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Genetic evidence of promiscuity in *Peromyscus leucopus*

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Summary. We collected pregnant female *Peromyscus leucopus* from natural populations during the summer of 1987 and 1988 and allowed these females to give birth to their field-conceived young in the laboratory. Blood samples were taken by suborbital puncture and phenotypes of five genetic loci (Esterase-1, trasferrin, hemoglobin, albumin and 6-phosphogluconate dehydrogenase) were studied using horizontal starch-gel electrophoresis to detect multiple paternity in single litters. Only esterase-1 was found to be highly polymorphic, with four alleles in samples of both years. One litter out of 29 in 1987 and 6 litters out of 32 in 1988 contained three different paternal alleles and served as direct evidence of multiple paternity in the field. The proportion of females engaging in multiple matings in natural populations of *P. leucopus,* assuming that all males were involved in every multiple mating, is 25%–100% (mean 68%). Because it is unlikely that all males are involved in every multiple mating, the actual proportion of females engaging in multiple matings should be greater.

Introduction

The prevailing mating system in wild populations of *Peromyscus leucopus* has been controversial during the last two decades. Myton (1974) suggested that the social organization in wild populations of *P. leucopus* consists of family groups with one adult female and several adult males, implying a mating system of simultaneous polyandry. Baccus and Wolff (unpublished, but cited in Wolff and Lundy 1985) found indications of multiple paternity in single litters in wild populations of *P. leucopus*, which is consistent with the social organization proposed by Myton (1974). On the other hand, Horner (1947), McCarty and Southwick (1977) and Hartung and Dewsbury (1979) documented paternal care in captive *P. leucopus,* and Mineau and Madison (1977) proposed pair activity involving one male and one female, both of which were radio-tracked. Mineau and Madison (1977) also noted the birth of one litter by the female when she was spatially associated with the male. As there were no males, other than the radio-tracked one, found in the home range of the radio-tracked female, the litter must have been sired by the radio-tracked male only. These studies appeared to support a monogamous mating system as they indicated joint parental care of both sexes, spatial association of one male and one female, and exclusivity of mating, which are three basic dimensions of monogamy (Dewsbury 1988).

Our previous studies on local *P. leucopus* populations demonstrated that males did not provide paternal care of any sort and that they stopped interacting with the females once the copulation was over (Xia and Millar 1988). We also revealed, by a field experiment, that females close to oestrus have more males nearby than females far from oestrus (Xia and Millar 1989). These results were consistent with our hypothesis that males in this species mate polygynously. The mating behaviour of female *P. leucopus* in the wild is unknown, although Xia and Millar (1989) interpreted Myton's (1974) evidence as consistent with polyandrous mating by females. The ultimate proof of effective polyandry lies in genetic evidence showing multiple paternity in single litters. In this study, we examine the genetics of multiple paternity in single litters and estimate the proportion of litters having multiple paternity in natural populations of *P. leucopus.*

Methods

The study was conducted in deciduous forests north of London, Ontario, Canada (43°N, 81°W). Adult female *P. leucopus* were sampled with grids of Longworth live traps in the summer of 1987 and 1988 and allowed to give birth to field-conceived young in the laboratory. Altogether 34 and 37 females gave birth to field-conceived young in 1987 and 1988, respectively. Blood samples

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of at least 15 µl were taken by suborbital puncture from each female and her young at weaning and examined for genetic polymorphism for five loci, which were found to be highly polymorphic in *P. leucopus* populations studied by Robbins et al. (1985), using horizontal starch-gel electrophoretic procedures described in Selander et al. (1971). Three young died before their blood was sampled, and 6 young had electromorphs that were difficult to score. We assumed in subsequent analysis that these 9 young constituted a random sample and did not bias our estimation of allelic and genotypic frequencies. The five loci examined were (1) esterase-1, (2) hemoglobin, (3) albumin, (4) 6-phosphogluconate dehydrogenase and (5) transferrin. Except for esterase-1 (Es-1), which was highly polymorphic, the other four were either monomorphic (hemoglobin, albumin and 6-phosphogluconate dehydrogenase) or only slightly polymorphic (transferrin, with an allelic frequency of 0.984 for one allele and 0.014 for the other). Only data for Es-1 were used to detect multiple paternity in this study. To test for possible phenotypic consistency in Es-1, in 1987, 28 mice of representative genotypes were kept alive in the laboratory until 1988 and their blood was scored together with blood from mice caught in 1988. The blood samples from these 28 mice also served as a reference for scoring electromorphs of blood samples taken from mice caught in 1988.

Detection of multiple paternity

Two methods were used to detect multiple paternity in single litters. The first one was simply to examine whether a litter contained at least three different paternal alleles. The second was based on differences in number of "homogenetic" and "heterogenetic" litters between monogamy and effective polyandry, a litter being homogenetic if all young in the litter have the same genotype and heterogenetic if otherwise. Let us illustrate the method with a simple example. Suppose a population with a polymorphic locus of two alleles, A and B, with corresponding allelic frequency P_a and P_b. Denote genotype frequencies P_{aa}, P_{ab} and P_{bb}. With monogamy, expected genotype frequency of young in a litter can be calculated given parental genotypes. For example, if two AA homozygotes mate, all young in the resulting litter will necessarily have the same genotype of AA, i.e. a homogenetic litter. If an AB male mates an AB female, then there are three possible genotypes in the resulting litter, AA, AB and BB, with corresponding probabilities 0.25, 0.5 and 0.25, respectively, i.e. the litter may be heterogenetic. We will show below that the number of homogenetic litters will decrease, and heterogenetic litters increase, with the number of males with which each female mates. For an intuitive understanding, one can imagine that a litter will necessarily be homogenetic if it results from a homozygous female mating with either an AA *or* a BB male, but a litter will have a non-zero probability of being heterogenetic if the same female mates with both an AA male *and* a BB male.

Females each mating with only one male. When a female is homozygous, then the probability of her litter being homogenetic is 1 if she mates with a homozygous male, and equal to $2 \cdot 0.5^n$ if she mates with a heterozygous male. So the probability of a homozygous female i having a homogenetic litter, given one locus with two alleles, can be expressed as

$$\text{Prob(homoF)}_i = (P_{aa} + P_{bb}) + 2 \cdot P_{ab} \cdot 0.5^n \quad (1)$$

where n is litter size.

If a female is heterozygous, then the probability of her litter being homogenetic is $2 \cdot 0.5^n$ when she mates with a homozygous male, and $(2 \cdot 0.25^n + 0.5^n)$ when she mates with a heterozygous male. Thus, the probability of a heterozygous female j having a homogenetic litter is

$$\text{Prob(HeteroF)}_j = (P_{aa} + P_{bb}) \cdot 2 \cdot 0.5^n + P_{ab} \cdot (2 \cdot 0.25^n + 0.5^n) \quad (2)$$

The expected total number of homogenetic litters is the sum of all probabilities, one probability for each female. The expected number of heterogenetic litters is simply the difference between total number of litters and the number of homogenetic litters.

Table 1. Calculation of expected number of homogenetic litters when two males are involved in multiple matings

Maternal[a] Genotype	Paternal Category	Prob_1[b]	Prob_2[c]
Homo.	1	$P_{aa}^2 + P_{bb}^2$	1
	2	$2 \cdot (P_{aa} \cdot P_{ab} + P_{ab} \cdot P_{bb})$	$0.75^n + 0.25^n$
	3	$P_{ab}^2 + 2 \cdot (P_{aa} \cdot P_{bb})$	$2 \cdot 0.5^n$
Hetero.	1	$P_{aa}^2 + P_{bb}^2$	$2 \cdot 0.5^n$
	2	$2 \cdot (P_{aa} \cdot P_{ab} + P_{ab} \cdot P_{bb})$	$0.375^n + 0.5^n + 0.125^n$
	3	$P_{ab}^2 + 2 \cdot (P_{aa} \cdot P_{bb})$	$2 \cdot 0.25^n + 0.5^n$

[a] Homo. = homozygote; Hetero. = heterozygote
[b] Prob_1 = probability of different combinations of males
[c] Prob_2 = probability of a resulting litter being homogenetic given maternal genotype, specific combination of males and litter size

Females each mating with two males. With two alleles and three genotypes, nine different combinations of two males are possible. These nine combinations can be grouped into three paternal categories: (1) two males of the same homozygotes, i.e. only one type of sperm is contributed (paternal category 1 in Table 1), (2) one homozygous male and one heterozygous male (paternal category 2 in Table 1), contributing two different types of sperm in the ratio of 3:1 and (3) two heterozygous males, contributing two types of sperm in the ratio of 1:1 (paternal category 3 in Table 1). The probability of a female of a certain genotype producing a homogenetic litter, therefore, equals the product of two probabilities: the probability of the female encountering different combinations of two males (Prob_1 in Table 1) and the probability of the female producing a homogenetic litter given Prob_1 and litter size (Prob_2 in Table 1). Using the same symbols as before, equations corresponding to (1) and (2) are:

$$\begin{aligned}\text{Prob(HomoF)}_i = {} & (P_{aa}^2 + P_{bb}^2) \cdot 1 \\ & + [2 \cdot (P_{aa} \cdot P_{ab} + P_{ab} \cdot P_{bb})] \cdot (0.75^n + 0.25^n) \\ & + [P_{ab}^2 + 2 \cdot (P_{aa} \cdot P_{bb})] \cdot (2 \cdot 0.5^n)\end{aligned} \quad (3)$$

where P_{aa}, P_{ab} and P_{bb} are genotype frequencies and n is litter size; and

$$\begin{aligned}\text{Prob(HeteroF)}_j = {} & (P_{aa}^2 + P_{bb}^2) \cdot (2 \cdot 0.5^n) \\ & + [2 \cdot (P_{aa} \cdot P_{ab} + P_{ab} \cdot P_{bb})] \cdot (0.375^n + 0.5^n + 0.125^n) \\ & + [P_{ab}^2 + 2 \cdot (P_{aa} \cdot P_{bb})] \cdot (2 \cdot 0.25^n + 0.5^n)\end{aligned} \quad (4)$$

Females each mating with all males. With all males involved in multiple matings, the frequency of allele A and B in sperm is the same as allelic frequencies of the parental population, i.e. P_a and P_b. Thus, the probability of a homozygous mother producing a homogenetic litter is

$$\text{Prob(HomoF)} = P_a^n + P_b^n \quad (5)$$

where n is litter size, and the probability of a heterozygous mother producing a homogenetic litter is

$$\text{Prob(HeteroF)} = (P_a/2)^n + [(P_a + P_b)/2]^n + (P_b/2)^n. \quad (6)$$

Clearly, the number of homogenetic litters decreases with the number of males a female mates with, being the largest when each female mates with a single male and smallest when the female mates with all males in the population.

To find out whether females mate monogamously or polyandrously, one needs to calculate the number of homogenetic and heterogenetic litters expected under monogamy by using Eqs. 1 and 2, and to test whether the observed number of homogenetic litters is significantly smaller, and heterogenetic litters greater, than the expected value. A one-tailed significance test is appropriate

because the prediction is directional. A χ^2-test or G-test is a poor choice since both are always two-tailed and because of their simplicity, statistical power is lost. A one-tailed test can be done by summing up the probabilities of obtaining the observed number of homogenetic and heterogenetic litters and all other frequencies representing a greater deviation from expectation. For example, if the observed number of homogenetic and heterogenetic litters is 20 and 80, respectively, and the number of homogenetic and heterogenetic litters expected under monogamy is 40 and 60, respectively, then the probability that the sampled litters are from a monogamous population is the sum of the probabilities of obtaining the observed values (20 and 80) and other more extreme values – such as 19 and 81, 18 and 82, 17 and 83, up to 0 and 100 – from a binomial distribution of $(0.4+0.6)^{100}$. The sum of the probabilities in this case is smaller than 0.0001. One can therefore conclude that the result is not compatible with monogamy.

As our study involves a locus with four alleles, A, B, C and D, the equations for finding the number of homogenetic and heterogenetic litters expected under monogamy are slightly more complex than Eqs. (1) and (2). Let P_{homo} and P_{hetero} be the frequency of homozygotes and heterozygotes, respectively. The expected probability of a homozygous female producing a homogenetic litter, given litter size n, is

$$\text{Prob(HomoF)} = P_{\text{homo}} + 2 \cdot 0.5^n \cdot P_{\text{hetero}}. \quad (7)$$

The expected probability of a heterozygous female IJ (where I, J = A, B, C, D; I ≠ J), producing a homogenetic litter is

$$\text{Prob(IJ female)} = 2 \cdot 0.5^n \cdot P_{\text{homo}} + (2 \cdot 0.25^n + 0.5^n) \cdot P_{\text{IJ}} + 4 \cdot 0.25^n \cdot (P_{\text{hetero}} - P_{\text{IJ}}) \quad (8)$$

Such calculation was carried out for each female and the sum of probabilities was the expected number of homogenetic litters under monogamy. We should mention that Hardy-Weinberg equilibrium is assumed in calculating paternal genotypic frequencies and that the test is conservative with inbreeding.

Estimating frequency of litters resulting from multiple paternity

Birdsall and Nash (1973) and Merritt and Wu (1975) introduced a method for estimating frequency of litters with multiple paternity for genetic loci of three different alleles, and we extended the method to include loci of four alleles. In order to identify a litter of multiple paternity with certainty, at least three different paternal alleles must be identified in the litter. The probability of finding such a litter depends on (1) probability of paternal males carrying three or four different alleles of the locus in question (Pr_1), which in turn depends on genotypic frequency and number of males involved in multiple matings and (2) the probability of these three or four different paternal alleles being present in the young (Pr_2), which in turn depends on the maternal genotype, litter size and allelic frequency of sperm population she receives, which in turn depends on number of males she mates.

The relationship between Pr_1, Pr_2, and the number of males involved in multiple matings requires special attention because it is impossible to know how many males were actually involved in multiple matings in the wild or whether all multiple matings involved the same number of males. For this reason, Pr_1 is calculated for two extreme situations: (1) when only two males are involved in multiple matings (henceforth referred to as two-male case) and (2) when all males in the population are involved in multiple matings (henceforth referred to as all-male case). Both Pr_1 and Pr_2 are at a minimum in the two-male case and a maximum in the all-male case. Correspondingly, expected number of litters with multiple paternity is at a minimum in the two-male case and a maximum in the all-male case. These two estimates (minimum and maximum) provide two reference points for comparison with the observed number of litters with multiple paternity. For example, if the observed number of litters with at least three paternal alleles is N_0, and the expected number of litters with at least three paternal alleles is N_{two} in the two-male case and N_{all} in the all-male case, then the proportion of litters resulting from multiple insemination from more than one male is N_0/N_{two} for the two-male case and N_0/N_{all} for the all-male case. The difficulty of the method, therefore, lies in the calculation of N_{two} and N_{all}, each of which requires separate estimation of Pr_1 and Pr_2.

Two methods can be used to calculate Pr_1, one deriving the probability from the observed genotypic frequency and the other from allelic frequency assuming Hardy-Weinberg equilibrium. For example, the probability of paternal males carrying four different alleles of Es-1 in the two-male case can be calculated either from the observed genotypic frequency as $2[(P_{ab} \cdot P_{cd}) + (P_{ac} \cdot P_{bd}) + (P_{ad} \cdot P_{bc})]$ or from allelic frequency as $24 \cdot P_a \cdot P_b \cdot P_c \cdot P_d$. In our study, we used the first method because it is less affected by deviations from Hardy-Weinberg equilibrium than the second. In the all-male case, each female will have available to her a sperm population that has the same allelic frequency as that of the male population as a whole.

Given that a female mates with males carrying three or four different alleles (Pr_1), the probability that at least three different paternal alleles are realized in the resulting litter (Pr_2) can be calculated as follows.

1. When only two males are involved in the multiple mating and the female is homozygous, then Pr_2 can take only two values, one when the female mates two males carrying only three different alleles and one when she mates two males carrying four different alleles. The first can be calculated by expanding the expression $(P_a+P_b+P_c)^n$ (where P_a P_b and P_c are allelic frequency of the sperm population contributed by the two males and equal 0.25, 0.5 and 0.25, respectively, and n is litter size) and summing up those terms which include P_a, P_b and P_c. The second can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where $P_a=P_b=P_c=P_d=0.25$, n=litter size) and summing up those terms which include any three of P_a, P_b, P_c and P_d.
2. When only two males are involved and the female is heterozygous, then Pr_2 can take five values; four values when males carry three different alleles and one value when males carry four different alleles. The last value can be calculated by expanding $(P_a+P_b+P_c+P_d+P_e)^n$ (where P_a is the probability of young sharing the same genotype as the mother, P_b and P_c are the probabilities of young being homozygous for each of the alleles carried by the mother, and P_d and P_e is the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother) and summing up those terms that include (1) P_a, P_d and P_e or (2) P_b, P_d and P_e or (3) P_c, P_d and P_e or (4) P_b, P_c and P_d or (5) P_b, P_c and P_e. The four values when two males carry three different alleles of a locus can be calculated as follows:

a. When the heterozygous female has the allele present twice in males and lacks only one allele present in males (e.g. AB female with AB, AC males), Pr_2 can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where P_a is the probability of the offspring sharing the same genotype as the mother, P_b and P_c are the probabilities of an individual being homozygous for each of the alleles carried by the mother, and P_d is the sum of the probabilities of an offspring carrying the allele not present in the mother) and summing up those terms that include P_b, P_c and P_d.

b. When 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), Pr_2 can be calculated by expanding $(P_a+P_b+P_c)^n$ (where P_b and P_c are the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother and $P_a=1-P_b-P_c$, n=litter size) and summing up those terms that include P_a, P_b and P_c.

c. When 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), Pr_2 can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$, where symbols mean the same as in a, and summing up those terms that include P_b, P_c and P_d.

d. When the heterozygous female lacks the allele present twice in males and possesses only one of the three paternal alleles, Pr_2 is the same as in b.

3. When all males are involved in the multiple mating and the female is homozygous, Pr_2 can be calculated by expanding $(P_a+P_b+P_c+P_d)^n$ (where P_a, P_b, P_c and P_d are gene frequencies of the population and n=litter size) and summing up those terms that include any three of P_a, P_b, P_c and P_d.
4. When the female is heterozygous, Pr_2 can be calculated by expanding $(P_a+P_b+P_c+P_d+P_e)^n$ (where P_a is the probability of young sharing the same genotype as the mother, P_b and P_c are the probabilities of young being homozygous for each of the alleles carried by the mother, and P_d and P_e is the sum of the probabilities of an offspring carrying each of the two alleles not present in the mother) and summing up those terms that include (1) P_a, P_d and P_e or (2) P_b, P_d and P_e or (3) P_c, P_d and P_e or (4) P_b, P_c and P_d or (5) P_b, P_c and P_e.

It should be mentioned that the above method assumes the absence of a null allele or presence of it at negligible frequency. Robbins et al. (1985) studied genetic polymorphism of Es-1 for 21 populations of *P. leucopus* over North America and no null allele was reported; the assumption of the absence of a null allele is likely justified. The method also assumes Hardy-Weinberg equilibrium and equal contribution of sperm by males participating in multiple-male matings. Inbreeding and unequal contribution of sperm by the participating males result in an underestimate of the frequency of multiple-paternity litters, while outbreeding results in an overestimate. More details concerning the assumptions of the method can be found in Birdsall and Nash (1973) and Merritt and Wu (1975).

Results

Samples of *P. leucopus* taken in 1987 and 1988 had the same four alleles and 10 potential genotypes (Table 2). Blood samples taken from the same mouse in 1987 and 1988 (N=28) did not show different band patterns, suggesting phenotypic consistency of Es-1 at different times in this population of *P. leucopus*. The observed genotypic frequencies fit closely to those expected under Hardy-Weinberg equilibrium (Table 2). The greatest discrepancy between the observed and expected genotypic frequencies occur in young sampled in 1988 (Table 2), but the probability of the discrepancy being due to chance is still greater than 0.05, based on a chi-square test of goodness-of-fit. There are some reasons that the population may indeed be in Hardy-Weinberg equilibrium.

Table 2. Genotypic frequencies for Es-1 of samples taken in 1987 and 1988

Genotype	1987				1988			
	Dam		Young		Dam		Young	
	O	E	O	E	O	E	O	E
AA	22	21.68	93	90.15	14	13.60	43	41.00
AB	3	4.25	19	21.06	10	12.01	51	63.89
AC	2	1.70	4	3.37	0	0.00	1	1.01
AD	2	1.70	5	9.27	5	3.79	25	16.10
BB	1	0.21	3	1.23	4	2.65	34	24.89
BC	0	0.17	0	0.39	0	0.00	1	0.78
BD	0	0.17	0	1.08	1	1.68	7	12.54
CC	0	0.03	0	0.03	0	0.00	0	0.01
CD	0	0.07	0	0.17	0	0.00	0	0.20
DD	0	0.03	3	0.24	0	0.26	0	1.58

O = observed; E = expected

First, alleles at Es-1 locus are usually considered neutral, so the effect of selection in relation to Hardy-Weinberg equilibrium may be ruled out. Second, adult females do not disperse (Wolff 1989), so the distribution of genotypic frequency in adult females should not be affected by migration. The last factor affecting Hardy-Weinberg equilibrium (excluding mutation, which should be trivial anyway) is the breeding system. If random mating did not hold for our population, then we should expect observed genotypic frequencies to deviate from Hardy-Weinberg equilibrium, which did not occur (Table 2). Thus, our assumption of Hardy-Weinberg equilibrium may be justified.

The result of a close fit between the observed and expected genotypic frequencies also lends support to our assumption of the absence of a null allele for the following reason. If a null allele were present, then individuals heterozygous for this null allele and a scorable allele would have been recorded as homozygotes for that scorable allele (false homozygotes). If the frequency of the null allele was as high as 0.05, then approximately 10% of individuals in the population would have been false homozygotes. These false homozygotes, added to those true homozygotes, would have resulted in an excess of homozygotes and a deficiency of heterozygotes to upset Hardy-Weinberg equilibrium, given our sample size. The fact that no significant departure from Hardy-Weinberg equilibrium was observed in both years suggests that the null allele, if present, must be at a very low level.

Detection of multiple paternity in single litters

One litter in 1987 and 6 litters in 1988 contained ≥ 3 different paternal alleles (Table 3), which implies that they resulted from multiple insemination by more than one male.

Table 3. Litters with multiple paternity in 1987 and 1988

Year	Maternal genotype	Offspring genotype
1987	AA	AB, AA, AD
1988	AA	AA, AB, AB, AD, AD
	AA	AA, AB, AB, AB, AC, AD
	AA	AA, AB, AB, AB, AD
	AA	AA, AA, AA, AB, AB, AD
	AA	AA, AA, AB, AB, AD
	AA	AA, AB, AB, AD, AD, AD

Table 4. Observed number of "homogenetic" litters (all young in the litter having the same genotype) and "heterogenetic" litters compared with expected values calculated with the assumption of monogamy

Year	Type of litter	Observed	Expected	P
1987	heterogenetic	15	10	0.0434
	homogenetic	15	20	
1988	heterogenetic	31	23	0.0008
	homogenetic	2	10	

Of 30 litters in 1987 and 33 litters in 1988 that had at least two scorable young, the observed number of homogenetic litters were smaller and heterogenetic litters greater than those expected under the assumption of monogamy (Table 4). A one-tailed significance test rejected the null hypothesis of monogamy ($P=0.0434$ in 1987 and 0.0008 in 1988).

Table 5. Probability of a female encountering different sperm pool given that only two males are involved in multiple matings and contribute equally to the sperm pool

Paternal alleles	Probability	
	1987	1988
2A,B,C	0.015603	0.005517
2A,B,D	0.033808	0.113097
2A,C,D	0.006761	0.001300
2B,A,C	0.001766	0.004017
2B,A,D	0.003827	0.082338
2B,C,D	0.000087	0.000689
2C,A,B	0.000353	0.000046
2C,A,D	0.000153	0.000011
2C,B,D	0.000017	0.000008
2D,A,B	0.001658	0.019402
2D,A,C	0.000332	0.000223
2D,B,C	0.000038	0.000162
A,B,C,D	0.001531	0.001893

Table 6. Probability of females of different genotypes obtaining sperm of different combinations of at least three different paternal alleles, assuming that only two males are involved in multiple matings

Maternal genotype[a]	Paternal alleles	Special case[b]	Probability	
			1987	1988
AA, BB	3		0.06440	0.22687
	4		0.00153	0.00189
AB	3	1	0.05501	0.20496
		2	0.00695	0.00198
		3	0.00201	0.01944
		4	0.00054	0.00040
	4		0.00153	0.00189
AC	3	1	0.02297	0.00688
		2	0.03383	0.11310
		3	0.00210	0.00424
		4	0.00561	0.10260
	4		0.00153	0.00189
AD	3	1	0.04266	0.13404
		2	0.01564	0.00568
		3	0.00408	0.08234
		4	0.00222	0.00476
	4		0.00153	0.00189

[a] Although there are ten possible genotypes with four alleles, only genotypes AA, AB, AC, AD and BB were found in mothers

[b] Special case: (1) the female has the allele present twice in males and lacks only one allele present in males; (2) the female has the allele present twice in males and lacks two other alleles present in males; (3) the female lacks the allele present twice in males but possesses the other two paternal alleles; (4) the female lacks the allele present twice in males and possesses only one of the three alleles in males

Estimating frequency of litters due to multiple paternity

1. Two-male case. The probability of a female encountering two mates carrying three or four different alleles (Pr_1) was calculated separately for 1987 and 1988 (Tables 5 and 6). Given Pr_1, the probability of a female producing a litter with at least three different paternal alleles present (Pr_2) was calculated for different combinations of maternal genotype and for litter size (Table 7). We then calculated the probability that mothers of different genotypes and litter sizes produced litters with at least three different paternal alleles. We will use mothers AA and AB of 1987, each producing a litter of five to illustrate the actual calculation. For mother AA, the probability of her mating two males carrying three different alleles was 0.0644 (Table 6) and, given such a mating, the probability of her having a litter carrying all three paternal alleles was 0.5273 (Table 7). The product of the two probabilities was 0.0340. The female

Table 7. Probability of a female producing a litter with at least three different paternal alleles present, given maternal genotype, number of paternal alleles and litter size, assuming only two males are involved in the multiple mating

Maternal genotype	Paternal alleles	Special case	Litter size	Probability
Homo.	3		3	0.18750
			4	0.375
			5	0.52734
			6	0.64453
			7	0.73315
	4		3	0.375
			4	0.65625
			5	0.82031
			6	0.90820
			7	0.95361
Hetero.	3	1	3	0.04688
			4	0.12988
			5	0.22705
			6	0.32730
			7	0.42161
		2	3	0.18750
			4	0.375
			5	0.52734
			6	0.64453
			7	0.73315
		3	3	0.04688
			4	0.11719
			5	0.19409
			6	0.27008
			7	0.34230
		4	3	0.18750
			4	0.375
			5	0.52734
			6	0.64453
			7	0.73315
	4		3	0.23438
			4	0.46875
			5	0.64819
			6	0.77271
			7	0.85516

Table 8. Calculation of the probability of a litter having at least three different paternal alleles given an AB mother in 1987 with a litter of five

Paternal alleles	Special case	Pr_1	Pr_2	$Pr_1 \cdot Pr_2$
3	1	0.05501	0.227051	0.0124901
	2	0.00695	0.527344	0.0036650
	3	0.00201	0.194092	0.0003901
	4	0.00054	0.527344	0.0002848
4		0.00153	0.648193	0.0009917

also had a probability of 0.0015 (Table 6) of mating two males carrying four different alleles and, given such a mating, the probability of her having a litter with at least three different paternal alleles present was 0.8203 (Table 7). The product of the two probabilities is 0.0013. The sum of these two products (=0.0352) is the probability of an AA mother, in the two-male case, producing a litter of five that contains at least three different paternal alleles. The calculation for the AB mother is much more complicated (Table 8), and the probability that she has a litter containing at least three different paternal alleles is equal to 0.0178, which is the sum of the last column, i.e. $Pr_1 Pr_2$, in Table 8. Such calculation was carried out for each female with at least three young in 1987 ($N=29$) and 1988 ($N=32$). The expected number of litters that contain at least three different paternal alleles was 3.6 (0.7 in 1987 and 2.9 in 1988) in the two-male case. The observed number of litters that contain at least three different paternal alleles was 7 (1 in 1987 and 6 in 1988). As 7 out of a sample of 61 (=29+32) has a lower limit of 2.6 at 0.95 confidence interval (Sokal and Rohlf 1981), we conclude that, given the two-male case, at least 72% (=2.6/3.6) of litters were sired by multiple fathers.

2. All-male case. Pr_1, the probability of a female mating with males carrying at least three different alleles, is now equal to 1, and the allelic frequency of sperm population a female received is the same as the allelic frequency of the population. Pr_2, the probability of a female producing a litter with at least three different paternal alleles present, given Pr_1 and gametic frequency, depends only on maternal genotype and litter size (Table 9). If a homozygous female produced a litter of five in 1988, then the probability of this litter containing at least three different paternal alleles was 0.338691; if a heterozygous female such as AB produced a litter of five in 1988, then the litter had only a probability of 0.163905 of containing at least three different paternal alleles (Table 9). The expected number of litters containing at least three paternal alleles was 2.0 in 1987 and 8.3 in 1988, as calculated from 29 mothers in 1987 and 32 mothers in 1988, with each of the mothers having at least three scorable young. Thus the frequency of litters due to multiple insemination involving all males was 50% (=1/2.0) and 72% (=6/8.3). Assuming that the degree of multiple paternity does not change over years, then the average of the frequency is 0.68 (=7/10.3). Because 7 out of a sample size of 61 (=29+32) litters had a lower limit of 2.6 at 0.95 confidence interval (Sokal and Rohlf 1981), the lower limit of frequency of litters due to multiple insemination involving all males was 25% (=2.6/10.3). The real value was almost certainly larger than 25% because it is hardly possible for a female to mate with all males in the population during one estrus due to temporal and spatial constraints.

Table 9. Probability of a litter with at least three different paternal alleles when all males are involved in multiple matings

Maternal genotype	Litter size	Probability	
		1987	1988
AA, BB	3	0.033733	0.120397
	4	0.065935	0.238503
	5	0.101480	0.338691
	6	0.140132	0.421466
	7	0.180779	0.490142
AB	3	0.011778	0.032046
	4	0.030048	0.090062
	5	0.053674	0.163905
	6	0.080859	0.244084
	7	0.110319	0.323934
AC	3	0.023795	N/A
	4	0.050067	N/A
	5	0.080251	N/A
	6	0.113502	N/A
	7	0.148709	N/A
AD	3	0.015707	0.035522
	4	0.036350	0.087701
	5	0.061501	0.144921
	6	0.089662	0.201102
	7	0.119617	0.253687

N/A = not applicable

Discussion

Our finding that females in wild populations of *P. leucopus* mate polyandrously provides an explanation for all previous, seemingly contradictory, findings. For example, Myton's (1974) observation of family groups with one adult female and several adult males is likely due to adult males clumping around an adult female near estrus. In an enclosure study, Xia and Millar (1988) found that the spatial relationship between males and females changes with the breeding status of the females, with males associating with females only when the latter were in estrus or close to estrus. Xia and Millar (1989) further demonstrated in a field study that adult females close to estrus had more males nearby than adult females far from estrus. These results were consistent with Xia and Millar's (1988, 1989) hypothesis that males tend to mate polygynously and that they adjusted their spatial relationship to females according to chance of mating. Thus, Myton's (1974) social groups were probably not permanent and males likely changed their "groupship" according to chances of mating. Mineau and Madison's (1977) observation of "pair activity" may involve more

than one male and one female, but only one male and one female out of a group of mice were radio-tracked. The paternal care documented by Horner (1947), McCarty and Southwick (1977) and Hartung and Dewsbury (1979) may represent abnormal behaviour caused by caging conditions (Xia and Millar 1988), and one should be extremely careful in generalizing from these laboratory results to field conditions (Hartung and Dewsbury 1979). The mating system in wild populations of *P. leucopus* appears to involve simultaneous polyandry by females and serial polygyny by males. At least 25% (the upper limit being 100%) of litters conceived under natural conditions appear to result from multiple insemination involving more than one male.

We have inferred polygynous matings by males on the basis of Xia and Millar (1988, 1989). The fact that females mate polyandrously confirms our inference, for the following reason. The sex ratio of adults is not male-biased in our population. The sex ratio at birth is 167:155 (male:female) according to our own data of field-conceived litters, and the 95% confidence interval for the percentage of males is 0.46–0.58. The survival of juveniles in the summer, as estimated by Harland et al. (1979), is 0.69 for males and 0.72 for females, i.e. juvenile females do not suffer higher mortality than juvenile males. Thus, there is no reason that the operational sex ratio should be male-biased in natural populations. Given that the operational sex ratio is not male-biased and that an average of 68% (25%–100%) of females mate polyandrously, the population would soon run out of sexually active males if each male mates with just one female. Thus, at least some males must mate with more than one female. In a test we carried out in the laboratory, none of 12 males, each of which had copulated with an estrous female 2 days earlier, refused to copulate with a second estrous female. It is our belief that the majority of males in natural populations of *P. leucopus* have a tendency to mate polygynously.

There are several possible reasons that males should mate polygynously. For purpose of illustration, let us start with a monogamous population with males providing paternal care and examine if mutant males that mate polygynously can increase in frequency in the population. Whether polygynous matings by males will be favoured depends on the fitness increment derived from paternal care relative to that derived from remating, assuming that a male cannot both mate polygynously and provide paternal care. It is thus worthwhile to consider factors that affect fitness increment derived from paternal care and remating. First, the probability of remating is high in wild populations of *P. leucopus* as there are estrous females throughout the entire breeding season. Second, no matter how tight the pair-bond may be, predation will necessarily result in some females losing their partners. As a consequence, selection will favour females that can rear the young single-handedly, leading to a reduced importance of paternal care. This would also favour males deserting their mates and seeking changes of remating. Third, monogamy with paternal care implies commitment of a male to a specific female. Unfortunate for this mating strategy, reproductive success of females in *P. leucopus* (Rintamaa 1976) varies widely, with some females producing no young in the entire breeding season and some producing as many as eight litters (Rintamaa et al. 1976). If a male bet his reproductive success on a single female, then his reproductive success will fluctuate more widely than that of a male that mates polygynously. For example, the variance of reproductive success of polygynous males mating four females is 2 ($=\sqrt{4}$) times smaller than that of monogamous males, other things being equal. It can be shown numerically that, in a finite population, a male is at a selective disadvantage if he bets his reproductive success on a single female. This argument assumes that males cannot predict future reproductive performance of females, but the relaxation of this assumption does not lead to monogamy. If males can predict female quality, then competition for the few good mothers, e.g. those producing eight litters in one breeding season, would be very intense and increase the probability of many males mating few females. Because all these three reasons appear to reduce the benefit of paternal care and increase the benefit of remating, it is not surprising that males should have polygynous tendency.

While it is not difficult to see the evolutionary advantage of polygynous matings by male *P. leucopus,* it is by no means easy to understand why females should mate polyandrously. One possible explanation involves pseudopregnancy in the species. Dewsbury (1984a, 1984b) found that male deer mice lose much of their inseminating power after a few consecutive copulations, but their vigor in copulation can stimulate pseudopregnancy (Conaway 1971). The duration of pseudopregnancy in *Cricetidae* is about 2 weeks (Conaway 1971; Dewsbury 1984a), which is a significant portion of a limited breeding season. As Conaway (1971) pointed out, a nonpregnant cycle in short-lived rodents appear to be a pathological luxury that should not be tolerated by natural selection. One strategy for females against pseudopregnancy would be to mate with more than one male so as to increase the likelihood of mating with at least one "fresh" male. In other words, a mutation predetermining its carriers to mate polyandrously is likely to spread and become fixed in natural populations.

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