

BRIEF COMMUNICATION

**PROBLEMS IN MEASURING AMONG-FAMILY VARIATION  
IN INBREEDING DEPRESSION<sup>1</sup>**

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Understanding the sources of variation in inbreeding depression within populations is important for understanding the evolution of selfing rates. At the population level, inbreeding depression is due to decreased heterozygosity caused by inbreeding, which decreases overdominance and increases the frequency of expression of recessive deleterious alleles. However, within individual families inbreeding has two distinct consequences: it reduces heterozygosity and it restricts the alleles present in offspring to those present in the parent. Outcrossing both increases heterozygosity and brings new alleles into a family (compared to the alleles present if the plant is self-pollinated). Both consequences of inbreeding affect offspring fitness, but the most common experimental design used to measure among-family variation in inbreeding depression cannot distinguish them. The result is that variance in inbreeding depression among families is confounded by genetic variation in the traits being measured. Also, correlations (among families) between measures of inbreeding depression or between inbreeding depression and mean trait values are confounded by genetic variation in the traits being measured. I conclude that more complex crossing designs that allow estimation of breeding values for individual families are required to accurately detect and measure among-family variation in inbreeding depression.

**Key words:** genetic variation; inbreeding depression; mating systems; outcrossing; selfing.

Inbreeding depression is a decline in mean phenotype, usually of fitness traits, with inbreeding (Lynch and Walsh, 1998). Understanding variation in inbreeding depression is important for understanding the evolution of selfing frequency in plants because it changes the dynamics of the evolution of self-pollination (Schultz and Willis, 1995; Kelly, 2005). Recently, numerous studies have demonstrated that families vary in the level of inbreeding depression (e.g., Agren and Schemske, 1993; Hamilton and Mitchell-Olds, 1994; Helenurm and Schaal, 1996; Culley et al., 1999; Dudash and Fenster, 2001; Picó et al., 2004; and references therein; see review in Kelly, 2005), suggesting that there is substantial genetic variation within populations in the effects of inbreeding on fitness. The approach used in most of these studies is to compare traits of offspring created by selfing with the traits of their siblings created by outcrossing. The standard coefficient of inbreeding depression is  $\delta = (W_o - W_s)/W_o$ , where  $W_o$  and  $W_s$  are the mean trait values of offspring from outcrossed and self-pollinated flowers, respectively (Lande and Schemske, 1985; Donohue, 1998). Negative numbers indicate outcrossing depression and positive numbers indicate inbreeding depression. Population level  $\delta$  can be calculated by either pooling all outcrossed vs. selfed offspring in a population or by estimating  $\delta$  separately for each family  $i$  and calculating  $\delta = \mu(\delta_i)$  (the mean of all families; Johnston and Schoen, 1994), though these estimates are not equivalent (their properties are discussed thoroughly by Johnston and Schoen, 1994). The variance in  $\delta_i$  among families is generally interpreted as a measure of the variation among families in inbreeding depression.

In the strictest sense, inbreeding depression is a conse-

quence of allelic interactions within loci (i.e., dominance; Crow and Kimura, 1970) and cannot occur when gene action is entirely additive (Lynch and Walsh, 1998). Inbreeding leads to increased homozygosity, which both reduces the incidence of overdominance (high trait values in heterozygotes) and increases the frequency with which recessive deleterious alleles are expressed (Carr and Dudash, 2003). In a large population, inbreeding does not affect average allele frequencies, only the frequency of heterozygotes, e.g., selfing reduces heterozygosity by 50% each generation. Thus, at the population level, the standard coefficient of inbreeding depression ( $\delta$ ) estimates the effect of changes in heterozygosity on mean phenotypes (see Johnston and Schoen, 1994, for a discussion of statistical considerations in estimating  $\delta$ ). However, within individual families allele frequencies are not the same for selfed vs. outbred offspring; selfing limits the alleles present in offspring to those present in the selfed parent, whereas offspring produced by outcrossing contain alleles from two parents. This difference in allele frequencies between selfed and outcrossed offspring contributes to the difference between selfed and outcrossed offspring in the traits being measured and thus contributes to the family level estimates of  $\delta$ , confounding two mechanistically different consequences of inbreeding.

Both the effect of inbreeding on heterozygosity and the effect on within-family allele frequencies influence the fitness consequences of inbreeding for individual plants and are important for the evolution of selfing rate. Many theoretical models of selfing-rate evolution thus consider both consequences simultaneously (e.g., Lande and Schemske, 1985; Schultz and Willis, 1995). However, the typical experimental design used to measure variation among families in  $\delta$  (in which we compare traits of offspring created by selfing with traits of their siblings created by outcrossing) cannot distinguish between the two sources of among-family variation in inbreeding depression. Whenever families vary genetically in the traits of interest (e.g., genetic variation in seed set) variance among families

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in  $\delta$  includes a component due to genetic variation in the measured trait, plus a component due to variation among families in the effects of homozygosity on the trait. Estimates of  $\delta_i$  are therefore not independent of trait means and variances; they will typically be correlated with family means for the measured trait. Here I describe two measures of family level inbreeding depression, one that is confounded by genetic variation among families ( $\delta'_i$ ) and one that is not ( $\delta_i$ ). I discuss the consequences of using one measure over the other and describe how to estimate variation among families in inbreeding depression without the confounding effect of genetic variation among families.

**The effect of experimental design on family-level estimates of inbreeding depression**—Let  $\mu$  be the overall population mean,  $B_i$  be the breeding value of an individual plant (twice the mean deviation of an individual's outcrossed progeny from the population mean; Falconer and Mackay, 1996),  $W_{Ei}$  be the expected mean trait of offspring from a family created from self-fertilization when there is no inbreeding depression, and  $W_{Si}$  be the mean value of a trait for offspring from the same family when there is inbreeding depression. If a plant pollinates itself, the expected mean phenotype of its offspring in the absence of inbreeding depression will be

$$W_{Ei} = \mu + B_i. \quad (1)$$

I define our index of inbreeding depression,  $\delta$ , to be the proportional reduction in a trait due to reduced heterozygosity caused by inbreeding, such that the expected mean phenotype for offspring from a selfed plant will be

$$W_{Si} = (\mu + B_i)(1 - \delta_i) = W_{Ei}(1 - \delta_i). \quad (2)$$

Rearranging, we find that the coefficient of inbreeding depression for each family  $i$  is

$$\delta_i = (W_{Ei} - W_{Si})/W_{Ei}, \quad (3)$$

which is equal to  $1 - (W_{Si}/W_{Ei})$ . Unfortunately, unless we already know  $\delta_i$ , we cannot measure  $W_{Ei}$  because families cannot simultaneously show inbreeding depression and not show inbreeding depression (i.e.,  $W_{Ei} = W_{Si}$  only when  $\delta_i = 0$ ; when  $\delta_i \neq 0$ , we only know  $W_{Si}$ ). The typical solution to this problem (e.g., Carr and Dudash, 1996; Culley et al., 1999; Daehler, 1999; Picó et al., 2004) is to self-pollinate some flowers on a plant to create the inbred families, while crossing other flowers from the same plant with pollen from a different (usually randomly chosen) plant to create the outcrossed families. This creates an outbred family with mean trait values of  $W_{Oi}$ . This outbred family is paired with an inbred (selfed) family, both sharing one parent in common.  $W_{Oi}$  is assumed to be an estimate of  $W_{Ei}$  such that  $\delta_i$  can be calculated as

$$\delta'_i = (W_{Oi} - W_{Si})/W_{Oi} = 1 - (W_{Si}/W_{Oi}). \quad (4)$$

However,  $W_{Oi}$  is not a good estimator of  $W_{Ei}$  because outbreds and inbreds differ in both their heterozygosity and in the alleles they carry (because outbred offspring receive alleles from two parents instead of only one). Instead,  $W_{Oi}$  will be intermediate between  $W_{Ei}$ , and the mean of the individuals chosen to provide pollen for outcrossing because half of the alleles in the outcrossed offspring come from the pollen donor. Thus, eq. 3 is a measure of inbreeding depression due only to changes in homozygosity within families (as inbreeding depression was historically defined in population and quantitative genetics; e.g., Lynch and Walsh, 1998; Haliburton, 2004), whereas

eq. 4 measures inbreeding depression due to both changes in heterozygosity and allele frequency differences between inbred and outbred families.

Assuming outcross parents are chosen at random from the population, the expected value of the outbred offspring is

$$W_{Oi} = \mu + B_i/2 = (W_{Ei} + \mu)/2. \quad (5)$$

Here we divide the breeding value ( $B_i$ ) by 2 because only half the genome comes from the parent to be inbred, whereas the other half is chosen at random from the population as a whole. In practice, parents that are chosen as pollen donors to create outbred families are a subsample of the entire population, such that

$$W_{Oi} = (W_p + \mu + B_i)/2 = (W_{Ei} + W_{Pi})/2, \quad (6)$$

where  $W_{Pi}$  is the mean trait value of the individual (or individuals) chosen to provide pollen for the outcrossing. In many experiments, all plants are pollinated with pollen from the same donors such that  $W_{Pi}$  is the same for all estimates of  $W_{Oi}$ ; if different pollen donors are used for each plant, then  $W_{Pi}$  will vary among plants increasing the among-family variance in  $\delta_i$ . Equation 6 is identical to eq. 5 except that  $W_{Pi}$  (the mean phenotype of the parents chosen as pollen donors for outcrossing plant  $i$ ) is substituted for  $\mu$ . Substituting  $(W_{Ei} + W_{Pi})/2$  for  $W_{Oi}$  in eq. 4, we find that

$$\delta'_i = 1 - [2W_{Si}/(W_{Ei} + W_{Pi})]. \quad (7)$$

In other words, our estimate of  $\delta$  for a specific family,  $i$ , is dependent on both the breeding values of the parent to be inbred ( $W_{Ei} = \mu + B_i$ ) and breeding value of the parent chosen as a pollen donor for outcrossing [ $W_{Pi} = \mu + B_p$ , where  $B_p$  is the breeding value of the pollen donor; i.e.,  $\delta'_i = 1 - [2W_{Si}/(2\mu + B_i + B_p)]$ ]. Remember that the amount of inbreeding depression due to reduced heterozygosity,  $\delta_i$ , is  $1 - (W_{Si}/W_{Ei})$ . When the expected mean of a family in the absence of inbreeding depression ( $W_{Ei}$ ) is the same as the expected mean of offspring chosen as pollen donors for the outbreds ( $W_{Pi}$ ; i.e., when  $B_i = B_p$ ), then  $\delta'_i = \delta_i$ . However, if  $W_{Ei} > W_{Pi}$  ( $B_i > B_p$ ) then  $\delta'_i$  underestimates  $\delta_i$ , whereas if  $W_{Ei} < W_{Pi}$  ( $B_i < B_p$ ), then  $\delta'_i$  overestimates  $\delta_i$ . We thus expect  $\delta'_i$  to overestimate the true  $\delta_i$  (i.e., the effect of inbreeding due to reduced heterozygosity) for families with high trait values (e.g., high fitness) whereas  $\delta'_i$  will underestimate the true  $\delta_i$  for families with low trait values. This effect is due to the difference in alleles present in outcrossed vs. selfed offspring.

**Consequences for interpreting family-level estimates of inbreeding depression**—There are two important consequences of this difference between  $\delta'_i$  and  $\delta_i$ . First, the expected value of  $\delta'_i$  is not 0 even when inbreeding depression is absent. Instead,  $\delta'_i = 0$  only when the expected mean of a family in the absence of inbreeding depression ( $W_{Ei}$ ) is exactly the same as the expected mean of offspring chosen as pollen donors for the outbreds ( $W_{Pi}$ ), a rare occurrence in real experiments. Second, any variation in mean trait values among families will necessarily generate variation in  $\delta'_i$  among families even when  $\delta_i$  (the inbreeding depression due to increasing homozygosity) is the same for all families. Figure 1 illustrates this; it shows an example of four families that differ only in their mean trait values. In this example,  $\delta_i = 0.2$  for all four families, meaning that selfed plants (open circles) have 20% lower fitness than do outcrossed plants (black circles). All four plants are fertilized with pollen from another plant randomly chosen to be a

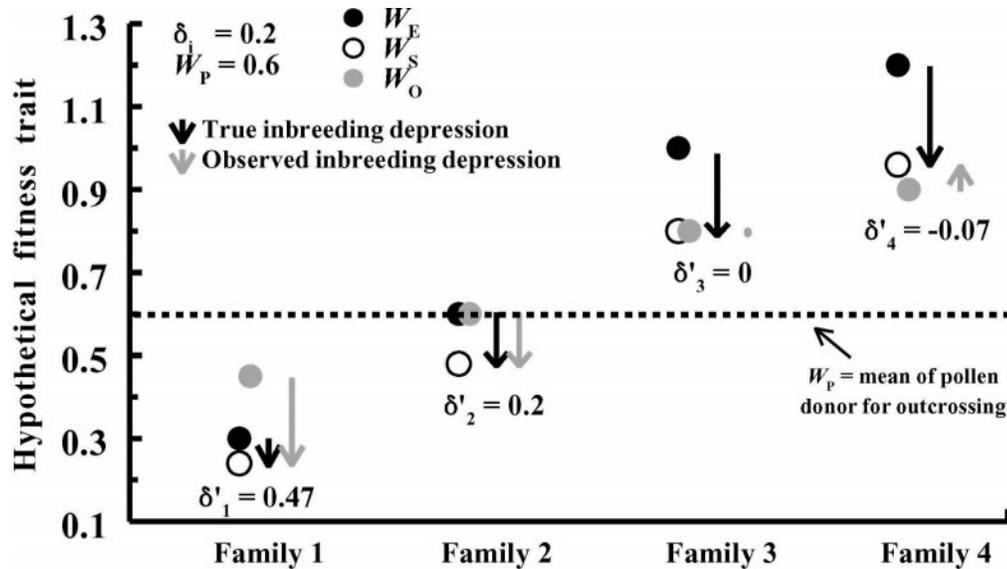


Fig. 1. A hypothetical example illustrating the effect of trait means on estimates of inbreeding depression for individual families ( $\delta'_i$ ). In this example, the true value of inbreeding depression ( $\delta_i$ ) does not vary among families;  $\delta_i = 0.2$  for all families.  $W_{Ei}$  is the expected mean trait of offspring from a self-pollination family when there is no inbreeding depression;  $W_{Si}$  is the mean value of a trait for offspring from the same self-fertilized family when there is inbreeding depression;  $W_{Oi}$  is the mean value of a trait for offspring created by outcrossing. Note that  $\delta'_i$  varies according to the mean of trait value. The degree to which  $\delta'_i$  over- or underestimates  $\delta_i$  depends on how much the family deviates from the population mean. Only when  $W_{Ei} = W_p$  does  $\delta'_i$  accurately estimate  $\delta$ . The value of  $W_{Si}/W_p$  at which  $\delta'_i = \delta_i$  depends on the value of  $\delta_i$ ; specifically,  $\delta'_i = \delta_i$  when  $W_{Si}/W_p = 1 - \delta_i$ .

pollen donor for outcrossing. This pollen donor has trait mean  $W_p = 0.6$  (for simplicity in the example, all four plants are pollinated by the same donor, so  $W_p$  is a constant). Grey circles are the expected fitness of progeny from a cross (e.g., eq. 6). Dark arrows indicate the true difference between the fitness of selfed plants without inbreeding depression and expected fitness of these plants for  $\delta_i = 0.2$ . Because all families have  $\delta_i = 0.2$ , there is no variance in  $\delta_i$  among families. Grey arrows indicate the observed difference between the fitness of selfed plants and the fitness of outbred plants; this observed difference reflects both the effect of inbreeding that is measured by  $\delta_i$  plus an effect of alleles received from the pollen donor on fitness (the offspring created from outcrossing only share half of their chromosomes in common with their inbred siblings). For families with mean traits lower than the average of the pollen donors, the observed inbreeding depression ( $\delta'_i$ ), which includes both the consequences of heterozygosity and genetic differences between the parents, overestimates the true inbreeding depression ( $\delta_i$ ; see Family 1). For families with higher mean trait values than the pollen donors, the observed inbreeding depression underestimates the true inbreeding depression (Family 3) and may even indicate outbreeding depression (e.g., Family 4); this outbreeding depression is due entirely to alleles obtained from the pollen donor and not a fitness consequence of heterozygosity. In general  $\delta'_i$  overestimates  $\delta_i$  for small-trait families by more than it underestimates  $\delta_i$  for large-trait families. Only when  $W_{Ei} = W_p$  does  $\delta'_i$  accurately estimate  $\delta_i$  (Family 2), but this will be true for very few families in the sample. In this example, we see substantial variation among families in  $\delta'_i$  even though  $\delta_i = 0.2$  for all families. Ågren and Schemske's (1993) estimator RP (relative performance of crosstypes), which is widely used as an alternative to  $\delta'_i$ , is likewise confounded. Estimates of  $\delta'_i$  are also sensitive to family size and have a variety of other statistical

problems that are discussed by Johnston and Schoen (1994) and Lynch and Walsh (1998).

Because the coefficient  $\delta$  only provides a measure of the magnitude of inbreeding depression, the typical statistical method to test for among-family variation in inbreeding depression is to use an analysis of variance in which trait value = cross type + family + cross type  $\times$  family; a significant cross type  $\times$  family interaction is assumed to indicate among-family variation in inbreeding depression (Ågren and Schemske, 1993; Hamilton and Mitchell-Olds, 1994; Helenurm and Schaal, 1996; Culley et al., 1999; Dudash and Fenster, 2001; Picó and Koubek, 2003; Picó et al., 2004). Although this analysis does not depend on estimates of  $\delta_i$ , it does not accurately assess the presence of among-family variation in inbreeding depression. Instead, it is affected by the genotype of the pollen donor for the same reason as  $\delta'_i$  and thus will detect variation in inbreeding depression when none is present; it tests for variation among families in the difference between  $W_{Oi}$  and  $W_{Si}$  and not for the differences between  $W_{Ei}$  and  $W_{Si}$ . Testing for a significant cross type  $\times$  family interactions also has a second problem—the analysis of variance tests whether the *difference* in a trait between self and outcrossed treatments varies among families and not whether  $\delta$  (a ratio) varies among families (Johnston and Schoen, 1994). Log-transformation of the traits eliminates this second problem (Johnston and Schoen, 1994), but not the former (see Kelly, 2005, for additional discussion of these analyses).

A few recent studies have examined how estimates of  $\delta'_i$  for different traits are correlated (among families), and whether they are correlated to reproductive traits (e.g., Picó et al., 2004). This type of analysis can shed much light on whether the mechanism and genetics of inbreeding depression are similar for different kinds of traits and whether there is a genetic correlation between mating system traits and the loci that con-

inbreeding depression. However, correlations calculated using  $\delta'_i$  are also confounded; traits that are positively genetically correlated will be biased toward positively correlated estimates of  $\delta'_i$ , and traits that are negatively correlated (such as seed size and seed number) will be biased toward negatively correlated estimates of  $\delta'_i$ . These correlations are thus difficult to interpret without understanding the underlying genetic relationships between the traits.

These two effects on estimates of inbreeding depression are difficult to disentangle in studies of inbreeding depression that compare offspring created through selfing with their half-sibs created by outcrossing. Such designs should not be used to quantify among-family variation in inbreeding depression until better statistical tools are available to disentangle these sources of variance. Other experimental designs, such as many factorial designs, allow us to accurately estimate breeding values ( $B_i$ ) and thus  $W_{Ei}$ . However, such designs are work intensive and thus are rarely performed. Thus, further studies of the statistical properties of  $\delta$ , and how best to estimate it, are necessary before we can interpret among-family variation in  $\delta'$  as evidence of variation in  $\delta$ .

**Conclusion**—The standard measure of inbreeding depression used in most experimental studies is  $\delta'_i = (W_{O_i} - W_{S_i})/W_{O_i}$  [which is  $1 - (W_{S_i}/W_{O_i})$ ; eq. 4], where  $W_{S_i}$  is the mean phenotype of offspring created by selfing and  $W_{O_i}$  is the mean phenotypes of their half-siblings created by outcrossing one of the parents. However, the difference in trait values (such as fitness) between inbred offspring and outbred offspring includes two effects; differences in heterozygosity between inbred and outbred offspring and differences in the alleles present in inbred vs. outbred offspring. When averaging inbreeding depression across all individuals in a population, the expected frequency of alleles in the population of offspring created by selfing does not differ from the expected frequency of alleles in the offspring created by outcrossing, and thus  $\delta = \mu(\delta'_i)$  is a good measure of inbreeding depression (but see Johnston and Schoen, 1994, for some statistical issues with this estimator). However, the differences in allele frequencies between inbred and outbred offspring contributes to the variation among families in  $\delta'_i$ , in addition to the variation among families in  $\delta'_i$  caused by changes in heterozygosity. Though both sources of variation can affect the evolution of mating systems, they can only be distinguished if breeding values ( $B_i$ ) can be accurately estimated for each family, allowing expected trait values in the absence of inbreeding ( $W_{Ei}$ ) to be accurately estimated. Estimates of breeding values require complex experimental designs.

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