Lecture 6

Inbreeding and Crossbreeding

Changes in the Mean

1. Inbreeding

Inbreeding (mating of related individuals) often results in a change in the mean of a trait compared with its value in a random-mating population. Its importance is that inbreeding is generally harmful and reduces fitness. In particular, inbreeding often causes a reduction of the mean value for quantitative traits associated with reproduction and viability.

Inbreeding is intentionally practiced to:

- create genetic uniformity of laboratory stocks
- produce stocks for crossing (animal and plant breeding)

Inbreeding is unintentionally generated:

- by keeping small populations (such as is found at zoos). Genetic drift is a special case of inbreeding. The smaller the population, the quicker inbreeding accumulates.
- during selection (which has the effect of reducing the population size relative to the no-selection case).

The critical parameter for describing inbreeding is F, *the probability that the two alleles at a locus in an individual are identical by descent.* In an individual inbred to amount F, a randomly-chosen locus has both alleles IBD with probability F and hence is a homozygote.

To deduce how inbreeding changes the mean value of a quantitative trait, consider a large number of inbred lines ($F > 0$) that were derived from an initial base population. The initial gene frequencies of alleles A_1 and A_2 at a single locus affecting the trait are p_0, q_0; these gene frequencies are expected to remain the same averaged over all inbred lines (we denote these averages by p and $q = 1 - p$).

To compute the genotypic probabilities under inbreeding, suppose we chose a locus at random. With probability F the two alleles are IBD, and hence this locus is always homozygous, with freq(A_1A_1) = p and freq(A_2A_2) = $q = 1 - p$. If the alleles are not IBD, then the genotypic frequencies follow Hardy-Weinberg. Thus, the expected genotypic frequencies under inbreeding become

Genotype	Alleles IBD	Alleles not IBD	Population frequency
A_1A_1	$F \cdot p$	$(1-F)p^2$	$p^2 + Fpq$
A_2A_1	0	$(1-F)2pq$	$(1-F)2pq$
A_2A_2	$F \cdot q$	$(1-F)q^2$	$q^2 + Fpq$

If the genotypes A_1A_1, A_1A_2, A_2A_2 have values of $a, d, -a$, then the mean under inbreeding becomes

$$\begin{aligned}\mu_F &= a \cdot (p^2 + Fpq) + d \cdot (1-F)2pq - a \cdot (q^2 + Fqq) \\ &= a(2p-1) + 2(1-F)pqd\end{aligned}$$

Noting that the mean character value in a random mating population ($F = 0$) is

$$\mu_0 = a(2p-1) + 2pqd,$$

the mean under inbreeding can be expressed as

$$\mu_F = \mu_0 - 2Fpqd$$

More generally, if there are k loci, then the mean is

$$\mu_F = \mu_0 - 2F \sum_{i=1}^{k} p_i \, q_i \, d_i = \mu_0 - B \, F$$

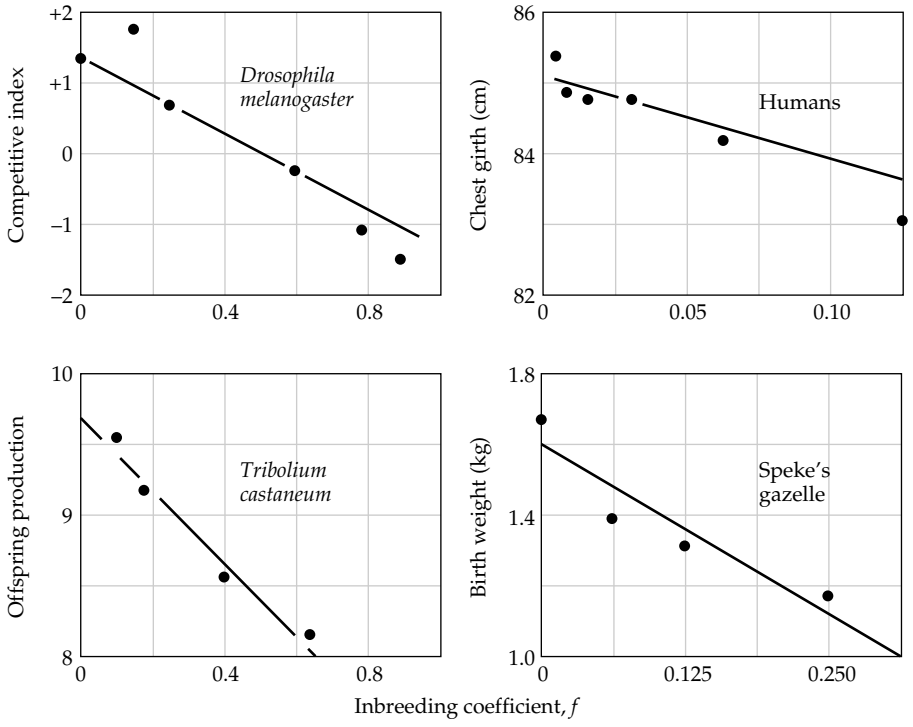
where $B = 2 \sum p_i \, q_i \, d_i$ is the reduction in the mean under complete inbreeding ($F = 1$).

Hence,

- there will be a change of mean value under inbreeding only if $d \neq 0$, i.e., dominance is present.
- for a single locus, if $d > 0$, inbreeding will decrease the mean value of the trait. If $d < 0$, inbreeding will increase the mean.
- with multiple loci, a decrease in the mean under inbreeding (**inbreeding depression**) requires **directional dominance**, with the dominance effects d_i tending to be positive.
- the magnitude of the change of mean on inbreeding depends on gene frequency, and is greatest when $p = q = 0.5$

Inbreeding Depression in Fitness Traits

Fitness-related traits (such as viability, offspring number and body size) often display inbreeding depression, as the following examples illustrate:



Computing the Inbreeding Depression Coefficient, B

In many cases, lines cannot be completely inbred due to either time constraints and/or because in many species lines near complete inbreeding are nonviable. In such cases, one must estimate the inbreeding depression from the changes in series of lines under partial inbreeding.

Recalling that

$$\mu_f = \mu_o - BF$$

it immediately follows that the slope of the regression of the population mean μ_f on the inbreeding coefficient F estimates the inbreeding depression coefficient B.

The above equation is true if loci combine additively (no epistasis), in which case the change in mean should be directly proportional to F (i.e., a linear function of F), the inbreeding coefficient. If epistasis is present, the change in mean can be a nonlinear (polynomial) function of F. Hence, if epistatis is absent, we expect a linear regression of mean on F to be an adequate fit of the data.

Why do traits associated with fitness show inbreeding depression?

Two competing hypotheses have been proposed:

- **Overdominance Hypothesis:** Genetic variance for fitness is caused by loci at which heterozygotes are more fit than both homozygotes. Inbreeding decreases the frequency of heterozygotes, increases the frequency of homozygotes, so fitness is reduced. Since some inbred lines have means for fitness traits equal to the base population, this explanation cannot be generally true.
- **"Dominance" Hypothesis:** Genetic variance for fitness is caused by rare deleterious alleles that are recessive or partly recessive; such alleles persist in populations because of recurrent mutation. Most copies of deleterious alleles in the base population are in heterozygotes. Inbreeding increases the frequency of homozygotes for deleterious alleles, so fitness is reduced.

While the dominance hypothesis is sufficient to account for inbreeding depression, even a very small fraction of overdominant loci will have a major effect on the level B. Hence, even though most loci that contribute to inbreeding depression may due to uncovering of deleterious recessives, the bulk of the contribution to inbreeding depression could theoretically come from a much smaller fraction of overdominant loci.

Minimizing the Rate of Inbreeding

Given the detrimental effects of inbreeding, breeders and zoo keepers go to great lengths to minimize the rate of increase in F. Inbreeding occurs in any finite population, but the larger the effective population size N_e, the slower the effects of drift. One standard way for maximizing N_e is to ensure that all individuals make an equal contribution of offspring to the next generation, as the effective population decreases as the variance in offspring number increases. Maximal N_e occurs when each male and female in the population leaves exactly the same number of offspring. Alas, in many breeding situations there is a very skewed sex ratio, often due to constraints of reproductive biology (a bull can leave effectively an infinite number of offspring, while a cow typically has only one a year).

Gowe et al (1959) suggest that when the sex ratio of contributing parents is r females to each male, every male should contribute (exactly) one son and r daughters, while every female should leave one daughter and also with probability $1/r$ contribute a son. Wang (1997) improved on this by imposing that a female contributing a son does not contribute a daughter, but instead that another female from the same male family contributes two daughters.

2. Line Crossing or Crossbreeding

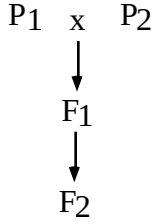
When inbred lines are crossed, the F_1 progeny show an increase in mean for characters that previously suffered a reduction from inbreeding. This increase in the mean over the average value of the parents is called **hybrid vigor** or **heterosis**. Fitness lost on inbreeding is restored by crossing.

To see how heterosis is inbreeding depression in reverse, imagine a large number of inbred lines derived from an outbred base population in which $F = 0$. The mean in each line declines with inbreeding, and the mean of all inbred lines is $\mu_F = \mu_0 - B\,F$. If all these lines are crossed at random, $F = 0$, and the mean of the crossbreeds = μ_0, the mean of the outbred population.

Heterosis can also arise in crosses between outbred (i.e., randomly mating lines), as we detail below.

Single Crosses

Consider the cross between two particular parental strains (P_1 and P_2), which may have no known common origin. In this case the heterosis depends on the difference in gene frequency between the lines, and the amount of heterosis changes from the F_1 to the F_2. Suppose the crossing scheme is:



We will define heterosis as the deviation from the midparental value, so that for the F_1,

$$H_{F_1} = \mu_{F_1} - \frac{\mu_{P_1} + \mu_{P_2}}{2}$$

Let the allele frequencies for a diallelic locus in populations 1 and 2, be p and $p + \delta p$, respectively. We assume the genotypes in P_1 and P_2 are in Hardy-Weinberg proportions (which also hold if the lines are completely inbred), giving the means as

$$\mu_{P_1} = (2p - 1)a + 2p(1 - p)d$$
$$\mu_{P_2} = \mu_{P_1} + 2(\delta p)a - 2(\delta p)^2 d$$

In the F_1, the probability of (say) an A_1A_2 locus is the probability of receiving an A_1 from P_1 and an A_2 from P_2 ($p\,[1 - (p + \delta p)\,]$) or an A_2 from P_1 and an A_1 from P_2 ($[\,1 - p\,]\,[\,p + \delta p\,]$). Considering the other two genotypes gives the mean of the F_1 (expressed in terms of the means for P_1) as

$$\mu_{F_1} = \mu_{P_1} + (\delta p)a,$$

giving a (mid-parental) heterosis (for this particular locus) of

$$H_{F_1} = \mu_{F_1} - \frac{\mu_{P_1} + \mu_{P_2}}{2} = (\delta p)^2 d$$

Hence, for this locus to show heterosis ($H > 0$), we require both a difference in allele frequencies between the populations ($\delta p \neq 0$) and positive dominance ($d > 0$). Note immediately that overdominance ($d > a$) is not required for heterosis. Summing over all loci, the heterosis produced by dominance is

$$H_{F_1} = \sum_{i=1}^{n} (\delta p_i)^2\, d_i$$

Hence,

- heterosis depends on dominance. $d = 0$ = no inbreeding depression and no heterosis. As with inbreeding depression, directional dominance is required for heterosis.
- H is proportional to the square of the difference in gene frequency between populations. H is greatest when alleles are fixed in one population and lost in the other (so that $|\delta_i| = 1$). $H = 0$ if $\delta = 0$.
- H is specific to each particular cross. H must be determined empirically, since we do not know the relevant loci nor their gene frequencies.

Heterosis in F_2

The F_2 generation is derived by mating the F_1 at random. The gene frequencies in the F_1 are the average of the two parents, so that freq$(A_1) = (p + p + \delta)/2 = p + \delta/2$. Since the F_2 is formed by random mating, and the genotype frequencies are in HW equilibrium with allele frequency $p + \delta/2$, the F_2 mean becomes

$$\mu_{F_2} = a([\,p + \delta/2\,]^2 - [\,1 - p - \delta/2\,]^2) + d2[\,p + \delta/2\,][\,1 - p - \delta/2\,]$$

A little algebra shows that

$$H_{F_2} = \mu_{F_2} - \frac{\mu_{P_1} + \mu_{P_2}}{2} = \frac{(\delta p)^2\, d}{2} = \frac{H_{F_1}}{2}$$

so that in the F_2, only half the advantage of the F_1 hybrid is preserved. Since (presumably) random mating also occurs in subsequent generations, the heterosis in future generations is the same as the F_2 heterosis, as the allele frequencies do not change and genotypes remain in Hardy-Weinberg frequencies.

Agricultural importance of heterosis

Heterosis is extremely important in world agricultural. Crosses often show **high-parent heterosis**, wherein the F_1 not only beats the average of the two parents (**mid-parent heterosis**), it exceeds the value of the best parent.

The importance of high-parent heterosis is illustrated by the following estimates of the world-wide contribution of heterosis to both yield and land savings. Here the percent hybrid advantage is the yield increase of the hybrid over the best single variety. (After Duvick 1999).

Crop	% planted as hybrids	% Hybrid yield advantage	Annual added yield Percent	tons	Annual Land savings
Maize	65	15	10	55×10^6	13×10^6 ha
Sorghum	48	40	19	13×10^6	9×10^6 ha
Sunflower	60	50	30	7×10^6	6×10^6 ha
Rice	12	30	4	15×10^6	6×10^6 ha

Change of Variance With Inbreeding

Inbreeding causes a re-distribution of genetic variance within and between lines. For completely additive loci, this can be expressed in terms of the genetic variance ($V_A = V_G$) present in the base population:

	General	$F = 1$	$F = 0$
Between Lines	$2FV_A$	$2V_A$	0
Within Lines	$(1-F)V_A$	0	V_A
Total	$(1+F)V_A$	$2V_A$	V_A

Analogous to the single locus case, inbreeding increases genetic variance between lines and decreases genetic variance within lines. With dominance, the expressions are not simple multiples of the base population genetic parameters, and depend on gene frequency, so there is no simple solution for the re-distribution of variance (see Chapter 3 of Walsh and Lynch).

The heritability within any one inbred line (assuming only additive variance) is

$$h_t^2 = \frac{(1-F_t)V_A}{(1-F_t)V_A + V_E}$$

or

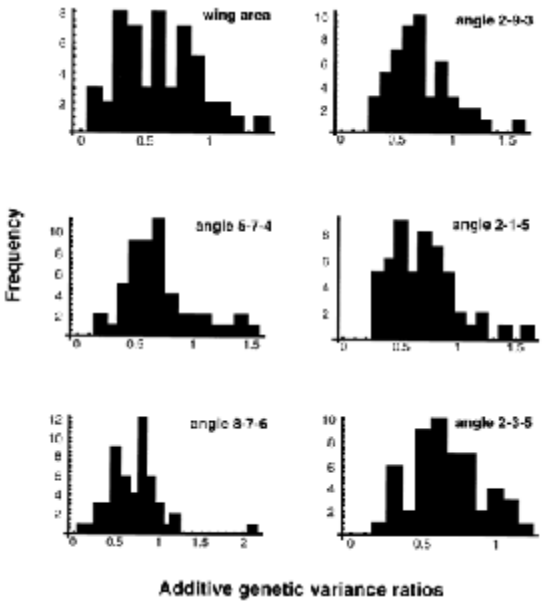
$$h_t^2 = h_0^2 \, \frac{1-F_t}{1-h_0^2 \, F_t}$$

when expressed in terms of heritability in the base population.

Example: Effect of Inbreeding on the Additive Genetic Variance

(M. Whitlock and K. Fowler, 1999, Genetics 152:345-353)

Whitlock and Fowler created 52 inbred lines of *Drosophila melanogaster* by passing each through a bottleneck of one pair ($F \simeq 0.25$). Parent-offspring regressions were used to estimate additive genetic variances for a set of wing dimensions in each line, as well as in the outbred base population from which the lines were derived. Results were expressed as the ratio of V_A in inbred lines to V_A in the base population.



On average, V_A in the inbred lines was 60-71% of original V_A, depending on the trait. The reduction in V_A was thus slightly greater than the theoretically predicted 25%. The authors attributed this to additional inbreeding that took place after the one-pair bottlenecks.

Notably, V_A for a given trait varied greatly among lines, with a few lines even showing significant increases in V_A. This is not surprising, because allele frequencies in each line will change randomly.

Change of Variance with Inbreeding and Mutation

Inbred lines will never completely lose all genetic variance, because new mutational variance is introduced each generation at rate V_M. In the long term the genetic variance within an inbred line will reach an equilibrium level at which point the variance gained each generation from mutation is exactly balanced by the variation lost by inbreeding. We consider the particular case where inbreeding is caused by genetic drift in a finite population, in which case the accumulation of inbreeding scales as $1/(2N_e)$, where N_e is the effective population size.

Assume:

- Strictly neutral mutations
- Strictly additive mutations
- Symmetrical distribution of mutational effects

Then at equilibrium

$$V_A = V_G = 2N_e V_M$$

Note that this is the same as that expected within a selection line in mutation-drift equilibrium. With $V_M = 10^{-3}V_E$ and $N_e = 2$ for full sib inbreeding, V_G at mutation-drift equilibrium is $4 \times 10^{-3}V_E$, and the heritability is

$$h^2 = \frac{V_A}{V_G + V_E} = \frac{4 \times 10^{-3}V_E}{4 \times 10^{-3}V_E + V_E} = 0.004$$

which is trivial.

Mutation also contributes to the increase in variance between sublines derived from a common inbred line. The variance among lines from new mutation after t generations is

$$V_B = 2V_M[\, t - 2N_e(1 - e^{-t/2N_e})]$$

At equilibrium the rate of divergence is $2V_M$ per generation and the total divergence expected is $2tV_M$, which is not negligible.

Additional References

A full treatment of the change in variance under inbreeding when dominance is present can be found in Chapter 3 of Walsh and Lynch, on the web at

http://nitro.biosci.arizona.edu/zbook/volume_2/chapters/vol2_03.html

Inbreeding and Crossbreeding Problems

1. Suppose that four genes, each with two alleles affecting body weight have been identified and are segregating in a random-bred strain of mice. The mean weights for the three genotypes (in grams) for each of the four genes are as follows:

Gene A		Gene B		Gene C		Gene D	
A_1A_1	22	B_1B_1	22	C_1C_1	22	D_1D_1	22
A_1A_2	21	B_1B_2	22	C_1C_2	20	D_1D_2	21
A_2A_2	20	B_2B_2	20	C_2C_2	20	D_2D_2	18
freq(A_2)	0.5	freq(B_2)	0.5	freq(C_2)	0.1	freq(D_2)	0.2

Calculate for each gene the inbreeding depression caused by it in the progeny of one full-sib mating, when the allele frequencies in the random-bred population are as shown. Recall that $F = 0.25$ after one generation of full sib mating. What is the total inbreeding depression? What is the value of B?

2. Suppose highly inbred lines of mice are crossed in pairs, and the adult body weights of the two lines crossed and of the F_1 are as shown below. Calculate: (1) the amount of heterosis shown by each cross and (2) the expected body weights in the F_2 generation if random breeding within each crosses was continued. Assume that there are no maternal effects on adult body weight.

	Line A	Line B	F_1
(i)	18	16	20
(ii)	22	16	21
(i)	18	18	24

3. The genetic variance of bristle number in random breeding populations of *Drosophila* is about 2. Assume that all the genes affecting bristle number act additively, and suppose that a random-bred population is inbred in a large number of lines by full-sib mating over 10 generations.

(1) Plot graphs showing the genetic variance (i) between lines, (ii) within lines, (iii) in total. (See F & M Table 5.1 for inbreeding coefficients with full sib mating.)

(2) Show on the graphs the limiting values if the lines were 100% inbred.

Solutions to Inbreeding and Crossbreeding Problems

1. The contribution to inbreeding depression from each loci is $2Fp_iq_id_i$

	Gene A	Gene B	Gene C	Gene D
d_i	0	1	-1	1
$2p_iq_id_i$	0	0.5	-0.18	0.32

$$B = \sum 2p_iq_id_i = 0.64, \qquad F \sum 2p_iq_id_i = 0.16$$

Hence, the inbreeding depression is 0.16.

2. Recall that Heterosis $H = F_1 - (\mu_A + \mu_B)/2$, while only half of this is retained in the F_2, with the mean of the F_2 being $H/2 + (\mu_A + \mu_B)/2$

	Line A	Line B	F_1	H	F_2 mean
(i)	18	16	20	3	18.5
(ii)	22	16	21	2	20
(i)	18	18	24	6	21

3. Recall that the variances are as follows: Between Lines $2FV_A = 4F$, Within Lines $(1 - F)V_A = 2(1 - F)$, Total $(1 + F)V_A = 2(1 + F)$.

Limiting values: Between-lines: 4, within lines: 0, total = 4.