 1, 0, 0, 0, 0, 0, 0}	{1, 0, 0, 0, 0, 0, 0, 0, 0, 0}	0.4	5.0	0.126
Type B error	{4, 3, 3, 2, 2, 2, 1, 1, 1, 1}	{2, 2, 1, 1, 1, 1, 1, 1, 1, 1}	0.8	1.7	0.035*

$X_{\text{offspring}}$ and $Y_{\text{offspring}}$ represent number of offspring for each parent in group X and Y, respectively. Ten parents for each group were assumed. The examples with type A error and type B error were created so that $(W_x - W_y)$ is reduced without changing W_x/W_y (type A error) or that W_x/W_y is reduced without changing $(W_x - W_y)$ (type B error). Statistical significance of the difference in fitness was tested by permutation with 100 000 replicates.

* $P < 0.05$.

can have a large effect on estimates of absolute or relative fitness, or on tests of difference in group fitness, may not be widely appreciated.

Conclusion

We have shown that minimizing either type A or type B error insures the minimum bias on an estimate of the RRS when all parents are sampled, and that type B error is critical when many offspring have missing parents from the data. However, type A error influences the estimate of RRS more than type B error, when all parents are sampled and both types of error are moderately high. Type A error also influenced the power for detecting a statistically significant difference in fitness. Based on the correction of the error effects, we proposed new methods to obtain an unbiased estimate of RRS and number of offspring whose parents are not sampled. One can apply these methods whenever assignment error rates are obtainable from the same data. Otherwise, one can minimize the error effects, finding an appropriate stringency of the assignment decision criterion, depending on a goal of study. Our methods for unbiased RRS and for number of offspring whose parents are not sampled will provide better insights for broad range of studies on population genetic structures and mating systems in wild populations, regardless of assignment method employed.

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Appendix I

Obtaining estimates of type A and type B errors from estimates of type I and type II errors

One way to estimate a and b without samples of known parentage is to use the simulated distribution of an assignment test statistic such as that provided by the program CERVUS (Marshall *et al.* 1998). Their test statistic, Δ , is the difference in LOD scores between the most likely parent and the second most likely parent. The null hypothesis in the test is that the most likely parent is not the true parent of the offspring. Thus, the test controls the proportion of incorrectly assigned offspring among the assigned offspring (type I error at the rate α). Type II error, β , would be the proportion of incorrect decisions among the unassigned offspring. The simulation in CERVUS provides $(1 - \alpha)$ (the 'confidence level') and the fraction of offspring assigned, $P_{\text{assigned}} = N_{\text{assigned}}/N_{\text{offspring}}$ (the 'success rate'), instead of β . Here we show how to estimate type A and B error rates from either type I and II error rates or type I error rate and P_{assigned} .

By definitions,

$$\alpha = \frac{b(xW_x a + yW_y a + zW_z)}{(1-a)(xW_x + yW_y) + b(xW_x a + yW_y a + zW_z)} \quad (\text{eqn 17})$$

and

$$\beta = \frac{a(1-b)(xW_x + yW_y)}{a(1-b)(xW_x + yW_y) + (1-b)zW_z} \quad (\text{eqn 18})$$

or

$$\beta = \frac{a(xW_x + yW_y)}{a(xW_x + yW_y) + zW_z} \quad (\text{eqn 19})$$

When all parents are sampled ($zW_z = 0$), α is simply $ab/(1-a+ab)$ and β is one, because all the offspring unassigned are made by incorrect decision. In this case, from equations 5 and 10 we see that

$$P_{\text{assigned}} = 1 - a + ab \quad (\text{eqn 20})$$

From these equations,

$$a = 1 - (1 - \alpha)P_{\text{assigned}} \quad (\text{eqn 21})$$

and

$$b = \frac{\alpha P_{\text{assigned}}}{1 - (1 - \alpha)P_{\text{assigned}}} \quad (\text{eqn 22})$$

Thus, both a and b can be estimated from α and P_{assigned} .

Now consider nonsampled parents. The proportion of offspring whose parents were sampled, $P_{\text{offspring}'}$ is defined as

$$P_{\text{offspring}} = \frac{xW_x + yW_y}{N_{\text{offspring}}} \quad (\text{eqn 23})$$

By equations 5 and 23, equations 17 and 19 become

$$\alpha = \frac{b(1 - P_{\text{offspring}} + aP_{\text{offspring}'})}{(1-a)P_{\text{offspring}} + b(1 - P_{\text{offspring}} + aP_{\text{offspring}'})} \quad (\text{eqn 24})$$

and

$$\beta = \frac{aP_{\text{offspring}'}}{1 - P_{\text{offspring}} + aP_{\text{offspring}'}} \quad (\text{eqn 25})$$

respectively. From these equations, a and b are estimated by the following equations;

$$a = \frac{\beta(1 - P_{\text{offspring}'})}{(1 - \beta)P_{\text{offspring}'}} \quad (\text{eqn 26})$$

and

$$b = \frac{\alpha(P_{\text{offspring}'} - \beta)}{(1 - \alpha)(1 - P_{\text{offspring}'})} \quad (\text{eqn 27})$$

($\alpha \neq 1$, $\beta \neq 1$ and $P_{\text{offspring}'} \neq 0, 1$). Although $P_{\text{offspring}'}$ is usually not known, one may assume that $P_{\text{offspring}'}$ is the same as the proportion of parents sampled, $P_{\text{parent}'}$ which is an input parameter of the simulation in the CERVUS program. In other words, if you can assume that missing parents have the same fecundity as sampled parents,

$$P_{\text{parent}'} = \frac{x + y}{x + y + z} = P_{\text{offspring}'} = \frac{xW_x + yW_y}{N_{\text{offspring}'}} \quad (\text{eqn 28})$$

Then we can substitute $P_{\text{offspring}'}$ with $P_{\text{parent}'}$ in equations 26 and 27 to estimate a and b .

The CERVUS program does not provide a direct estimate of the type II error rate, but we can estimate a and b using P_{assigned} ('success rate' in CERVUS) instead of β . Here, by definition of $P_{\text{assigned}'}$

$$P_{\text{assigned}'} = 1 - \frac{(1-b)(xW_x a + yW_y a + zW_z)}{N_{\text{offspring}'}} \quad (\text{eqn 29})$$

and from equations 5, 10, 23 and 29 we obtain

$$a = 1 - \frac{(1 - \alpha)P_{\text{assigned}'}}{P_{\text{offspring}'}} \quad (\text{eqn 30})$$

and

$$b = \frac{\alpha P_{\text{assigned}'}}{1 - (1 - \alpha)P_{\text{assigned}'}} \quad (\text{eqn 31})$$

b is now independent of $P_{\text{offspring}'}$ and can be estimated from the confidence level $(1 - \alpha)$ and the proportion of offspring assigned ($P_{\text{assigned}'}$). This estimation method is easily applied to any categorical assignment method and program in which type I and type II error rates or the proportion of offspring assigned ($P_{\text{assigned}'}$) are available. An empirical method to estimate a and b is described in the main text.

Appendix II

Proof of the equal power of the permutation test for the difference in the fitness between groups and for the RRS test

Here we provide a proof that the power of the permutation test to detect a statistical significance of the difference in average absolute fitness between two groups is exactly the same as of the permutation test to detect a statistically significant departure of the RRS from one.

Assume the observed difference and relative ratio of two groups, X and Y, as D_{obs} and R_{obs} , respectively ($D_{\text{obs}} \geq 0$ and $R_{\text{obs}} \geq 1$). Here we represent those obtained from a permutation (sampling without replacement) as D_{per} and R_{per} , respectively. The hypothesis to be proved here is that $D_{\text{obs}} \leq D_{\text{per}}$ when $R_{\text{obs}} \leq R_{\text{per}}$ and $R_{\text{obs}} \leq R_{\text{per}}$ when $D_{\text{obs}} \leq D_{\text{per}}$. From the definitions,

$$D_{\text{obs}} = \hat{W}_{x[\text{obs}]} - \hat{W}_{y[\text{obs}]} \quad (\text{eqn 32})$$

$$R_{\text{obs}} = \hat{W}_{x[\text{obs}]} / \hat{W}_{y[\text{obs}]} \quad (\text{eqn 33})$$

$$D_{\text{per}} = \hat{W}_{x[\text{per}]} - \hat{W}_{y[\text{per}]} \quad (\text{eqn 34})$$

$$R_{\text{per}} = \hat{W}_{x[\text{per}]} / \hat{W}_{y[\text{per}]} \quad (\text{eqn 35})$$

where $\hat{W}_{x[\text{obs}]}$, $\hat{W}_{y[\text{obs}]}$, $\hat{W}_{x[\text{per}]}$ and $\hat{W}_{y[\text{per}]}$ are the average fitness observed in the group X and Y, and those obtained for the group X and Y from a permutation, respectively ($\hat{W}_{x[\text{obs}]}$, $\hat{W}_{y[\text{obs}]}$, $\hat{W}_{x[\text{per}]}$ and $\hat{W}_{y[\text{per}]} = 0$). Because the permutation (without replacement) holds the total number of offspring produced by the two groups,

$$n_x \hat{W}_{x[\text{obs}]} + n_y \hat{W}_{y[\text{obs}]} = n_x \hat{W}_{x[\text{per}]} + n_y \hat{W}_{y[\text{per}]} \quad (\text{eqn 36})$$

where n_x and n_y are the number of parent in the group X and Y, respectively.

First, we prove $D_{\text{obs}} \leq D_{\text{per}}$ when $R_{\text{obs}} \leq R_{\text{per}}$. From $R_{\text{obs}} \leq R_{\text{per}}$, and (33), (35) and (36), we obtain

$$\hat{W}_{x[\text{obs}]} \leq \hat{W}_{x[\text{per}]} \quad (\text{eqn 37})$$

and

$$\hat{W}_{y[\text{obs}]} \geq \hat{W}_{y[\text{per}]} \quad (\text{eqn 38})$$

From (32), (36), (37) and (38), $D_{\text{obs}} - D_{\text{per}}$ becomes

$$D_{\text{obs}} - D_{\text{per}} = (\hat{W}_{x[\text{obs}]} - \hat{W}_{x[\text{per}]}) + (\hat{W}_{y[\text{per}]} - \hat{W}_{y[\text{obs}]}) \leq 0 \quad (\text{eqn 39})$$

Thus $D_{\text{obs}} \leq D_{\text{per}}$ is true when $R_{\text{obs}} \leq R_{\text{per}}$.

The proof of $R_{\text{obs}} \leq R_{\text{per}}$ when $D_{\text{obs}} \leq D_{\text{per}}$ can be obtained in the same manner. From $D_{\text{obs}} \leq D_{\text{per}}$ (32), (34) and (36), we again obtain (37) and (38), and hence $R_{\text{obs}} \leq R_{\text{per}}$ becomes

$$R_{\text{obs}} - R_{\text{per}} = \frac{1}{\hat{W}_{y[\text{obs}]} \hat{W}_{y[\text{per}]}} (\hat{W}_{x[\text{obs}]} \hat{W}_{y[\text{per}]} - \hat{W}_{x[\text{per}]} \hat{W}_{y[\text{obs}]}) \leq 0 \quad (\text{eqn 40})$$

So $R_{\text{obs}} \leq R_{\text{per}}$ is true when $D_{\text{obs}} \leq D_{\text{per}}$. In either case, the equality is obtained only if $\hat{W}_{x[\text{obs}]} = \hat{W}_{x[\text{per}]}$ and $\hat{W}_{y[\text{obs}]} = \hat{W}_{y[\text{per}]}$. These results confirm that $D_{\text{obs}} \leq D_{\text{per}}$ is true whenever a permutation results in $R_{\text{obs}} \leq R_{\text{per}}$ and *vice versa*, and that a permutation test measuring the proportion of $D_{\text{obs}} \leq D_{\text{per}}$ is statistically equivalent to that measuring the proportion of $R_{\text{obs}} \leq R_{\text{per}}$. Namely, they have exactly the same power. Because permutations for testing R_{obs} occasionally produce null output because of 0 in the denominator, one should apply a test of D_{obs} .