Theor. Appl. Genet. 49 (1977) 181-185

The Use of Gene Frequencies in Estimating the Mean Number of Mates in a Multiple-Mate and Stored-Sperm System of Mating

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Summary. As is the case with single-mate systems, random mating within a multiple-mate and stored-sperm system of mating generates a number of predictable mate-encounter patterns. Under a random mating model, the proportions of homozygous recessive females producing offspring in the distinct classes: i) dominant only, ii) dominant and recessive, and iii) recessive only, are shown to be a function of the mean number of matings per female. Accordingly, the validity of hypothesized mean numbers of matings per female may be tested through a comparison of observed and expected genotypic distributions. A number of mate-encounter patterns have low expected frequencies and may even fail to occur without producing significant changes in gene frequencies.

Introduction

The generality of random mating in explaining genetic equilibria is well known; however, as far as multiple-mate systems are concerned, the requirements for random mating have rarely been discussed. Random mating for genetic purposes consists of the random union of gametes and the Hardy-Weinberg law will be shown to operate in the case of a multiple-mate and stored-sperm system of mating. Under this property, the proportions of homozygous recessive females producing specific classes of offspring are shown to provide an estimate of the mean number of matings per female. The pertinent points requiring recognition are:

i) Individuals within many species may mate several times.
ii) Repeat matings can involve either single or multiple mates.
iii) Within the multiple-mate class, repeat matings may or may not involve the same mate or its genetic equivalent.

The latter case is most typical of natural populations and examples, including the feature of stored sperm, are the terrestrial isopods, *Armadillidium vulgare* (Howard 1940) and *Venezillo evergladensis* (Johnson 1976b), and the snail, *Cepaea nemoralis* (Murray 1964).

Structure of Random Mating

The well-known expression of random mating for a two-allele, autosomal locus within a single-mate system is given in Table 1. It is noted from Table 1 that the contributions to the total progeny by the genotypes AA, Aa, and aa, for either parental sex, are p^2, 2pq, and q^2, respectively. In the single-mate system, the contribution to total offspring by the genotype AA, for a given parental sex, is the sum of the frequencies of 3 possible mate encounters. In turn, the frequency of each mate encounter is determined by the frequencies of the 2 genotypes of the mates participating in the en-

Table 1. Frequencies of mating combinations in a large random-mating population with gene frequencies A = p and a = q

Mating Combination	Frequency of Mating
AA × AA	p^4
AA × Aa	$2p^3q$
AA × aa	p^2q^2
Aa × AA	$2p^3q$
Aa × Aa	$4p^3q^2$
Aa × aa	$2pq^3$
aa × AA	p^2q^2
aa × Aa	$2pq^3$
aa × aa	q^4
	$(p + q)^4 = 1$

Table 2. Mate-encounter patterns, under random mating, of females with the same genotype in a multiple-mate system with mean number of mates = 3

Encounter pattern (mating combination)	Male genotypes ($p^2 \equiv$ AA, $2pq \equiv$ Aa, $q^2 \equiv$ aa)	Possible orders (permutations)
1	(p^2, p^2, p^2)	1
2	$(p^2, p^2, 2pq)$	3
3	(p^2, p^2, q^2)	3
4	$(p^2, 2pq, 2pq)$	3
5	$(p^2, 2pq, q^2)$	6
6	$(2pq, 2pq, 2pq)$	1
7	$(2pq, 2pq, q^2)$	3
8	$(2pq, q^2, q^2)$	3
9	(q^2, q^2, p^2)	3
10	(q^2, q^2, q^2)	1

Table 3. Frequencies of male-encounter patterns when females are classified by the combination of genotypes of 3 male mates

Male-Encounter	Females		
Pattern	$p^2 \equiv$ AA	$2pq \equiv$ Aa	$q^2 \equiv$ aa
1	p^8	$2p^7q$	p^6q^2
2	$6p^7q$	$12p^6q^2$	$6p^5q^3$
3	$3p^6q^2$	$6p^5q^3$	$3p^4q^4$
4	$12p^6q^2$	$24p^5q^3$	$12p^4q^4$
5	$12p^5q^3$	$24p^4q^4$	$12p^3q^5$
6	$8p^5q^3$	$16p^4q^4$	$8p^3q^5$
7	$12p^4q^4$	$24p^3q^5$	$12p^2q^6$
8	$6p^3q^5$	$12p^2q^6$	$6pq^7$
9	$3p^4q^4$	$6p^3q^5$	$3p^2q^6$
10	p^2q^6	$2pq^7$	q^8
Sum*	p^2	$2pq$	q^2

* Represents the contributions to the next generation by that particular female genotype

counter. The contribution to total offspring by the genotype AA in a multiple-mate system is obtained in a similar way except:

i) More than 3 possible mate encounters can be involved, and

ii) The frequency of each encounter is determined by more than 2 genotypic frequencies.

If multiple matings are restricted to males, an unequal adult sex ratio is likely. For example, a 1 male: 3 female ratio may operate with each male and female averaging 3 and 1 matings, respectively. Random encounter of female genotypes by the males achieves random mating but such systems usually do not involve stored sperm. Multiple matings by both sexes, with females storing sperm, may exist in equal or unequal sex ratios without affecting randomness. In the latter system, a mating is a pooling of gametes from one female and some mean number of males.

Given random mating in a multiple-mate system, each successive mate may have any of 3 possible genotypes. Depending upon the number of genotypes and the number of multiple matings, several mate-encounter patterns exist. Furthermore, if successive mates have different genotypes, their order in the mating sequence must be taken into account. Like mating frequencies in the single-mate system, the frequency of each derives from the Hardy-Weinberg genotypic frequencies for the mates, together with the number of permutations of the mating combination. Without loss of generality, assume that the multiple-mate system has a mean of 3 matings per female and that a two-allele autosomal locus is being scored. Then, under random mating, the mating pattern of those females all with the same genotype, say AA, should reflect the expansion $(p^2+2pq+q^2)^3$ as detailed in Table 2. Clearly, the frequency of a mating pattern is the overall product of 4 parental genotypic frequencies (1 female, 3 male) and the number of permutations of the particular pattern. The frequencies of the 10 mating patterns given in Table 2, for each of the 3 female genotypes, are listed in Table 3. It will be observed from the bottom of Table 3 that, as in the case of a single-mate system, the contributions to the total progeny by the genotypes AA, Aa, and aa, for either parental sex, are p^2, $2pq$, and q^2, respectively. Clearly, this is to be expected since random mating was assumed to be operating within both systems. In any exercise where estimating the mean number of matings per female is contemplated, the following points should be considered:

i) Fertilization may begin before the mean number of matings has been achieved.

ii) Random mortality relative to genotype may reduce the number of matings for some females below the mean number.

iii) Random differences in fertility with age may occur.

However, provided that:

i) The order of male genotypes in a mating sequence is random, and

ii) There is an absence of differential viability of sperm with respect to genotype, the estimate of the mean should be free of bias.

Moreover, systematic error is avoided if the investigator's sampling method is random and confined to one generation. The last requirement is discussed below.

Multiple-mate systems, particularly in the isopod examples cited above (Howard 1940; Johnson 1976b) frequently display unequal adult sex ratios. The evolution of sex ratios suggest this condition develops from higher, but random, mortality in one sex (Johnson 1976a). Nevertheless, the frequencies of the mate-encounter patterns detailed in Tables 2 and 3 are not sensitive to changes in the adult sex ratio.

Mean Number of Matings

Many, perhaps most, species engaging in multiple matings and storing sperm also live for rather long periods. The isopods cited above frequently live and store sperm for well over a year and comparable data exist for the snail, *Cepaea nemoralis* (Murray 1964). In addition, the young are produced in distinct broods and individuals from their parents' first brood may themselves produce broods while their own sibs (probably half-sibs) are still being produced. An overlap of generations develops and comparable-aged individuals in a sample may not represent the same generation. A sample may well possess a higher number of young than mature specimens. If differential survival is an important component of selection, estimates of gene frequency obtained from a sample of mated and mature individuals are likely to differ from estimates based on the whole population. On this basis, though without this explanation, I suggested a sample of homozygous recessive females of the snail *Cepaea nemoralis*, studied by Murray (1964), may have offspring not in equilibrium for gene frequencies estimated from the whole population (Johnson 1976a). Murray used the proportion of phenotypes in successive broods from each female to estimate the mean number of matings per female. He concluded a value of 3 matings was most consistent with the data, but gave no test for the estimate.

In a single-mate system, the proportions of dominant and recessive offspring among all offspring from homozygous recessive females are: $(p^2q^2+pq^3)/q^2$ and $(pq^3+q^4)/q^2$ or p and q, respectively. Provided all the possible mate encounters in a multiple-mate system occur, the proportions remain the same, p and q. The population gene frequencies were estimated by Murray as $p = 0.1$ and $q = 0.9$. The observed offspring numbers, from 35 homozygous recessive mothers, in the dominant and recessive classes were 262 and 1,157, respectively. Based on the population's gene frequencies, the expected numbers of dominant and recessive offspring are 141.9 and 1,277.1, respectively. A χ^2 comparison of these distributions reveals a $P < .01$ for the deviation to arise by chance. This discrepancy could have resulted from:

i) The estimates of p and q obtained from the sample of females being different from the corresponding values for the entire population, or

ii) Higher reproductive success amongst recessive (yellow) mothers inseminated by dominant (pink) mates.

If the gene frequencies were not different, then the proportions of homozygous recessive mothers producing only dominant, both dominant and recessive, and only recessive offspring differ between these systems. The expected proportions of homozygous recessive females producing the three classes of offspring for the singlemate system and for 2, 3, and 4 multiple mates are given in Table 4. The observed distribution obtained from the 35 females,

Table 4. Proportions of homozygous recessive females producing the different classes of offspring

	Class of Offspring		
System of Mating	Dominants only	Dominants and Recessives	Recessives only
Single Mate	p^2	$2pq$	q^2
Multiple-2-Mate	p^4	$1-(p^4+q^4)$	q^4
Multiple-3-Mate	p^6	$1-(p^6+q^6)$	q^6
Multiple-4-Mate	p^8	$1-(p^8+q^8)$	q^8

Table 5. Homozygous recessive mothers grouped by class(es) of offspring produced

	Class of Offspring			
Distribution	Pink	Pink&Yellow	Yellow	χ^2
Observed	0.0	17	18	--
Expected:				
Mean = 2	3.5×10^{-3}	12	22.9	2.570
3	3.5×10^{-5}	16.4	18.6	.001
4	3.5×10^{-7}	19.9	15.1	.919

together with expected distributions corresponding to differing hypothesized mean numbers of matings per female, are given in Table 5. With an hypothesized mean of 3 matings per female, the resulting expected distribution fits the observed distribution rather well. However, the estimates of p and q obtained from the offspring do differ from the whole population either due to selection as suggested above or due to the frequencies of p and q in the parents differing from the whole population. In either case, the proportions of homozygous recessive females producing only dominant, both dominant and recessive, and only recessive offspring give a biased estimate of the number of mates as the Hardy-Weinberg conditions do not exist.

Stability of Gene Frequencies

The structure of random mating in a multiple-mate and stored-sperm system depends on a larger number of mate encounters than exists for single-mate systems. The frequency of certain mate encounters may be quite low and in a finite number of matings, some may fail to occur. If $p = q$, the low-frequency encounters are equally distributed among the matings of AA and aa females and the loss of such matings would resemble a balanced polymorphism where the homozygous classes suffer a selective loss. If $p > q$, a larger portion of low-frequency matings exists for the aa females. A loss of such matings would push q to still lower values. It is easy to demonstrate, however, that in practice this effect is unlikely to be important.

If $p = 0.9$ and $q = 0.1$ and if numerical values for mate-encounter patterns are computed from expressions in Table 3, then for a mean of 3 mates, the frequencies for 23 of the 30 encounter patterns are $< .01$. These frequencies are identified in Table 6. If all encounter patterns with an expected frequency $< .01$ fail to occur, then only 7 encounter patterns will contribute to the next generation. The frequencies for these 7 encounter patterns may be distributed over the appropriate offspring genotypes. For instance, AA females each mating with 2 AA and 1 Aa males do so at a frequency of $6p^7q$ and they produce AA and Aa offspring in a ratio of 5:1 respectively. The fre-

Table 6. Frequencies of 30 male-encounter patterns (3 random encounters per female) when $p = .9$ and $q = .1$

Male-Encounter	Females		
Pattern	p^2	$2pq$	q^2
1	$p^8 = .430$	$2p^7q = .096$	$p^6q^2 < .01$
2	$6p^7q = .287$	$12p^6q^2 = .064$	$6p^5q^3 < .01$
3	$3p^6q^2 = .016$	$6p^5q^3 < .01$	$3p^4q^4 < .01$
4	$12p^6q^2 = .064$	$24p^5q^3 = .014$	$12p^4q^4 < .01$
5	$12p^5q^3 < .01$	$24p^4q^4 < .01$	$12p^3q^5 < .01$
6	$8p^5q^3 < .01$	$16p^4q^4 < .01$	$8p^3q^5 < .01$
7	$12p^4q^4 < .01$	$24p^3q^5 < .01$	$12p^2q^6 < .01$
8	$6p^3q^5 < .01$	$12p^2q^6 < .01$	$6pq^7 < .01$
9	$3p^4q^4 < .01$	$6p^3q^5 < .01$	$3p^2q^6 < .01$
10	$p^2q^6 < .01$	$2pq^7 < .01$	$q^8 < .01$

quency of the encounter is then distributed to the AA and Aa offspring as $5p^7q$ and p^7q respectively. If this operation is performed for all the 7 patterns predicted to exist and the genotypic frequencies for the offspring are adjusted to a total of one, the frequencies become: AA, 0.8267, Aa, 0.1662; and aa, 0.0079. In the case where the low-frequency mate encounters are not lost, the frequencies are: AA, 0.81; Aa, 0.18; aa, 0.01. Clearly, the differences are negligible. Moreover, the number of matings required to produce such a loss is < 100 which, in most populations, is a very low number of matings per generation.

Received June 2, 1976
Communicated by L.D. VanVleck

Note added in proof: The p and q values provided by proportions of dominant and recessive offspring from recessive female parents, respectively, are estimates of p and q in male parents only. Where sexes are separate (unlike *Cepaea*) and where adult males and females may differ in gene frequencies, only male gene frequencies are valid in estimating the mean number of mates.

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