Mating system of the meadow vole, *Microtus pennsylvanicus*

Previous studies on parental and spacing behavior of *Microtus pennsylvanicus* suggest a promiscuous mating system, but attempts to find multiple paternity in single litters have been unsuccessful. In this paper we present evidence of multiple paternity in single litters conceived in the wild early in the breeding season. The proportion of litters sired by multiple males was estimated, by a conservative method, to be 33.1%. We argue that the presence of promiscuity, rather than polygyny, in *M. pennsylvanicus* is the result of two factors. First, overwintered breeding males are similar in age and size, resulting in small variation in competitive ability among males. This reduced variation in competitive ability reduces the possibility that some males defend several females and others defend none. Second, the habitat structure of the meadow vole makes it difficult for a male to detect other males nearby, and this reduces the possibility that one male excludes others from mating when a female comes into estrus. *Key words:* competitive ability, mating systems, meadow voles, *Microtus pennsylvanicus*, multiple paternity. *[Behav Ecol 4:83–89 (1993)]*

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Different mammalian species, or different animal populations within the same species, often exhibit a diverse array of mating systems (Clutton-Brock, 1989; Eisenberg, 1981; Lott, 1991; Terborgh and Janson, 1986). It has been hypothesized that the necessity of male assistance in rearing young and defendability of females by males are the most important determinants of mating systems (Clutton-Brock, 1989; Davies, 1991). The defendability of females is suggested to be related to female spacing behavior (Clutton-Brock, 1989; Ims, 1987, 1988a; Ostfeld, 1985). If paternal assistance is not important in rearing young, and if females are clumped spatially, the above hypothesis would predict greater potential for polygyny, resulting in single males defending more than one female (Emlen and Oring, 1977).

The defendability of females by males, however, does not depend on female spacing behavior alone, but also depends on variation in competitive ability among males and on habitat structure. In large mammals in which breeding males differ greatly in age and size, variation in competitive ability is expected to be large. Thus, some males can monopolize groups of females, leading to a polygynous mating system. Morever, males of large mammalian species usually can see the approach of other males to their territory or their mates, which makes it difficult for physically weaker males to adopt a sneaker strategy such as observed in bull frogs (Howard, 1978), sunfishes (Gross, 1982), and coho salmon (Gross, 1985). On the other hand, breeding males of many small rodent species, especially early in the breeding season when breeding is restricted to those born in the previous year, are similar in age and size. Thus, variation in competitive ability is expected to be smaller, and it should be more difficult for some males to monopolize groups of females, whereas other males of similar age and

size may have no female at all. For this reason we would expect mating resources (estrous females) to be more equally allocated among males in small mammals than in large mammals. To assess this prediction, we need detailed knowledge of mating systems and reproductive success of males in many small mammalian species.

The mating systems of voles and mice are difficult to study because of the secretive habits of these animals, most of which are nocturnal or crepuscular and defy direct behavioral observation. Three indirect methods have been employed in previous studies to infer the mating system of natural populations of these species: (1) laboratory or enclosure observation of parental and mating behavior (e.g., Hartung and Dewsbury, 1979a,b; Xia and Millar, 1988); (2) field monitoring and manipulation of spatial relationships between adult males and females using traps and radiotelemetry (e.g., Boonstra and Rodd, 1983; Ims, 1988b; Lambin and Krebs, 1991; Madison, 1980; Ribble and Salvioni, 1990; Xia and Millar, 1989); and (3) genetic analysis of multiple paternity in single litters (e.g., Birdsall and Nash, 1973; Foltz, 1981; Kawata, 1988; Ribble, 1992; Xia and Millar, 1991).

The meadow vole, *Microtus pennsylvanicus*, is one of the most intensively studied rodent species in North America. Paternal behavior in the species has been observed in the laboratory by Hartung and Dewsbury (1979b), but comparative studies (McGuire and Novak, 1984, 1986) showed that male *M. pennsylvanicus* spent much less time in the nest than male pine voles, *M. pinetorum*, or prairie voles, *M. ochrogaster*, two presumably monogamous species (Fitzgerald and Madison, 1983; Getz and Hofmann, 1986; Getz et al. 1987; Thomas and Birney, 1979). There is no indication that the presence of a male meadow vole is important in rearing young. Male *M. pennsylvanicus* are not territorial, but fe-

Table 1

Protein loci that were scored consistently in *Microtus pennsylvanicus*

Enzyme/protein	Symbol	Tissue
Monomorphic		
Amylase-1	*AMY*-1	Saliva
Malate dehydrogenase-2	*MDH*-2	Kidney
Lactate dehydrogenase	*LDH*	Kidney
Albumin	*Alb*	Plasma
Fumarate hydratase	Fumarase	Kidney
Polymorphic		
Isocitrate dehydrogenase-1	*IDH*-1	Kidney
Mannosephosphate isomerase	*MPI*	Kidney
Aminopeptidase (cytosol)	*LAP*	Plasma
Transferrin	*Trf*	Plasma
Amylase-2	*AMY*-2	Saliva
Phosphoglucomutase-1	*PGM*-1	Kidney
Phosphoglucomutase-2	*PGM*-2	Kidney
Phosphoglucomutase-3	*PGM*-3	Kidney

Table 2

Allelic frequencies of males for *PGM*-3 and *AMY*-2

Loci	A	B	C	D
PGM-3	0.083	0.754	0.116	0.046
AMY-2	0.042	0.740	0.218	

males are (Boonstra and Rodd, 1983; Madison, 1980; Webster and Brooks, 1981). Using radiotelemetry, Madison (1980) located 100 voles more than 14,000 times and found no indication of pair bonding. Males do not have a fixed home range, and their activity range may change daily and overlap those of several other males as well as those of several females, with the greatest overlap occurring in the vicinity of estrous females (Getz, 1961, 1972; Webster and Brooks, 1981).

These findings appear consistent with a promiscuous mating system characterized by (1) females practicing simultaneous polyandry, (2) males practicing serial polygyny, and (3) lack of pair bonding, which distinguishes serial polygyny from serial monogamy. Paradoxically, Sheridan and Tamarin (1986, 1988) found no evidence of multiple paternity in single litters in *M. pennsylvanicus*. However, their sample sizes were small, and their probability of detecting multiple paternity was low (see Discussion). In this study, we examined paternity in a large number of litters, found evidence of multiple paternity in single litters, and estimated the proportion of litters having multiple paternity in a natural population of *M. pennsylvanicus*.

MATERIALS AND METHODS

The study area was located on abandoned grassland adjacent to the Pearson International Airport near Toronto, Canada (43°41' N, 79°38' W; Boonstra and Rodd, 1983). A live-trapping grid (1.48 ha) of 13 by 15 stations was set up on 2 March 1983 and trapped at weekly intervals from 9 March to 21 May 1983. Trap spacing was 7.6 m, and one Longworth trap was placed at each trap point and covered with a board to protect the trap from snow and rain. All traps were baited with oats, provided with cotton for warmth, and locked open when not in use. We eartagged voles when first caught, and on each capture we recorded the tag number, location, weight, sex, and sexual condition.

We brought pregnant females back to the laboratory and allowed them to give birth. Close to the end of the sampling period, we also removed 152

breeding males to the laboratory. Seventy-eight pregnant females weaned 465 young in the laboratory. Three females had litter sizes of two and were excluded from subsequent estimation of expected number of litters carrying three or more different paternal alleles (a litter of two has zero probability of carrying three different paternal alleles for a given locus). Tissue samples taken from males, mothers, and their young were subjected to electrophoresis. A preliminary electrophoretic survey included 38 loci from tissues of the brain, hemolysate, kidney, liver, muscle, saliva, and heart. Enzyme and protein loci that could be scored consistently are listed in Table 1. Of the eight polymorphic loci (Table 1), *PGM*-3 and *AMY*-2 have four and three alleles (Table 2), respectively, and form the genetic basis for this study.

The inheritance pattern for *AMY*-2 was determined to be Mendelian by Sheridan and Tamarin (1985). We reached the same conclusion for *PGM*-3 based on two lines of evidence. First, a breeding colony was set up to look at inheritance patterns. One of the two kidneys of each adult was removed using surgical procedures described in Pavone and Boonstra (1984), which allowed us to determine genotype of these animals for the *PGM*-3 locus. These nephrectomized animals were then paired. When the resulting young were at least 2 weeks old, one kidney was also removed from each animal for electrophoresis. Because of the difficulty of breeding the meadow voles in the laboratory, not all genotypes bred, and hence the resulting data are incomplete. A total of 56 pairs produced young, of which 42 were of *BB* × *BB*, yielding 136 *BB* offspring; 11 were of *BB* × *BC*, yielding 15 *BB* offspring and 21 *BC* offspring; 1 was of *BC* × *CC*, yielding 1 *BC* offspring; 1 was of *BB* × *AC*, yielding 3 *BC* offspring and 2 *AB* offspring; and 1 was of *BB* × *BD*, yielding 2 *BB* offspring and 2 *BD* offspring. These data, though weak by themselves, are consistent with an inheritance pattern of codominant, autosomal alleles. Second, the electromorphs of the 78 mothers and their 465 field-conceived young were also consistent with our interpretation of 1 locus with 4 codominant alleles.

Multiple paternity was detected by identifying litters with three or more paternal alleles. The method for estimating proportion of litters with multiple sires is presented in detail in the appendix.

RESULTS

There were 11 litters with at least 3 different paternal alleles (Table 3), and these serve as direct evidence of multiple paternity in single litters. We therefore proceeded to estimate proportion of litters sired by multiple males. The rationale of the estimation is as follows. First, we calculate the expected number of litters (N_E) carrying at least three different paternal alleles when 100% of females are

Table 3

Litters with multiple paternity

Loci	Mother	Mother genotype	Offspring genotype									
PGM-3	B0363	BB	BB	BB	BC	BC	BC	BD				
	B0843	BB	AB	AB	AB	BB	BC	BD				
	B0895	BB	AB	AB	BB	BB	BD	BD				
	B0341	AB	AB	AD	AD	AD	BC	BC	BC			
	B0896	BB	AB	BB	BB	BB	BC	BC	BD			
	B0035	BB	BB	BC	BC	BC	BC	BD	BD	BD		
	B0844	BB	AB	AB	AB	BB	BB	BC	BC	BC		
	B0953	BB	BB	BB	BC	BC	BC	BC	BC	BD	BD	
AMY-2	B0878	AC	AA	AB	AC	AC	AC	CC	CC			
	B0959	BB	AB	AB	AB	AB	BB	BC	BC			
	B0808	BB	AB	BB	BB	BB	BB	BB	BC	BC		

polyandrous. If the observed number of litters carrying at least three different alleles is N_o, then the proportion of litters with multiple sires is N_o/N_E.

Because we do not know how many males were actually involved in each multi-male insemination, N_E was estimated for two extreme cases: (1) when only two males were involved in each multi-male mating ($N_{E,2}$) and (2) when all males were involved in each multi-male mating ($N_{E,all}$). These two extreme estimates were obtained for both PGM-3 and AMY-2. For PGM-3, $N_{E,2}$ and $N_{E,all}$ are 7.49 and 22.61, respectively; the observed number of litters with three or more different paternal alleles (N_o) is eight. For AMY-2, $N_{E,2}$ and $N_{E,all}$ are 3.27 and 10.61, respectively, and N_o is 3.

On the basis of the result above, if only two males were involved in multi-male mating, then virtually all litters would have had multiple sires. If all males were involved in each of the multi-male matings, then the proportion of litters sired by multiple males is 35.4% as estimated from PGM-3 and 28.3% as estimated from AMY-2. Apparently, given a population with a certain proportion (p) of litters sired by multiple males, an estimate of this proportion based on one locus is unlikely to be identical to an estimate of this proportion based on a different locus, unless sample size is infinite and the electrophoresis and subsequent scoring of electromorphs are absolutely error-free. For this reason, it is desirable to obtain the mean and variance of estimates of p calculated from data of different loci. With data from the two loci, we obtain the mean and variance of our two estimates of p using the maximum likelihood method.

Let p be the proportion of females engaging in multi-male matings. The Poisson parameter, λ, is then equal to $22.61 \cdot p$ for PGM-3 and $10.61 \cdot p$ for AMY-2. The probability of obtaining our results (i.e., eight multipaternity litters for PGM-3 and three multipaternity litters for AMY-2) is then

$$\text{Prob} = \frac{(22.61p)^8 e^{-22.61p}(10.61p)^3 e^{-10.61p}}{8!3!} \quad (1)$$

$$= \left(\frac{22.61^8 10.61^3}{8!3!}\right) p^{11} e^{-33.22p}. \quad (2)$$

Taking the natural logarithm of Prob, we obtain

$$L = \ln(\text{Prob})$$

$$= 11 \ln(p) - 33.22p + \text{constant}. \quad (3)$$

Taking the derivative of L and setting it to 0, we have

$$\frac{dL}{dp} = \frac{11}{p} - 33.22 = 0, \quad (4)$$

$$p = 11/33.22 = 33.1\%. \quad (5)$$

This equation for p agrees with our expectation because 11 (=8 + 3) is the sum of observed number of litters with multiple paternity and 33.22 (=22.61 + 10.61) is the sum of expected number of litters with multiple paternity. To obtain the variance of p (V_p), we take the second derivative of L in Equation 5 and the negative reciprocal of the second derivative gives the variance of p, i.e.,

$$\frac{d^2L}{dp^2} = -\frac{11}{p^2} = -\frac{1}{V_p}, \quad (6)$$

$$V_p = \frac{p^2}{11} = 0.01, \quad (7)$$

which is the variance of the estimate when using different loci. We conclude that females in our population of *Microtus pennsylvanicus* mate polyandrously, with 33.1% of litters sired by multiple males. Because this estimate is reached by assuming that each mating involved all males in the population, which is unlikely, the actual percentage of litters sired by multiple males must be higher.

DISCUSSION

Sheridan and Tamarin (1988) considered *M. pennsylvanicus* a nonpolyandrous species because they found no evidence of multiple paternity in their population, whereas we found good evidence of multiple paternity. There are three reasons for the discrepancy between their results and ours. First, Sheridan and Tamarin (1986, 1988) examined only 24 litters with litter size larger than 2, whereas we examined 75 litters. Second, Sheridan and Tamarin's litter size was limited by the number of young recruited into their trappable population, and loss between birth and recruitment probably occurred in most litters. Our loss was minimized because females gave birth in the laboratory. The average litter size of their 24 litters was only 4.3 and that for our 75 litters was 6.1. Third, Sheridan and Tamarin examined only blood and saliva from living

animals for polymorphic loci, whereas we examined blood, saliva, brain, kidney, liver, muscle, and heart for polymorphic loci. As a parallel example of the problem of low sample size, Kawata (1985) examined 12 litters in another microtine species, *Clethrionomys rufocanus*, and found no evidence of multiple paternity. He concluded that multiple paternity, if present, must be rare in the species. When sample size was increased in a subsequent study (Kawata, 1988), 5 out of 29 litters were found to have 3 or more different paternal alleles.

The number of species in voles and mice in which genetic analysis has been applied to studying mating systems is now six. The deer mouse, *Peromyscus maniculatus* (Birdsall and Nash, 1973), the white-footed mouse, *P. leucopus* (Xia and Millar, 1991), the red-backed vole, *Clethrionomys rufocanus bedfordiae* (Kawata, 1988), and the meadow vole, *M. pennsylvanicus* (this study) show a mating system characterized by simultaneous polyandry by females, serial polygyny by males, and lack of pair-bonding. The old-field mouse, *Peromyscus polionotus* (Foltz, 1981), and the California mouse, *P. californicus* (Ribble, 1992), have a monogamous mating system. In comparison to a polygynous mating system, these polyandrous and monogamous mating systems found in voles and mice imply that variance of reproductive success among males should be relatively small because mating resources are shared more equally among males. Consistent with this line of reasoning is Sheridan and Tamarin's (1988) evidence that the variance of reproductive success in male *M. pennsylvanicus* is smaller than that of females. This contrasts with higher variance among males than among females in typical polygynous species, such as the red deer (Clutton-Brock et al., 1982).

There is good reason for these short-lived rodents living in a seasonal environment to have mating systems that lead to roughly equal partitioning of mating resources among males. Short life span combined with a seasonal environment implies that breeding males will be of similar age (mostly over-wintered animals) and size. These males may therefore have similar competitive ability in gaining mating resources. In contrast, males in large mammalian species, such as the red deer, vary greatly in age and size among breeding males. As a consequence, these males will differ greatly in competitive ability, which leads to more unequal allocation of mating resources among males and a more polygynous mating system.

Thus, in analyzing and predicting a mating system of a population or a subpopulation, one should pay special attention to variation in competitive ability (which indicates ability to defend resources breeding females need, to provide parental care, to dominate over other individuals of the same sex, etc.) that is available in the population. Competitive ability depends on three factors: genetic differences (which are under strong selection and should have little additive variance), environmental differences (due to experience, accidents, maternal effects caused by differential nutrition, etc.), and age differences among breeding animals. Thus, if one population has little variance in age among breeding males while another population has great variation in age among breeding males, we should expect that mating resources should be allocated more

equally among males in the former population than in the latter, everything else being equal.

Although the reduced variation in age and size among breeding males may explain the lack of a polygynous mating system in the meadow vole, other factors may also play a role. In their natural habitats of grasslands and meadows, the meadow vole is mainly nocturnal or crepuscular. This implies that their vision is limited and that they probably rely more on hearing and smell to receive information about their environment (Wolff, 1985), and hearing and smell are not accurate for identifying approaching competitors. In addition, predation risk may also inhibit overt aggressive behavior required for territorial defense. Thus, habitat structure may limit the ability of a male to confront and drive away other males and monopolize mating access to several females.

The mating system of *M. pennsylvanicus* can also be partially inferred from the association pattern of certain types of territoriality with certain kinds of mating systems. Monogamous species are typically associated with territoriality involving both males and females, and polygynous species are typically associated with male territoriality. In both of these mating systems, males monopolize mating access to either one or several females through territoriality. Males in *M. pennsylvanicus* are not territorial, and, without male territoriality, it is difficult to imagine how males can monopolize mating access to any estrous female. The result is that several males will have access to an estrous female, leading to multi-male insemination and multiple paternity.

But why should females mate with multiple males? Three adaptive explanations have been proposed. First, Wolff (1985) argued that the polyandrous mating by the female is a strategy against infanticide by males because a mated male is less infanticidal than an unmated male. However, if infanticidal inhibition caused by mating takes place immediately after mating but fades away within 21 days (the average gestation period in the meadow vole), then such inhibition only benefits young of other females, not young of the female who has mated with the male. Second, a female mates with multiple males to reduce the chance of pseudopregnancy (Conaway, 1971). Dewsbury (1984a,b) documented a reduction of fertility in male deer mice, *Peromyscus maniculatus*, after six or fewer ejaculations. Third, females mate with multiple males rather than just one male because repeated ejaculations by one male may reduce the proportion of normal sperm (i.e., increase proportion of abnormal sperm), leading to an overall decrease in semen quality (Gibson and Jewell, 1982; Simpson and Edey, 1979; Tomkins and Bryant, 1976). One strategy against poor semen quality caused by repeated ejaculations from one male would be to sample the first few ejaculations from many males.

In summary, the meadow vole, *Microtus pennsylvanicus*, has a promiscuous mating system with females accepting multi-male insemination and producing litters of multiple paternity. That males fail to monopolize mating access to estrous females may be due to reduced variance in competitive ability among males, predation, and habitat structure. That females accept multiple insemination may be adaptive in terms of reducing male infanticide, avoiding sperm-depleted males or sperm of poor quality,

and/or increasing genetic diversity in progeny. More experimental studies are required to evaluate relative importance of these hypotheses.

APPENDIX

Statistical method for estimating frequencies of litters with multiple sires for a locus with four alleles

This appendix explains in detail our method of estimating frequencies of litters with multiple sires using a locus of four alleles. For loci with three alleles, refer to Birdsall and Nash (1973), as well as to Merritt and Wu (1975), who corrected some errors in Birdsall and Nash (1973). We have some prototype computer programs for carrying out the bulk of calculations.

Because we do not know how many males participate in each multi-male mating, the estimation was carried out for two extreme cases: (1) when only two males were involved in each multi-male mating and (2) when all males were involved in each multi-male mating. The rationale of the estimation is as follows. First, we calculate the expected number of litters (N_E) carrying at least three different paternal alleles, when 100% of females are promiscuous. If the observed number of litters carrying at least three different alleles is N_o, then the proportion of litters with multiple sires is N_o/N_E.

Two-male case

Whether a female produces a litter containing at least three different paternal alleles depends on (1) her probability of mating with two males carrying three or four different alleles (Pr_1) and (2) given Pr_1, the probability of the three or four different paternal alleles being realized in the litter (Pr_2), which in turn depends on female genotype and allelic frequencies of the three or four different paternal alleles in the sperm received by the female. The probability of a female mating with two males carrying three and four different alleles is

$$Pr_1(3 \text{ alleles}) = 12(P_a^2 \cdot P_b \cdot P_c + P_a^2 \cdot P_b \cdot P_d$$
$$+ P_a^2 \cdot P_c \cdot P_d + P_a \cdot P_b^2 \cdot P_c$$
$$+ P_a \cdot P_b^2 \cdot P_d + P_b^2 \cdot P_c \cdot P_d$$
$$+ P_a \cdot P_b \cdot P_c^2 + P_a \cdot P_c^2 \cdot P_d$$
$$+ P_b \cdot P_c^2 \cdot P_d + P_a \cdot P_b \cdot P_d^2$$
$$+ P_a \cdot P_c \cdot P_d^2 + P_b \cdot P_c \cdot P_d^2)$$

and

$$Pr_1(4 \text{ alleles}) = 24 \cdot P_a \cdot P_b \cdot P_c \cdot P_d,$$

respectively, where P_a, P_b, P_c, and P_d are frequencies of the four alleles, respectively. From our data of PGM-3 locus with P_a, P_b, P_c, and P_d equal to 0.083, 0.754, 0.116, 0.046, respectively, a female had a probability of 0.160 of mating with two males carrying three different alleles, and a probability of 0.008 of mating with two males carrying four different alleles.

Homozygous females. When a homozygous female mates with two males carrying three different paternal alleles, her probability of having the three different paternal alleles realized in her litter (Pr_2)

can be calculated by expanding $(P_1 + P_2 + P_3)^n$ (where P_1, P_2, and P_3 are allelic frequencies of the sperm pool contributed by the two males and equal to 0.25, 0.5, and 0.25, respectively, and n is litter size) and adding those terms which include P_1, P_2, and P_3. The resulting value, if $n = 6$, is 0.64453, which is exact.

When the same homozygous female mates with two males carrying four different alleles, her Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3 + P_4)^n$ (where $P_1 = P_2 = P_3 = P_4 = 0.25$, $n =$ litter size) and summing up those terms that include any three of P_1, P_2, P_3, and P_4. The resulting value, if $n = 6$, is 0.9082. Thus, the probability of a homozygous female producing a litter of six with at least three different paternal alleles is $(0.160 \cdot 0.64453) + (0.008 \cdot 0.9082) = 0.111$. Such calculations were carried out for each homozygous female in our population and we designate the sum of these resulting probability values as $N_{E,homo}$.

Heterozygous females. When a heterozygous female mates with two males carrying three different paternal alleles, there are four different situations: (1) the heterozygous female carries the allele present twice in the two males and lacks only one allele present in males (e.g., AB female with AB, AC males), (2) the heterozygous female has the allele present twice in males and lacks two alleles present in males (e.g., AB female with AC, AD males), (3) the heterozygous female lacks the allele present twice in males but possesses the other two paternal alleles (e.g., AB female with AC, BC males), and (4) the heterozygous female lacks the allele present twice in males and possesses only one of the three paternal alleles (e.g., AB female with AC, DD males). The probability of an AB female encountering each of these four situations is 0.103, 0.037, 0.012 and 0.009, respectively, the sum of which equals 0.160. The calculation of Pr_2 for these four different situations is as follows.

For situation 1, Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3 + P_4)^n$ (where P_1 is the probability of the offspring sharing the same genotype as the mother, P_2 and P_3 are the probabilities of an individual being homozygous for each of the alleles carried by the mother, and P_4 is the sum of the probabilities of an offspring carrying the allele not present in the mother) and adding those terms that include P_2, P_3, and P_4.

For situation 2, Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3)^n$ (where P_2 and P_3 are the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother and $P_1 = 1 - P_2 - P_3$, $n =$ litter size) and adding those terms that include P_1, P_2, and P_3.

For situation 3, Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3 + P_4)^n$ (where variables are the same as in situation 1) and adding those terms that include P_2, P_3, and P_4. For situation 4, Pr_2 is the same as in situation 2. For a female producing a litter of six, her Pr_2 for each of these four situations is 0.32730, 0.64453, 0.27008, and 0.64453, respectively.

When a heterozygous female mates with two males carrying four different paternal alleles, the probability of her litter containing at least three different paternal alleles can be calculated by expanding $(P_1 + P_2 + P_3 + P_4 + P_5)^n$ (where $P_1 = 0.25$ is the probability of young sharing the same genotype as

the mother, $P_2 = P_3 = 0.125$ is the probability of young being homozygous for each of the alleles carried by the mother, and $P_4 = P_5 = 0.25$ is the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother, n is litter size), and adding those terms that include P_1, P_4, and P_5; P_2, P_4, and P_5; P_3, P_4, and P_5; P_2, P_3, and P_4; or P_2, P_3, and P_5. The resulting value, if $n = 6$, equals 0.77271. Thus, the probability of an AB female producing a litter of six with at least three different paternal alleles is

$$[(0.103 \cdot 0.32730) + (0.037 \cdot 0.64453)$$
$$+ (0.012 \cdot 0.27008) + (0.009 \cdot 0.64453)]$$
$$+ [0.008 \cdot 0.77271]$$
$$= 0.072.$$

Such calculations are carried out for each female, and we will designate the sum of the resulting probability values as $N_{E,heter}$. Now $N_E = N_{E,homo} + N_{E,heter}$. In our case, $N_E = 7.49$.

All-male case

When each female mates with all males in the population, the allelic frequency in the sperm received by the female is the same as allelic frequency in the population and Pr_1 will therefore be 1. There are two Pr_2 values to be calculated.

When the female is homozygous, Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3 + P_4)^n$ (where P_1, P_2, P_3, and P_4 are allelic frequencies of the population and n is litter size) and adding those terms that include any three of P_1, P_2, P_3, and P_4. The resulting value, if $n = 6$, equals 0.139.

When the female is heterozygous, Pr_2 can be calculated by expanding $(P_1 + P_2 + P_3 + P_4 + P_5)^n$ (where P_1 is the probability of young sharing the same genotype as the mother, P_2 and P_3 are the probabilities of young being homozygous for each of the alleles carried by the mother, and P_4 and P_5 are the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother) and adding those terms that include P_1, P_4, and P_5; P_2, P_4, and P_5; P_3, P_4, and P_5; P_2, P_3, and P_4; or P_2, P_3, and P_5. The resulting value for an AB female with a litter of six equals 0.088. The sum of these values calculated for each of the homozygous and heterozygous females was N_E and equalled 22.61.

We had 75 litters, of which 8 carried at least 3 different paternal alleles (i.e., $N_o = 8$). The 95% confidence interval of 8/75 is 4.84 − 19.99. In the absence of information from other loci, we conclude that (1) if promiscuous females each mated with two males, at least 61% (=4.84/7.94) of females were promiscuous and (2) if promiscuous females each mated with all males, at least 21% (=4.84/22.61) of females were promiscuous.

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