

INBREEDING AND HOMOZYGOSIS IN BEES*

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TWO discoveries in recent years have made controlled mating in the honey bee (*Apis mellifera* L.) a practical possibility. These are (1) suitable techniques for artificial insemination, and (2) the use of carbon dioxide to stimulate egg-laying by virgin and artificially inseminated queens. (For techniques and references, see MACKENSEN and ROBERTS 1948.) In controlled breeding experiments it is desirable to have formulae for estimating the changes in homozygosity and relationship which follow various systems of mating.

The genetic consequences of various kinds of inbreeding procedures have been worked out by JENNINGS (1912, 1914, 1916), ROBBINS (1917), and especially by WRIGHT (1921, 1933). But because of ploidy differences in male and female some modification is required if the formulae are to be applicable to bees. KALMUS and SMITH (1948) have recently reported on the amount of increase in homozygosity to be expected under three systems of mating. In this article we shall, using WRIGHT's methods, verify and extend these results, and give procedures for determining coefficients of inbreeding and relationship from irregular bee pedigrees. The formulae are, of course, applicable to *Habrobracon* or other haploid-diploid organisms.

Since the male bee is haploid, the genetic composition of every gamete produced by a drone is the same and is identical to that of the unfertilized egg from which the drone developed. Therefore, the correlation between the gamete giving rise to a male and a gamete produced by that male is one. With this slight modification in the value of the coefficients in a path diagram, the procedures devised and used by WRIGHT (1921, 1933) become directly applicable. The situation is analogous to matings involving sex-linked or homozygous autosomal loci in diploids and in some cases the results have already been worked out by WRIGHT.

GENERAL FORMULAE

The symbols and basic formulae used here as well as the method of diagramming mating systems are taken directly from the work of WRIGHT (1921a, 1921b, 1933, 1934). The reader is referred to these papers for a general discussion of WRIGHT's method of path coefficients and its application to breeding problems. The formulae relevant to this discussion are as follows and refer to the diagrams in the figures:

(1) Squared path coefficient, zygote to gamete $b^2 = (1+f')/2$

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- (2) Squared path coefficient, gamete to zygote $a^2 = 1/2(1+f)$
- (3) Correlation between uniting egg and sperm $f = 1 - 2p$
- (4) Proportion of heterozygous loci $p = (1-f)/2$

Symbols with primes refer to the previous generation. The formula for p assumes that, with random mating, $p = .5$, which would be true in the special case of two alleles with equal frequency. If the proportion of heterozygosity in a randomly mating population is some other value, p should be multiplied by twice this value to give the true proportion of heterozygosity.

MOTHER-SON MATING

In this procedure the unmated queen is first stimulated to lay unfertilized eggs. Later she is inseminated with sperm from a drone which has developed from one of these eggs, and the whole process is repeated with the females that have developed in the next generation. KALMUS and SMITH (1948) have called the step from one female to the next a "double generation" and we shall follow that terminology here.

From figure 1 and formulas (1) and (3)

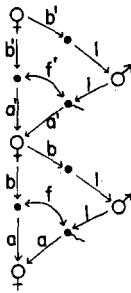


FIGURE 1. Mother-son mating.

$$f = b^2 = (1 + f')/2$$

$$p = p'/2$$

The proportion of heterozygosity in females is halved each double generation as has been shown by KALMUS and SMITH. This system of mating is mathematically equivalent in a double generation to a single generation of self-fertilization in diploid organisms.

With present techniques of artificial insemination of bees the best results are obtained by pooling the semen from three or four drones for a single insemination of a queen and repeating the process after 48 hours. Fortunately this does not change the rate of increasing homozygosity under this system of mating. As long as all the males used are sons of the queen which they are used to inseminate the proportion of heterozygosity is reduced by 50 percent each double generation (equivalent to 29.3 percent each single generation).

BROTHER-SISTER MATING

From the diagram in figure 2

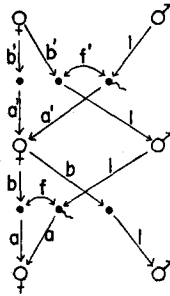


FIGURE 2. Brother-sister mating.

$$f = ba'b'^2 + ba'f' = 1/4 + f'/2 + f''/4$$

$$p = p'/2 + p''/4 = p' - p''/8$$

This formula is equivalent to the formula, $h = h' + (1 - h'')/8$ where h is the proportion of homozygous loci, derived by KALMUS and SMITH by an entirely different procedure. Also the mathematically equivalent case, that of brother-sister mating with sex-linked genes was originally worked out by WRIGHT (1933). The limiting rate of change per generation (obtained by setting $p/p' = p'/p'' = x$ and solving for $1 - x$) is 19.1 percent.

As in the case of self fertilization the use of pooled semen from several brother drones does not alter these results.

AUNT-NEPHEW MATING

From figure 3

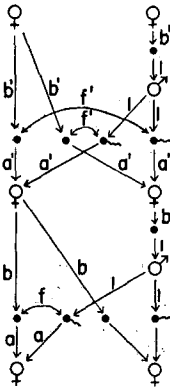


FIGURE 3. Aunt-nephew mating.

$$f = b^2a'^2b'^2 + b^2a'^2 + 2b^2a'f' = 3/8 + f'/2 + f''/8$$

$$p = p'/2 + p''/8$$

This is equivalent to the formula, $h = 3h'/4 + (9 - h'')/32$, obtained by another procedure by KALMUS and SMITH (1948). The limiting percentage reduction in heterozygosity is 31.7 percent per double generation, or 17.0 percent per single generation.

In order for the heterozygosity to be reduced at this rate it is necessary that a single male be used to inseminate the queen. This precludes the use of pooled semen which with present techniques is necessary for a high percentage of fertilized eggs and therefore makes this system of mating less useful than it would be otherwise.

COUSIN MATING

In bees there is no distinction between cousin and double cousin mating because of the fact that the male has only one parent. A system of continuous

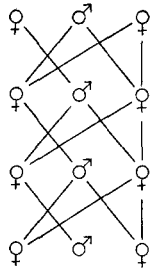


FIGURE 4. A system of continuous cousin mating.

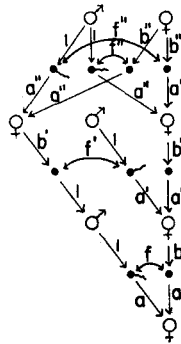


FIGURE 5. Cousin mating.

cousin mating is diagrammed in figure 4. From the path diagram in figure 5,

$$\begin{aligned}
 f &= ba'f' + 2(a'b' b''a''f) + a''b''a'b + a''b''a'bb''^2 \\
 &= 3/16 + f'/2 + f''/4 + f'''/16 \\
 p &= p'/2 + p''/4 + p'''/16
 \end{aligned}$$

This is the same formula as that obtained by WRIGHT for mating of half-brothers and sisters in diploid organisms. The limiting rate of decrease of heterozygosity (obtained by setting $p/p' = p''/p' = p'''/p'' = x$ and solving for $1-x$) is 13 percent per generation as compared with eight percent for double first cousins in diploids.

In this system of mating, as in aunt-nephew mating, it is necessary to use sperm from only a single drone if this analysis is to be applicable.

is illustrated by the first three generations of the pedigree in figure 8. This is equivalent to repeated backcrossing to the original female with the drones acting as successive gametes. The situation then becomes identical to the same mating system in diploids which has been worked out by JENNINGS (1916) and WRIGHT (1921). The heterozygosity formula is:

$$p = 1/8 + p'/2$$

and if the initial percentage of heterozygosity is assumed to be 50 percent the series 3/8, 5/16, 9/32, 17/64, etc. is obtained.

This series does not approach 0 as a limit, but rather 1/4 as may be seen by setting $p = p'$. Nevertheless, the increase in homozygosity in the first few generations is quite rapid, being initially the same as brother-sister mating. The chief merit of this system of mating comes from the fact that females mature faster than males. The time from egg to time of mating for queens is about 22 days and for drones about 38 days. For this reason heterozygosity is reduced more rapidly at first by this system than by brother-sister mating. Also this procedure allows repeated backcrossing to an outstanding queen.

The use of pooled semen does not affect the accuracy of the calculations in this system.

CONCLUSIONS CONCERNING INBREEDING SYSTEMS

The formulae from the previous sections together with the proportion of heterozygosity in successive generations under each system of mating are given in table 1. These proportions are shown graphically in figure 7. The

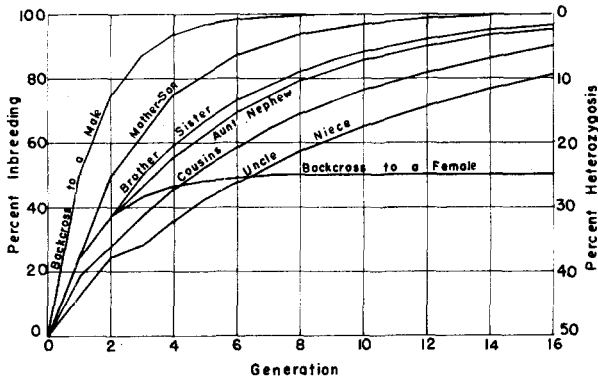


FIGURE 7. Percentage of inbreeding (100 f) and percentage of heterozygosity (100 p, assuming the initial value to be 50 percent) in successive generations of various systems of inbreeding.

initial value of the heterozygosity is assumed to be 50 percent, which would be true for a randomly mating population with the two alleles equally frequent. If the initial fraction is not 50 per cent multiply each figure in the table by twice this fraction.

Among those systems of mating which are practical with present techniques the most rapid decrease in heterozygosity is by mother-son mating. Next most rapid over a long period is brother-sister mating. For a few generations,

TABLE 1
Change in heterozygosity under various systems of mating.

SYSTEM OF MATING	HETEROZYGOSITY FORMULA	APPROXIMATE DECREASE IN HETEROZYGOSITY PER SINGLE GENERATION %	PROPORTION OF HETEROZYGOUS LOCI IN SUCCESSIVE GENERATIONS (STARTING WITH .500)										
			0	1	2	3	4	5	6	10	16	∞	
Backcross to σ^1	$p = \frac{p'}{2}$	50	.500	.250	.125	.062	.031	.015	.008	.000	.000	.000	0
Mother-son	$p = \frac{p''}{2}$	29.3	.500	—	.250	—	.125	—	.062	.015	.002	0	
Brother-sister	$p = \frac{p' + p''}{2 + 4}$	19.1	.500	.375	.312	.250	.203	.164	.133	.057	.016	0	
Aunt-nephew	$p = \frac{p' + p''}{2 + 8}$	17.4	.500	—	.312	—	.219	—	.148	.069	.022	0	
Cousins	$p = \frac{p' + p'' + p'''}{2 + 4 + 16}$	13.0	.500	.406	.359	.312	.271	.236	.206	.118	.051	0	
Uncle-niece	$p = \frac{p'' + 3p'''}{4 + 8 + 8}$	9.6	.500	.438	.375	.359	.320	.285	.262	.175	.095	0	
Backcross to φ	$p = \frac{1 + p'}{8 + 2}$	—	.500	.375	.312	.281	.266	.258	.254	.250	.250	.250	

* Formula given for double generations.

because of the shorter period from egg to time of mating in females, back-crossing to a female through successive matings with her sons is more rapid. With any of these three systems pooled semen from brother drones may be used without changing the formulae.

All other practical systems are considerably slower than these. However, if it becomes a practical procedure to make successive back crosses to a male by collecting semen from the spermatheca of previously fertilized females, this will be the most rapid system of all. The heterozygosity would be decreased by 50 percent each generation and the generation cycle would be short because of the short life cycle of females.

If it is desired to compute the probability that a bee, after a certain number of generations of inbreeding, will be homozygous for N independent factors or for a certain chromosome length, the formulae developed by HALDANE (1936) and applied by KALMUS and SMITH (1948) may be used.

COEFFICIENTS OF INBREEDING AND RELATIONSHIP

Since it is usually not possible to adhere to a strict system of mating for a great length of time, it is necessary to assess the consequences of inbreeding that follow the irregular patterns so often observed in pedigrees. For such purposes WRIGHT'S (1922) coefficients of inbreeding and relationship are regularly used. With very slight modifications these formulae are directly applicable to bee pedigrees.

The path diagram is drawn in the usual way and the inbreeding coefficient is computed by the formula

$$F = \Sigma (1/2)^{n-1} (1 + F_A)$$

where n is the number of generations from the individual in question to a common ancestor and back again through the other parent. However, all steps involving a male are counted as $1/2$ rather than 1 . F_A is the coefficient of inbreeding of the common ancestor, considered to be 0 in a male.

Exactly the same modification is made in the formula for coefficient of relationship

$$R_{xy} = \Sigma (1/2)^n \frac{1 + F_A}{\sqrt{(1 + F_x)(1 + F_y)}}$$

where n is the number of generation steps (again counting all steps involving a male as half a step) from one individual through a common ancestor to the other. F_x and F_y are the inbreeding coefficients of the individuals whose relationship is being measured. As in the previous formula the inbreeding coefficient of a male is considered to be 0 .

Figure 8 shows a pedigree of bees from our stocks. The inbreeding coefficients of the various females in the pedigree are as follows:

C. $F = (1/2)^{3-1} = .25$

D. $F = (1/2)^{3-1} + (1/2)^{4-1} = .375$

E. $F = (1/2)^{3-1}(1 + .25) + (1/2)^{4-1} + (1/2)^{5-1} = .5$

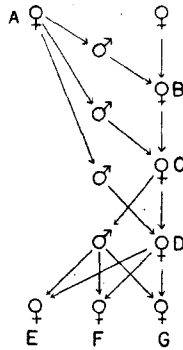


FIGURE 8. A bee pedigree.

Thus the sisters E, F, and G have on the average only one-half as many heterozygous loci as if they had been produced by random mating.

The relationship coefficient between E and G is

$$R = \frac{1}{\sqrt{(1 + .5)(1 + .5)}} [(1/2) + (1/2)^2(1 + .375) + 2(1/2)^3(1 + .25) + 2(1/2)^4 + 2(1/2)^5] = .896.$$

In the first term in brackets the father is the common ancestor, in the second term D, in the third C, and in the fourth and fifth A.

It is interesting to note that in bees full sisters have a coefficient of relationship of .75 if the female parent is not inbred as compared with .50 for diploid organisms.

If pooled semen is used the formulae for inbreeding and relationship coefficients still apply provided that the drones are all brothers and that the female thus inseminated is represented by only one daughter in the pedigree.

An alternative, and in many cases simpler, procedure for determining inbreeding and relationship coefficients is to draw the pedigree diagram omitting the males entirely, regarding them as equivalent to gametes from their mother. With the pedigree drawn in this way the usual formulae of WRIGHT are directly applicable. This simple method cannot be used if a male is represented by more than one daughter in the pedigree.

Recent work of MACKENSEN (unpublished) indicates that the *Habrobracon* type of multiple allelic sex determining system is at work in bees, except that homozygous diploids are lethal. This means, of course, that there would be strong selection for heterozygosity of genes closely linked to the sex determining locus and that the increase in homozygosity of such genes would be much slower in any inbreeding system.

SUMMARY

Formulae are given for determining the change in heterozygosity with various systems of inbreeding in honey bees. These formulae and numerical data are in table 1.

By making a slight modification (counting all steps involving a male as only half a generation) in WRIGHT's formulae for coefficients of inbreeding and relationship these become applicable to bee pedigrees.

ACKNOWLEDGMENT

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NOTE ADDED IN PROOF: We have learned that a similar article by POLHEMUS, LUSH, and ROTHENBUHLER in which many of the same conclusions are reached will appear in an early issue of the *Journal of Heredity*.

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